Hydrogels for central nervous system therapeutic strategies

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Introduction

As reported in the literature, the central nervous system (CNS) shows a limited capacity to regenerate spontaneously. For this reason, different regenerative strategies and treatment options for patients with CNS diseases and injuries have been analyzed and proposed.[1]

Anyway, as a consequence of the CNS complexity, it is difficult to individuate successful therapeutic strategies, and the use of the common delivery methods (i.e. intravenous and oral) is strongly restricted by the limited diffusion of drugs and biomolecules across the blood–brain barrier (BBB). Hydrogel-based materials have been already proposed and widely studied in the biomedical field.

In this context, this work will report many examples of several strategies adopted for CNS diseases. A brief overview of different approaches will be first presented and then the design of injectable hydrogels for in situ drug or cell release will be reported as a minimally invasive and interesting solution in the development of successful treatments for CNS neurodegenerative disorders, also focusing the attention on the possibility to properly optimize the rheological or mechanical and functional features of such devices.[1–10]

Cell-based therapy

As reported in the literature, cell-based therapies and delivery of bioactive molecules (i.e. small molecules, growth factors and antibodies[11,12]), also involving the use of hydrogels and nanoparticles (NPs) as platform for cell, growth factor or drug release, are employed for promoting tissue regeneration after injury. Cell-based therapies aim at replacing damaged cells and/or at maintaining cell viability, also promoting tissue regeneration. Cells have to be integrated into the host tissue in a direct way or through the secretion of factors for neurogenesis or neuroprotection.[13] Somatic stem cells (i.e. neural stem cell), embryonic stem cells (ESCs), CNS progenitor cells and cells derived from induced pluripotent stem cells (iPSs) may provide potential

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therapeutic effects.[14,15] Human ESCs (hESCs) provided a potentially endless specialized source of neural progenitor cells when employed as models to study human development.[14,16] Uncontrolled growth and teratoma formation have been often associated with platforms based on hESCs, and consequently, prior to clinical testing, effectiveness and safety must be demonstrated through further studies.[14,17,18] Taking into account the potential of generating patient-specific cells for autologous use, a great attention has been recently devoted to iPSs, with the aim to possibly avoid the need for immunosuppression.[14,19,20] The potential risk of tumor formation associated with the use of cells derived from iPS and hESC sources strongly limits their clinical impact.

Neural stem cells as somatic stem cells also represent tissue-specific or adult-derived stem cells. Human neural stem cells (hNSCs), which can be isolated from either mature or developing human brain tissue, are able to self-renew and differentiate into oligodendrocytes, astrocytes and neurons. Once properly isolated and expanded, fluorescence-activated cell-sorted (FACS) hNSCs will be able to maintain their ability to re-initiate neurosphere formation.[14,21,22] In addition, some studies on such obtained cells have highlighted how they display a normal karyotype, do not require specific factors for pre-differentiation and are able to retain multipotentiality, without forming tumors in vivo. Furthermore, even though safety and preclinical testing needed for gene-modified stem cells are more complex than for nonmodified ones, the use of neural stem cells for gene therapy has also been proposed, and this approach could be useful in specific disease cases.[14,22–27]

Some studies on preclinical disease models have highlighted that mesenchymal and umbilical cord blood stem cells are a very promising therapeutic tool for neurodegenerative or traumatic disorders affecting CNS, even if they differ from the neural stem cells in terms of action mechanism, cell survival and potential differentiation.[28–32]

It has also been analyzed the possibility to release many different protective soluble factors produced by mesenchymal stem cells (MSCs) from a nanostructured hydrogel to the brain parenchyma. A tailored nanostructure would play an important role in modulating the viscoelastic properties of the materials before and after the injection through clinical needles (see section "Injectable hydrogels"), as well as the release kinetics of specific biofactors, however influencing the behavior of cells. As an example, the use of bone marrow stem cells has provided interesting results in the brains of Alzheimer's disease (AD) mice, selectively producing the chemoattractant factor CCL5 and promoting the activation of endogenous microglia.[33] Furthermore, in vitro and in vivo studies demonstrated that human umbilical cord blood-derived MSCs secrete a soluble intracellular adhesion molecule-1 reducing amyloid-β plaques.[34]

With regard to treatment of Parkinson's disease (PD), the paracrine effect of MSCs and the release of glial-derived neurotrophic factor (GDNF) have been studied by Whone et al.[35] Such research evidenced some beneficial effects which were probably mediated by GDNF release, as the soluble factors produced by native human mesenchymal stem cells (hMSCs) were able to protect cultured monoaminergic perikarya and monoamine neurotransmitter transporter function.

Neurotrophic factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), neurotrophin-3 (NT-3), ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF), as well as extracellular matrix (ECM) proteins, such as fibronectin, laminin, collagen I or III and collagen IV represent the most widely considered factors in the clinical approach of cell-based therapies.[1,36–38] Different studies[1,39] performed on stroke-injured brain of human patients have shown no adverse effects concerning MSC transplantation[1,40] or an immortalized cell line of human teratocarcinoma-derived neurons.[1,41] However, poor cell survival together with uncontrolled differentiation and ineffective integration into the host tissue has strongly limited the efficacy of cell transplantation techniques.

Release of bioactive molecules

Several bioactive molecules have been demonstrated to promote tissue regeneration (i.e. neurogenesis, axonal regeneration, plasticity and neuroprotection). As an example, strategies based on intraventricular sequential release of epidermal growth factor (EGF) and erythropoietin (EPO) into stroke-injured brains (i.e. rat model) showed an improved migration of endogenous neural stem or progenitor cells (NSPCs) to the injury site, promoting neurogenesis and functional recovery.[1,42] In this field, specific growth factors[43,44] have been shown to be neuroprotective and promote axonal outgrowth. However, some delivery strategies are limited because of the low permeability of the BBB and blood–spinal cord barrier,[45] which require high systemic doses for reaching therapeutic concentrations at the injury site, often inducing local delivery strategies or cytotoxicity. Moreover, systemic administration can provide undesired side effects (i.e. tumor, fibrosis)[46] as it also leads to off-target distribution of therapeutic agents.

Current methods based on direct drug delivery to the CNS involve bolus injection and continuous infusion through a catheter or minipump system. With regard to the bolus injection into the intrathecal space for delivery to the spinal cord, the continuous cerebrospinal fluid flow disperses the injected drug throughout the CNS, thus resulting in a minimization of the local release.[11,47] The use of the catheter or minipump system requires invasive surgery. Consequently, cell and tissue death around the insertion site and a great risk of infection from the external minipumps are usually

caused.[48–50] The strongly limited diffusion of therapeutics from the ventricular surface into the brain parenchyma hinders the intraventricular infusion.[51] To target the retina, the protective ocular barriers are penetrated by applied drops with difficulty. As a consequence, invasive delivery techniques are normally employed often causing retinal detachment, vitreous hemorrhage and infection.[52]

NP-based drug delivery

Many efforts have been made to develop strategies for improving drug permeability across the BBB, also including drug delivery via NPs or liposomes.[53] Basically, many techniques for the synthesis of polymer-based NPs,[54,55] as well as for the encapsulation of several drugs and bioactive molecules, proteins, peptides or nucleic acids, have been proposed and analyzed. Several biodegradable polymer-based NPs have been widely considered because of some peculiar features such as biocompatibility, subcellular size, controlled-sustained release property, stability in the blood, nontoxicity and nonthrombogenicity.[54–58] Different nanosystems for drug delivery through ocular, oral and nasal administration as well as several strategies of synthesis and encapsulation of specific biomolecules have been also reported in the literature.[55,59] Several polymers have been considered for the NP synthesis, taking into account degradation rates and mechanism, according to the specific application.

It has also been suggested that polymeric NPs without surface modification show a limited ability to cross the BBB. For this reason, surface modification using ligands or surfactants may improve receptor-mediated endocytosis. However, the presence of positive charges may improve adsorptive-mediated endocytosis.[60]

In this context, polyesters such as poly-ε-caprolactone (PCL), poly(glycolic acid) (PGA) and poly(lactic acid) (PLA) as well as their copolymer poly(lactic-*co*-glycolic acid) (PLGA) have also been investigated, considering the interesting results obtained in the field of medicine.[54,60–64] It is well reported that the above-mentioned polymers are biocompatible and biodegradable, and the by-products degradation can be easily removed.

NPs may also be formed by chitosan, which is a biocompatible and biodegradable polysaccharide.[60,65] With regard to the preparation of chitosan NPs, many different methods have been described (i.e. ionotropic gelation, emulsification solvent diffusion, microemulsion and polyelectrolyte complex).[66] Wang et al.[67] have shown that intranasal delivery of estradiol-loaded chitosan NPs can lead to a significant amount of estradiol within the CNS.

The delivery of peptides, caspase inhibitors and dopamine to the CNS has been studied and optimized.[68–70] Furthermore, to possess a variety of ligands for BBB bypass, chitosan NPs may be suitably surface modified.[71] Nagpal et al. also demonstrated that at pH lower than 6, the amino groups of chitosan are protonated. Thus, it is positively charged and is an attractive one for nucleic acid delivery.[65]

Like chitosan, poly(ethylenimines) (PEIs) are cationic polymers may be employed for nucleic acid delivery. For example, disulfide-linked PEI NPs have been reported to deliver micro-RNAs to the CNS.[72,73]

Even though gelatin is widely employed in food and medical products, it is also an attractive one for drug delivery applications as it is biodegradable, nontoxic and bioactive. Specifically, it is a polyampholyte possessing both anionic and cationic groups along with hydrophilic group. Its swelling behavior, thermal and mechanical properties depend on the cross-linking degree.[54] With regard to the preparation methods, gelatin NPs can be produced through desolvation or coacervation[74] or emulsion,[75] and they have been used to encapsulate different peptide sequences to be delivered in a diffusion-controlled manner.[76]

Gelatin–siloxane (GS) NPs have been suitably modified with trans-activating transcriptor (TAT) peptide improving plasmid DNA transfection efficiency and the efficiency of SynB-poly(ethylene glycol) (PEG) NPs decorated with GS (SynB-PEG-GS) was investigated through in vitro and in vivo analyses by Tian et al.,[77] using brain capillary endothelial cells, a co-cultured BBB model and a normal mouse model. As for SynB-PEG-GS NPs, an efficient brain capillary endothelial cell uptake and an improvement in the BBB were obtained. In addition, it was demonstrated that the modification with the SynB peptide could enhance the efficiency of the NPs in crossing the BBB.[77] Didanosine-loaded and mannan-coated gelatin NPs were prepared using the double desolvation technique and then incubated with a mannan solution. Drug release and effects on cell behavior were properly analyzed.[78–80]

However, gelatin microspheres were also employed as a carrier during instrastriatal administration, providing an enhancement in the neuroprotective effects of osteopontin. Anyway, because of their size, gelatin microspheres have difficulties in entering the brain parenchyma via intranasal administration. For this reason, the delivery of an osteopontin peptide through gelatin NPs has been studied, especially in the case of ischemic stroke treatment, evidencing a significant decrease in mean infarct volume and suitably extending the therapeutic window of such intranasally administered peptide. Thus, gelatin NPs may be considered as a promising drug delivery system for the intranasal ischemic stroke treatment and, eventually, other neurologic disorders.[80]

In general, NPs could also play a key role in MSC tracking. A matter of great debate is represented by the fate of injected or transplanted MSCs into the body in animal or human models.[81] The cytotoxicity of superparamagnetic iron oxide nanoparticles (SPIONPs) employed as a contrast agent in magnetic resonance imaging for tracking labeled cells after transplantation in vivo and labeling cells in vitro, as well as the effects

on the neural differentiation of human amniotic membrane-derived MSCs (hAM-dMSCs) have been studied.[82]

In a further research, the effective concentration of SPIONPs to track MSCs has been analyzed through the evaluation of labeling toxicity and influence on multiple differentiated MSCs. The results demonstrated that at low concentrations of SPIONPs, cells were effectively labeled maintaining their proliferation and differentiation capacity.[83]

Injectable hydrogels

Biodegradable polymer-based devices have been considered as drug depots for sustained delivery.[84,85] However, these devices often require invasive surgical techniques for the implantation, and an appropriate alternative may be represented by injectable in situ gelling hydrogels. Cell-based therapy, release of bioactive molecules and NP-based drug delivery have been already discussed in the previous sections, and a combination of strategies involving bioactive molecules, cells and biomaterials should represent an interesting strategy to improve cell survival and integration as well as to achieve local delivery to the brain.[11,86,87] Advanced delivery vehicles for therapeutic molecules should provide a sustained and tunable drug release profile, thus avoiding multiple and high-dosage treatments.[88] Such delivery vehicles may also provide physical support for cells. In this scenario, injectable hydrogels also loaded with NPs (i.e. nanocomposite hydrogels) for in situ cell or drug release may be considered a minimally invasive solution for improving the effectiveness of potential therapeutic strategies for the treatment of severe neurodegenerative disorders, such as PD and AD.[89–92]

For this reason, several formulations of injectable materials able to form gels in situ have been recently proposed. For example, polymers such as chitosan or alginate form gels due to ionic interactions, either through changes in pH or addition of salts,[93] whereas other materials may gel as a consequence of temperature increase if they have a lower critical solution temperature (LCST) below body temperature. A light-induced hydrogel formation may also be obtained through the use of an appropriate photoinitiator in the presence of monomers.[94]

Among the natural polymers, agarose, which is a polysaccharide of D-galactose and 3,6-anhydro-L-galactopyranose, may gel as temperature is decreased, and it has been widely investigated for drug delivery applications.[95] Agarose gels through hydrogen bonding upon cooling, and if unmodified, it gels very slowly at body temperature,[96] and in order to overcome this limitation, an external liquid nitrogen cooling system has also been developed, thus inducing a quick gel formation in situ.[95]

Experimental analyses on rat model with a dorsal over-hemisection injury at T10, involving the injection of agarose solutions with BDNF-loaded lipid microtubules into the intrathecal space, have highlighted interesting results. A reduction in the astrocyte reactivity and in the production of chondroitin sulfate proteoglycans was achieved as a consequence of BDNF release, however improving the number of regenerating fibers that entered the hydrogel chondroitinase ABC-loaded lipid microtubules to the injured spinal cord.[46,97]

Anyway, even though there should be potential side effects, an approach where the liquid nitrogen is also delivered onto the gel has also been contemplated.[98,99] An intriguing strategy in the field of CNS should be represented by the use of MSCs for designing cell or biohybrid constructs. In this context, two strategies can be considered for regenerative or replacement therapies, since MSCs may be employed as a reservoir of trophic factors or may be properly differentiated toward a neuronal phenotype.

For instance, undifferentiated MSCs from the autogenous adipose tissues were embedded into an alginate hydrogel. Then, the cell construct was placed into an expanded poly(tetrafluoroethylene) tube for repairing a facial nerve lesion.[100,101] As a result, a well-organized neural tissue was formed within the tube-like systems after 12 weeks. In addition, interesting results were also obtained in terms of nerve conduction velocity, which was greater if compared to the control group. However, this study clearly suggests the possibility to use similar approaches involving different biocompatible materials.

A three-dimensional (3D) device able to support MSC proliferation or differentiation was also developed considering macroporous cellulosic hydrogels for inducing the neuronal differentiation of hMSCs.[102] The number of hMSCs increased by more than 14-fold after 1 week, and differentiation into neurons and glial cells could be evident after 2 weeks. Potential functionalization and/or nanostructuration of such hydrogels may also improve the promising results already obtained.

Chitosan, which is a natural polysaccharide, can also be employed to design injectable drug depot as gels can be prepared by ionic cross-linking (i.e. sodium tripolyphosphate) or covalent cross-linking (i.e. glutaraldehyde).[103,104] Beta-glycerophosphate (BGP) cross-linked chitosan was employed to deliver ellagic acid for the treatment of brain cancer.[105] With regard to the above-mentioned materials, gelling occurred within 3 min at body temperature, and they exhibited a linear release profile for 14 days. As for the preparation, a mildly acidic aqueous solution is required, thus representing an attractive strategy for the encapsulation of biomolecules, which are stable under the considered conditions.

Cross-linking of thrombin-activated fibrinogen by factor XIII in the presence of Ca^{2+} plays an important role in the formation of fibrin gels. Fibrin gels have been used as tissue sealant in wound healing as well as for drug delivery applications. In this case, drug release was also tailored through reversible binding, when bi-domain peptides were incorporated into the fibrin matrix.

It was also demonstrated that by properly incorporating a peptide which contained a heparin-binding domain into the fibrin matrix, the release of a heparin-binding protein (i.e. fibroblast growth factor) was slowed down.[106–108] Such system may be injected and polymerized in situ,[109,110] and the controlled delivery of NT-3, NGF and BDNF has also been analyzed. Furthermore, improvements have been reported in terms of neural fiber sprouting in rats.[111]

Considering that collagen represents the main component of connective tissues and type I collagen as the most abundant protein in humans,[112] it has been widely used in different applications, such as drug delivery.[113] Collagen is a great candidate for the development of in situ gelling systems, taking into account its thermal gelling properties.[114] However, as collagen gels are quite weak, many strategies have been proposed to improve their durability (i.e. cross-linking). In this context, genipin was employed to obtain collagen gels with enhanced properties.[115] An in vitro study on NSPCs has demonstrated an improvement of survival, growth and proliferation using a collagen gel were able to release CNTF.[116] For this reason, an efficient drug delivery device may be properly designed benefiting from cross-linking and chemical modification methods and drug and gene release strategies.[117]

Hydrogels consisting of gelatin–hydroxyphenylpropionic acid (Gtn-HPA) conjugate were synthesized through specific oxidative coupling of HPA moieties, and the stiffness of such materials could be properly tailored.[118] Experimental analyses evidenced that the rate of hMSCs proliferation increased as the stiffness of the hydrogels decreased. Specifically, with regard to cell cultures after 3 weeks, much more neuronal markers were expressed using hydrogel-based materials with the lower stiffness in comparison to the stiffer matrices.

Amphiphilic diblock copolypeptide hydrogels consisting of poly-L-lysine, poly-L-homoarginine, poly-L-leucine and poly-L-glutamate were properly analyzed in order to tailor also the gelation time by varying the ratio of hydrophilic to hydrophobic residues. Similar to injections of physiological saline solution, results from in vivo tests on mouse forebrain demonstrated that these materials were able to promote inflammation, gliosis and toxicity to neurons, axons and myelin. They also provided blood vessel and limited nerve in-growth over time.

Self-assembling peptides (SAPs) represent a further interesting strategy, and such molecules may form self-assembling scaffolds as a consequence of changes in temperature, pH or salt concentration. In rat and mouse models of spinal cord injury (SCI), promising results have been obtained through functionalization with specific active sequences (i.e. Ile-Lys-Val-Ala-Val, IKVAV).[119–121]

Hyaluronan (HA) is widely employed in the field of tissue engineering, as it is present in high levels in the ECM of epithelial, connective and neural tissues. It plays an important role in cellular processes such as cell proliferation, inflammation, morphogenesis and wound repair, however interacting with cells through specific surface receptors (i.e. CD44, receptor for HA-mediated motility (RHAMM)).[122] Anyway, the attention has been focused on the possibility to make HA more suitable for drug delivery applications[123,124] as it alone does not gel, and in the body, it is rapidly degraded by the enzyme hyaluronidase.

The design of an injectable hydrogel for drug delivery consisting of a physical blend of HA and methylcellulose (MC), known as HAMC, has also been proposed. Both HA and MC clearly contribute to the overall properties of HAMC, such as shear thinning behavior, injectability, gelling temperature, biocompatibility, bioresorbability and the ability to attenuate inflammation in the CNS.[125,126]

Although HAMC alone has been used as injectable material providing interesting results,[126] it has been also considered for designing a drug delivery device for the release of growth factors to the stroke-injured brain and to the injured spinal cord.[126–128] Considering that therapeutic agents (i.e. hydrophilic proteins) can diffuse through the HAMC, they can be loaded into polymeric nanospheres (i.e. PLGA) dispersed within the HAMC to properly extend the release profile. This diffusion strategy may also lead to a linear release profile with a low burst release.[129]

The presence of MC increases the solubilization of hydrophobic drugs,[130] allowing an extended release profile which can be modulated by varying the size of the drug particles. With regard to the SCI, Park et al.[131] developed a further and complex 3D biomimetic hyaluronic acid–based scaffold. Three components were basically used, a matrix metalloproteinase peptide cross-linker, BDNF and an IKVAV peptide derived from laminin. The obtained results evidenced that hyaluronic acid–based hydrogels containing BDNF and IKVAV create microenvironments which promote the differentiation of hMSCs along the neural cell lineage, thus suggesting their use for nerve regeneration after SCI.

Exhibiting a low viscosity at 23°C and forming a soft gel through salt addition at 37°C, MC has also been investigated as an injectable scaffold to repair brain defects.[132] Furthermore, it was demonstrated that the presence of MC did not negatively affect the size and stability of the injury cavity.

Pluronic F127 is an ABA block copolymer consisting of poly(propylene oxide) and poly(ethylene oxide). It exhibits inverse thermal gelling, and Pluronic gels were employed for lentiviral delivery of the green fluorescent protein gene to the CNS,[133] also highlighting no toxic effects in 293T cells and no decrease in transduction efficiency when compared to traditional transduction. Anyway, a limited biocompatibility of such material was suggested as a result of an increase in activated macrophages and partial tissue damage. However, with regard to the treatment of retinal diseases, a further study on this material has demonstrated the possibility

to locally deliver dexamethasone across the human sclera.[134]

Another interesting approach is the design of a temperature responsive drug delivery system based on poly(*N*-isopropylacrylamide) (PNIPAAm).[135–137] PNIPAAm exhibits a LCST lying between room temperature and body temperature. Consequently, at room temperature, it is soluble and gels at body temperature. Although PNIPAAm homopolymer gels exhibit poor elastic recovery and hold little water at physiological temperatures, it is possible to tailor the mechanical or viscoelastic and swelling properties combining PNIPAAm with PEG.[138] By mixing the drug with the PNIPAAm-PEG at room temperature, such materials were employed as a device to deliver BDNF for the repair of an incomplete lesion in SCI model.[139,140]

Even though PLA is widely employed to design microparticles or NPs for drug delivery applications, it may also be used to develop hydrogels with specific features. As an example, PLA-PEG-PLA triblock copolymers were analyzed for the delivery of NT-3 to the injured spinal cord in rat models. Specifically, by means of a light source and a photoinitiator, the PLA-PEG-PLA macromer was cured (in situ polymerization).[141] Furthermore, it was also demonstrated that PLA-PEG-PLA may represent an interesting platform for the delivery of GDNF and BDNF to the brain.[142]

Systems consisting of PEG hydrogels combined with soluble factors demonstrated how neural cell composition may be influenced by the hydrogel-based environment. In this context, a range of soluble factors useful to generate neuronal-enriched populations was properly indicated for specific hydrogel-based environments.[29,143] The chemical modification of synthetic hydrogels using biologically active molecules may clearly represent a strategic route to enhance biomaterial–cell interactions.

As a route to modify the properties of commonly used nonadhesive PEG-based hydrogels, the monomer 2-methacryl-oxyethyl trimethylammonium chloride (MAETAC) was employed obtaining a tethered neurotransmitter acetylcholine-like functionality with a complete 2-acetoxy-*N,N,N*-trimethylethanaminium segment.[144] The results from this research evidenced that MAETAC in the hydrogels could promote neuronal cell attachment and differentiation as a function of concentration.

To develop a scaffold for the treatment of SCI made of highly porous hydrogels, poly(2-hydroxyethyl methacrylate) (PHEMA) suitably modified with cholesterol was used.[145] In a rat model, these PHEMA-based hydrogels showed interesting adhesive properties in vivo and bridged a spinal cord lesion, also supporting adhesion and proliferation of rat MSCs in vitro. The 3D biohybrid cell–hydrogel constructs were also proposed for such application, however considering injectable hydrogels with tailored nanostructure.[146] Primary astrocytes, MSCs and glial populations were analyzed, and the results demonstrated cell survival within this hydrogel. Hejčl et al.[147] also focused the attention on SCI repair in rat models analyzing the effect of MSC seeding and of a functionalization with Arg-Gly-Asp (RGD) sequences on the performances of a hydrogel based on 2-hydroxypropyl methacrylamide in the case of a chronic lesion. As a result, an enhancement was observed for the rats after the implantation of the cell-loaded hydrogels, preventing tissue atrophy; in addition, the therapeutic strategy consisting in synergistic approaches combining appropriate hydrogels and MSCs was supported by the obtained results. However, from an engineering point of view, the design of injectable hydrogels with adequate structural or functional properties, which should be able to maintain their characteristics after the injection according to the specific application, always represents a great challenge.

Indeed, the injection of hydrogels through clinical needles may alter their rheological behavior and viscoelastic properties (storage or elastic modulus—G′ and viscous or loss modulus—G″). For example, a decrease in the storage modulus (Figure 1) and a potential alteration of the gel-like behavior could be caused by the injection of the hydrogels through clinical needles as a consequence of a total or partial disruption of the polymeric network.

The viscoelastic properties of acellular and cell-laden hydrogels may be enhanced through the inclusion of appropriate microparticles or NPs as a reinforcement without altering the gel-like behavior.[148] Even though after the injection through clinical needles G′ and G″ may decrease, the inclusion of NPs may provide values of the viscoelastic properties which are still suitable for the specific application.[149–154] The amount of NPs clearly represents a crucial factor. The storage modulus and the viscosity usually increase up to a threshold concentration of NPs. If the concentration of NPs is further increased beyond such limit, G′ dramatically decreases and the NPs act as "weak points" instead of a reinforcement for the composite system. However, it is possible to predict and optimize the rheological behavior of the injectable composite devices integrating

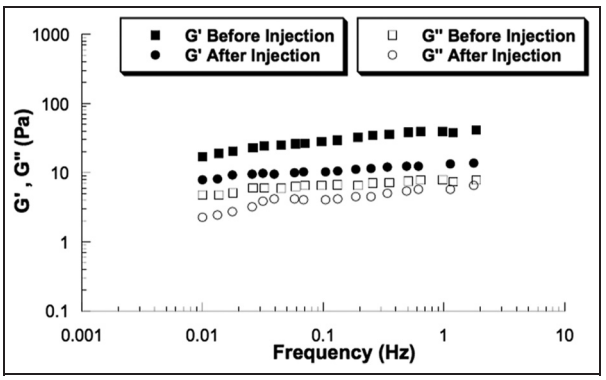

Figure 1. Storage modulus (G′) and loss modulus (G″) as a function of frequency for a collagen-based composite hydrogel: typical effect of the injection through a clinical needle.

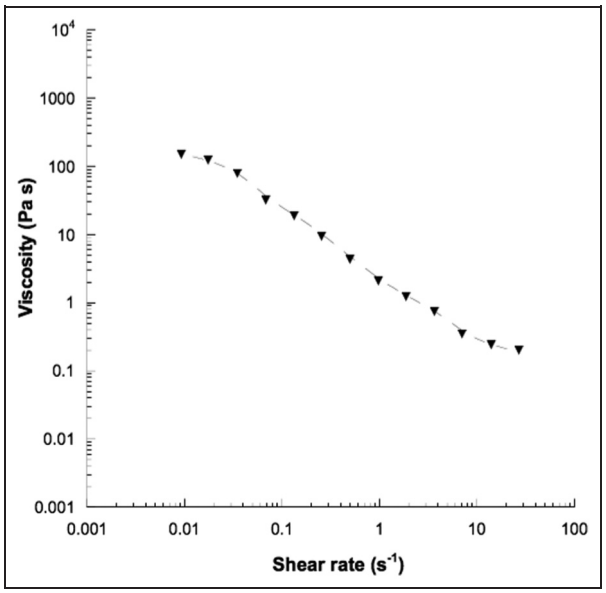

Figure 2. Viscosity as a function of shear rate for a collagen-based composite hydrogel.

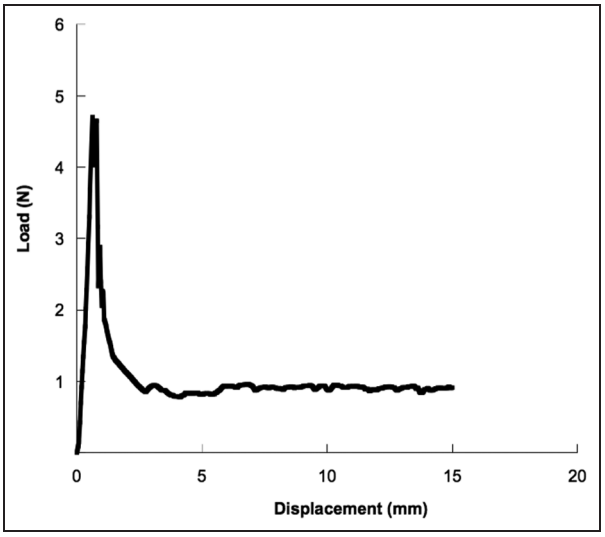

Figure 3. Typical load–displacement curve obtained from an injectability test performed on a collagen-based composite hydrogel.

mathematical models and experimental tests.[151] The rheological features also play an important role in the analysis and optimization of cell-laden composite gels. Specifically, small amplitude oscillatory shear tests may be carried out at different time points after cell seeding to assess the dynamic moduli (G′ and G″) over time, to understand the effect of cell behavior on the viscoelastic properties and to eventually optimize the cell density.[148] It is expected that at each time point, the values of the loss factor (G″/G′) are greater than those obtained from the corresponding acellular gels, and they increase over time.

Steady shear measurements may be performed to evaluate the viscosity as a function of the shear rate and, hence, the possibility to inject the material (i.e. shear thinning behavior; Figure 2). Anyway, the strains and rates induced by oscillatory rheometry are usually different than those achieved during injection-based applications and, hence, less likely to alter the structure of the developed devices.[155,156] For this reason, it could be difficult to simulate shear strains and rates occurring in the clinical practice (i.e. the materials are usually injected using syringes with suitable needles) by simply carrying out small amplitude oscillatory shear tests and steady shear measurements.

Accordingly, an adequate injection-based experimental setup is needed to assess the injectability properties. In particular, according to the specific application, a syringe equipped with a needle can be filled with the developed hydrogel and then mounted on a testing machine. The syringe piston is driven at a constant and fixed speed, and the material is injected into and through the needle, thus evaluating the characteristic load values (Figure 3). An empty syringe must be also tested to assess the friction between the piston and the syringe walls. An appropriate analysis clearly requires a comparison between acellular and cell-laden hydrogels at different time points after seeding to evaluate the effect of the cells on the flow behavior over time.

Furthermore, taking into account the principles of the capillary extrusion rheometry, the results from the injectability tests could be also used to obtain some rheological or functional features. In designing multifunctional and advanced hydrogel-based materials, a further crucial aspect is related to their biphasic nature. As a consequence, confined compression stress–relaxation tests may be also carried out on both cell-laden and acellular hydrogels, the aim being to measure functional parameters, such as the zero-strain permeability, the zero-strain compressive modulus, the nonlinear stiffening coefficient and the nonlinear permeability coefficient which provides a measure of the sensitivity to the deformation.

As an example, in the case of uniaxial confined compression, different equations are considered, spanning from the constitutive law for the extra-stress tensor to the relationship between the axial deformation and the hydraulic permeability, to obtain a nonlinear partial differential equation.[155,156] The deformation process is controlled by the fluid flow through the porous solid matrix, and permeability plays a key role in terms of transport properties.[157–159] Using a nonlinear biphasic model, the strain-dependent permeability can be evaluated by fitting the experimental data from the confined compression tests.[157,158]

Recently, collagen-PEG semi-interpenetrating polymer networks were also developed for brain injection in neurodegenerative disorders. Their viscoelastic properties, flow behavior, functional injectability and in vitro or in vivo biological performance were strictly analyzed, providing interesting information. The obtained results could clearly represent an important starting point for

the design of injectable hydrogel-based tools for novel drug or cell-based therapeutic strategies against brain-related neurodegenerative pathologies.[160]

Conclusion

Concepts such as injectable hydrogels, cell and drug delivery systems may be properly combined to design an appropriate therapeutic strategy for CNS diseases. At different levels, an accurate analysis on drugs or therapeutic agents and innovative biomaterial vehicles should provide a first crucial step toward a complex design.

The potential to design injectable hydrogel-based devices for in situ drug or cell release with tailored and enhanced rheological or mechanical and functional features, as a minimally invasive and interesting solution in the development of successful treatments, was stressed, without making comparisons between synthetic and natural materials or drug delivery and tissue engineering approaches. In particular, as in the literature, several CNS diseases and therapeutic strategies, together with a range of materials according to the specific disease, were already reported; after a brief overview of different approaches, this work aimed at summarizing the engineering process and basic concepts in the design of injectable hydrogels, focusing on the rheological and injectability features, as well as on the importance of other functional parameters (i.e. zero-strain permeability, zero-strain compressive modulus, nonlinear stiffening coefficient and nonlinear permeability coefficient), which will also influence the release kinetics of specific biofactors and the behavior of cells. Accordingly, taking into account the rheological, injectability and transport properties, it is possible to develop hydrogels with optimized characteristics, whose integration with biological expertise could be fully exploited to pursue novel and advanced CNS therapeutic strategies.

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