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# The application of hydrogels to spinal cord injury

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*Review*

**Abstract:** Spinal cord injuries (SCI) are very traumatic to the body; they can lead to permanent loss of motor and sensation. The injury also causes inflammation and the formation of glial scars, which inhibit neurite growth. It is thus very difficult to treat SCI's due to the inhospitable environment at and around the lesion site. This paper explores hydrogels as a desired tool for treating SCI's due to their ability to mimic the structure of the extracellular matrix (ECM), which supports cells and promotes their proliferation migration differentiation, as well as their ability to guide implanting neural tissue to restore and replace damaged tissue. The implantation of cells and molecules via hydrogels is promising and has been explored for SCI regeneration.

**Key words:** Spinal cord injury; hydrogels; tissue regeneration; cell-based transportation; molecule-based transportation.

## Introduction

Spinal cord injuries (SCI), which lead to permanent motor and sensory deficits below their lesion site, are among the most challenging injuries to treat in clinics. Many treatments have been explored, but they remain ineffective (1). Primary spinal cord injuries result in se-condary damages that include edema, vasospasm, excito-toxicity, inflammation, free radical production, ischemia, and demyelination (2). These secondary damages lead to the loss of neurons and glial scar formation (3), which leads to the creation of an impermeable barrier that axons cannot regenerate across (4). The Central Nerve System (CNS) has limited capabilities for axon regeneration, re-quiring the removal of scar tissue and the reconstruction of the injury site with glial cells, mesenchymal, blood ves-sels, extracellular matrix (ECM) and nervefibers (5, 6). Cell-based and molecule-based treatment strategies are a hopeful future cure for SCIs. However, traditional admi-nistrations, such as oral and intravenous, are limited by the blood-brain barrier (BBB) and blood cerebrospinal fluid barrier (BCSFB). Low diffusion makes these conventio-nal methods ineffective (6, 7). Furthermore, an ideal de-livery system to achieve local and sustained delivery of cells and molecules is needed.Hydrogels are a promising alternative because they have properties that mimic the ECM to provide substrates for neurons' adhesion prolife-ration and differentiation. This review will highlight the advantages of hydrogels applied to cell-based and mole-cule-based treatment strategies for SCI.

## Current Methods of Delivery

### Oral and intravenous administration

The blood-brain barrier (BBB) and blood cerebrospi-nal fluid barrier (BCSFB) protect the central nerve sys-tem from systemic circulation. Unfortunately, BBB and BCSFB also make conventional administrations (oral and intravenous) ineffective due to low diffusion, making it difficult to deliver therapeutic cells and molecules for de-generative disease (7).

### Intrathecal pump/carther

Intrathecal injection deliver molecules into CNS bypass BBB and BCSFB. Diffusion pumps/carther allow sustained release through surgical methods. Although they have been used to treat infections and pains, there are risks associated with the treatments. Repeated injections lead to infections, dehiscence of suture lines, pressure ulcers,the development of seroma, inversions of pump baclofen overdose and catheter failure (8, 9).

### Liposomes

Houwyer et al. demonstrated that liposomes can de-liver molecules across the BBB, due to the similarity of liposomes to the cellular membrane. Target-treatment can be achieved by the modification of liposomes with spe-cial monoclonal antibodies (mAbs) (10). A drawback of this treatment is that liposomes can be uptaken by macro-phages from the reticuloendothelial system (RES) (11) and render molecule delivery ineffective.

### Properties of ideal biomaterials

Because the above- mentioned administrations have li-mitations, an ideal system that can achieve local and sus-tained delivery is needed.Ideal delivery systems should have the following properties : Biocompatibility: The transplantation of materials should not cause immune response and should reduce rejection (12) between host tissue and grafted tissue, which is common in organ transplantation, and has no bad influence to grafted cells and molecules. After transplantation, grafted cells and

molecules maintain their own bioactivity to ensure suc-cessful regeneration of tissue. Representative example is poly(vinyl alcohol) hydrogel(PVA-H) (13). Stability: Materials are stable enough to avoid dispersing by envi-ronmental elements, such as temperature and power of hydrogen (PH). This stability provides long-term support for tissue regeneration. It is necessary that these materials are capable of protecting encapsulated cells and molecules under mild conditions to make sure that the cells and mo-lecules are delivered to the lesion site. Won et al. add a small quantity of graphene oxide to Pluronic copolymers to enhance stability of Pluronic gel (14). Degradation: Im-plantation materials must be degradable or their residue may cause immune response or inflammation at the le-sion site. Unwanted byproducts may also swell and lead to compression of the spinal cord. Materials must there-fore degrade without producing undesired byproducts. There must also be no chemical activations that degrade damaging host or grafted tissue. The rate of degradation must also be regulated; Degradation of materials should be slow enough to allow neuron growth differentiation or the production of neurophic factors with the help of mate-rials. If degradation occurs too quickly, there may not be adequate time for regeneration and the spinal cord may be compressed. If degradation occurs too slowly, on the other hand, the materials may increase the risks of immune res-ponse and inhibit the growth of neurons. Degradation of materials should be controlled and adjustable (15). Jiang et al. demonstrated that after being implanted, poly(ethylene glycol)-grafted-chitosan (PEG-g-CS) hydrogel maintains its integrity for two weeks and in the third week, collapses, merging into the tissue (16). No cell toxicity: Materials used for transplantation to the spinal cord should be safe for injection and should not harm the host tissue or other organs. These materials should be tested before transplan-tation to meet safety requirements. This requires material, as well as their additives, to be purified and ensured to be safe for encapsulated cells and molecules . There should be no toxic reactions between materials and their addi-tives. Gupta et al. explored crosslinked pullulan nanopar-ticles encapsulating bioactive molecules for drug and gene delivery.Cell adhesion/viability assay demonstrated that the pullulan nanoparticles are non-toxic to cells and do not cause any distinct harm to cells (17). Efficiency: Ma-terials should be designed to have desired outcomes with low concentration.A high concentration of materials may lead to spinal cord compression due to the limited capa-city of CNS and also increase the risks of side infections. Li et al. found a thermosensitive hydrogel which could improve the matrix modulus-induced cardiac differentia-tion efficiency of human mesenchymal stem cells (18). Bioactivity: Bioactivity of biomaterials can be determined by various factors including porosity, mechanical proper-ties and other soluble and insoluble cues.The proper size of biomaterial nanopores are important for cells' growth, migration and diffusion of bioactive molecules. The 3-di-mensional (3D) structure of these pores can mimic the ECM and provide growth-permissive substrates for cells' interactions, which determine cells' survival and growth. The mechanical properties of biomaterial should be simi-lar to those of the host tissue, which gives the materials the structure needed to bear the forces of the surrounding tissue. It is known that the stiffness of biomaterials has an effect on cells' behavior (15). Lawen Flynn et al. de-monstrated that size and arrangement of polycaprolactone (PCL) fibers dispersed in poly (2-hydroxyethyl methacry-late)( pHEMA) gels can control the diameter and number of hydrogel pores. These pores provide bridging support and contacting guidance for neurons (19). Peter Krsko et al. engineered surfaces that can control ascension and growth of cells and cell processes by controlling hydrogel patterns such as lines and arrays. Furthermore, the spaces between the individual hydrogels determine the adhesion of different kinds of cell populations (20). In conclusion, pores and channels give biomaterials the ability to enable axon regeneration after SCI by influencing cells' adhe-sion, growth and other processes. Furthermore,electros-pun nanofibers can not only mimick ECM by porosity but also by nanoscale morphology,high surface area and fibrous morphology. Malkoc et al. designed a microdevice on a collagen coated gelatin/PCL nanofiber mat which promote neuron-like PC12 cells' adhesion, differentiation, and neurite outgrowth compared to controls. Malkoc et al. demonstrated that electrospun nanofibers promote neu-rite outgrowth, produce nanofiber based nerve guidance conduits and provide mechanical and biochemical cues to stimulate stem cells differentiate into progeny (21,22).

**Introduction of hydrogels**

Hydrogels are natural or synthetic water-content polymer networks that can be designed to be biodegra-dable and release molecules at a controlled rate though cross-link modification (23). Hydrogels hold great pro-mise as desirable carriers for cell and molecule delivery. They are promising biomaterials in regeneration medi-cine. Hydrogels have been explored for decades for tissue engineering and have been applied to SCI, which is one of the most complex diseases due to the multiple growth inhibition and difficulties of replacing damaged tissue it causes. Moreover, hydrogels can mimic the native ECM structure (15, 24). The structure and properties of enginee-red hydrogels allow localized cell-mediated regeneration that enable cells' survival, spreading and migration (24, 25). Hydrogel-based delivery systems can also be de-signed to influence cells' processes. The ability of hydro-gels in histocompatibility between host tissue and graf-ted tissue contributes to tissue regeneration. The future application of hydrogels demand easy synthesis, stability, safety and efficacy.

Hydrogels were first described as a colloidal gel, then reported as water-swollen cross-linked networks. The first generation of hydrogels had a simple network structure that gave hydrogels basic properties such as solute diffu-sivity and cross-link density. Representative first gene-ration hydrogels are PHEMA, poly vinyl alcohol (PVA) and polyethylene glycol (PEG). Compared with first ge-neration hydrogels , second generation pays attention to the abilities of hydrogels to respond to changes in envi-ronment. These environmental changes trigger hydro-gels' special events ( i.e gel formation or molecule re-lease). Temperature and PH sensitive hydrogels were then created by modification of hydrophobic interactions and hydrogen bonding (26, 27). The third generation was fur-ther designed to have ability to tune mechanical, thermal and degradable properties. Currently, the most advanced hydrogels are "smart hydrogels", which have proper-ties such as mechanical stability and release kinetics to

achieve desired delivery (28).

Hydrogels can be modified by both physical and che-mical cross-linkers. Each type of cross-linked hydrogels have their own advantages and disadvantages. Chemically cross-linked hydrogels can survive for long term periods, but demand the fast formation of covalent bonds to get rid of elimination. The hydrogels also should have no cy-totoxic agents. On the other hand, physical cross-linked hydrogels appear to be sensitive to environmental stimu-lations (temperature and PH). The lack of covalent bonds in this hydrogel also makes it easier to eliminate after in-jection. .A high concentration is require, which leads to high osmatic pressure compression of spinal cord. Com-bined cross-linked strategies also show great promise for tissue regeneration (29). For example, Malgsia M et al. designed a physical and chemical cross-linked hydrogel xylene monochloride (XMC), which is injectable and is also long-lasting and safe to achieve minimally invasive surgeries and sustained biomolecule delivery (29).

**Ways to measure properties of hydrogels**

There are various ways to measure different properties of hydrogels. The mechanical properties are tested by dy-namic mechanical analysis under both wet and dry condi-tion. Porosities are tested by using micro-computed tomo-graphy and electron microscopy. The toxicity of hydrogels is analyzed by motor neuron survival and neuron growth. The functional recovery behavior is tested by Basso Beac-tie and Breshahan test(BBB test) and Dynamic Weight Bearing test(DWB test). Histological analysis reveals the presence of neurons and new blood vessels, glial scar for-mation, inflammation and myelination. Finally, the degra-dable property of hydrogels is analyzed by measurement of mass loss (30).

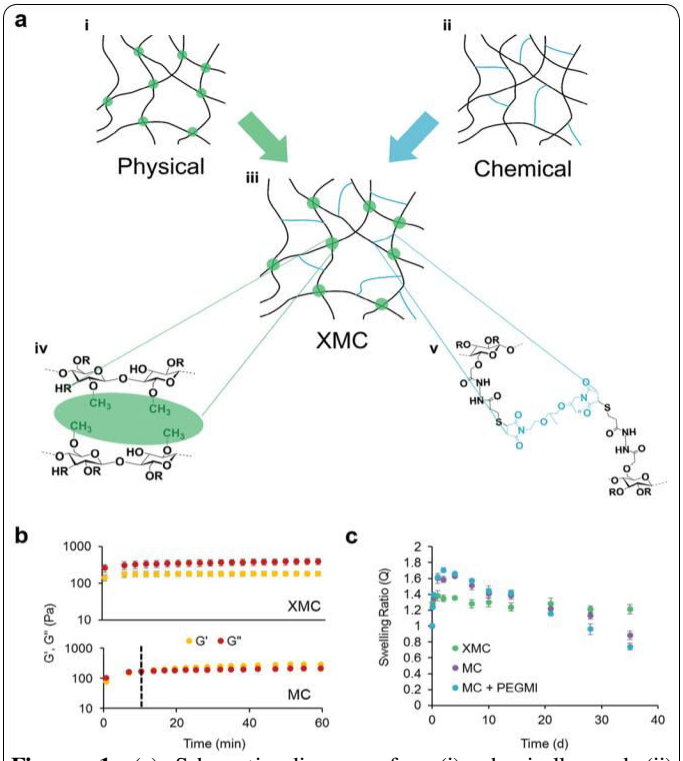


**Figure 1.** (a) Schematic diagram of a (i) physically and (ii) chemical-ly crosslinked hydrogel. (iii) Our crosslinked methylcellulose (XMC) is a hybrid hydrogel that is both physically and chemically crosslin-ked. (iv) The physical crosslinks consist of hydrophobic interactions between the methylcellulose chains while (v) the chemical crosslinks are formed by reaction of a thiol-modifi ed MC with a PEG-bisma-leimide crosslinker. (b) Shear storage and loss moduli ( $G'$, $G''$) for XMC and MC hydrogels. Top panel: XMC hydrogel (5 wt% MC, 0.1 μmol thiol/100 μL, 0.75:1 ratio maleimide–thiol, $n$ = 5) over time at 37 °C after 10 min of equilibration at 4 °C. Bottom panel: MC hy-drogel (5 wt% MC) over time at 37 °C after 10 min of equilibration at 4 °C. Dotted line marks the gelation point ( $G' > G''$).(c) Swelling ratio of the XMC hydrogel, MC hydrogel, and MC hydrogel with added PEG-MI 2 over time at 37 °C ( $n$ = 3). This also indirectly depicts stability of the hydrogel over a 35 d period (mean ± standard deviation plotted) (29).

**Cell-based strategies and molecule-based strategies**

Hydrogels support injured spinal cords as well as prevent scar formation by creating a hospital environment for tissue regeneration. 3-D structure pores and channels of hydrogels provide substrates for tissue ingrowth, com-bined with therapeutic cells and molecules (31). Hydro-gel-based treatment gives us hope to explore a desired effective strategy for SCI.

**Cell-based strategies**

Delivering therapeutic cells to lesion sites has always been one of the most widely regenerative strategies for de-cades. This method not only stimulates nerve regeneration and restores lost cells, but also provides trophic factors in-cluding anti-inflammatory and growth factors. These cell processes can modulate and promote recovery after SCI.

Among these cell-based treatments, stem cell therapy seems to be a promising approach to regeneration. Expe-riments which combine human fetal neural stem cells and hydrogels has been explored. Human fetal neural stem cells (hNSCs) is a type of stem cell that can have multi-ple potential differentiation properties and is suitable for human transplantation. Jason R. Thonhoff et al. explored three kinds of hydrogels for delivery of hNSCs: Pluronic F-127, Matrigel and PuraMatrix. Among these hydrogels, PuraMatrix is proven to be the most optimal hydrogel for hNSCs (32) .Růžička J et al. combined hNSCs with se-rotonin-modified poly (2-hydroxyethyl methacrylate) hy-drogel (pHEMA-5HT) and demonstrated that this kind of hydrogel provide a supportive environment to stimulate hNSCs differentiation both in vitro and vivo for regene-ration (33).

Adult neural stem/progenitor cells (NPCs) are promi-sing grafted cells due to their ability to self-renew and to differentiate to oligodendrocytes, astrocytes and neu-rons. Andrea J et al. explored survival and efficacy of brain stem/progenitor cells injected to a subacute SCI model of rats combined with hyaluronan and methyl cel-lulose(HAMC) modified with rat platelet derived growth factor-A (rPDGF-A). This strategy demonstrated signifi-cant reduction in cavitation, improved survival of grafted cells and behavioral improvement, and increased differen-tiation of oligodendrocytic (34).

Induced pluripotent stem cell-derived neural stem cell (iPSC-deviced NSCs) transplantation for treatment of SCI has shown therapeutic potential by differentiating into neurons and glia to stimulate new tissue across injury sites. Pre-clinical experiments demonstrated that trans-

plantation of iPSC-deviced NSCs shows promising survival, differentiation and therapeutic effects (35). Carla Christina Medalha et al. transplanted NPCs composed of

neuro-restricted and glia-restricted progenitors to partial lesion models and showed great survival and ability to ge-nerate neurons. However, when transplanted to a complete transection model of rats, they found poor survival grafted cells using different kinds of matrices and lesion methods (36). For regenerative medicine, recently it was found that rather than going through an IPSC stage, a nonviral deter-ministic transfection yields efficient reprogramming and ability to control complexity of induced neurons (37).

Olfactory ensheathing cells (OECs) delivery is another promising treatment for SCI. OECs can obtain the ability of neurogenesis throughout their life in olfactory system. Zhang Ling-Ling et al. engineered a new peptide hydro-gel scaffold named GRGDSPmx to provide cell growth guidance like natural ECM by mixing the pure RADA16 (AcN-RADARADARADARADA-CONH2) and de-signer peptide RADA16-GRGDSP ( H-Gly-Arg-Gly-Asp-Ser-Pro-OH) solution. Analyses showed increased proliferation and less apoptosis of OECs. Furthermore, GRGDSPmx provide desired environment for OECs (38).

Schwann cells (SCs) transplantation is also a promi-sing treatment strategy for SCI. It has been shown to re-duce neurons' loss, stimulate neurons' regeneration and promote myelination. It is demonstrated that transplanta-tion combining SCs with matrices can achieve long-term survival of SCs and promote grafted vascularization and axonal ingrowth. Matrigel and pure matrices have been applied to the delivery of SCs and promote survival of SCs and enhance locomotor improvement (39).

**Molecule -based strategies**

SCI is very complex; trophic factors play a crucial role in tissue regeneration. These trophic factors have func-tions of promoting angiogenesis, inhibiting scar forma-tion, reducing inflammation, and, increasing NPCs survi-val. Delivery of these trophic factors holds great promise when combined with hydrogels to achieve local and sus-tained release.

Chondroitinase ABC (ChABC) is one of these trophic factors that degrade the glials and lead to the regrowth of neurons. The regrowth and tissue repair is caused by the degradation of chondroitin sulfate proteoglycans (CSPGs), which is a kind of axon growth inhibiting factor promo-ting glial scar formation. ChABC is thermally unstable, so it is difficult to deliver. Malgosia M et al. engineered an af-finity-based modified methylcellulose(MC) hydrogel that achieves sustained release of bioactive ChABC. The MC hydrogel modified with an SH-3 bind peptide can control the release rate of ChABC by changing the binding stren-gth of SH3-protein/ SH3-peptide pair (40).

Neurotrophin-3 is a trophic factor that modulates the survival and function of tyrosine kinase C-positive neu-rons. Jason C et al. achieved sustained and local release by encapsulating NT-3 in poly(lacticco- glycolic acid) (PLGA) dispersed in an injectable hydrogel of hyaluronan and methylcellulose (HAMC) (41). J Piantino et al. esta-blished a biodegradable injectable hydrogel that achieved long-term NT-3 release over 2 weeks. The hydrogel/NT-3 treated animals showed great axon growth and functional improvement (12).

Delivering vascular endothelial growth factor (VEGF) to SCI has been explored as a promising therapeutic strategy. VEGF is a factor that has been applied to spinal cord regeneration by combining it with hydrogels and en-

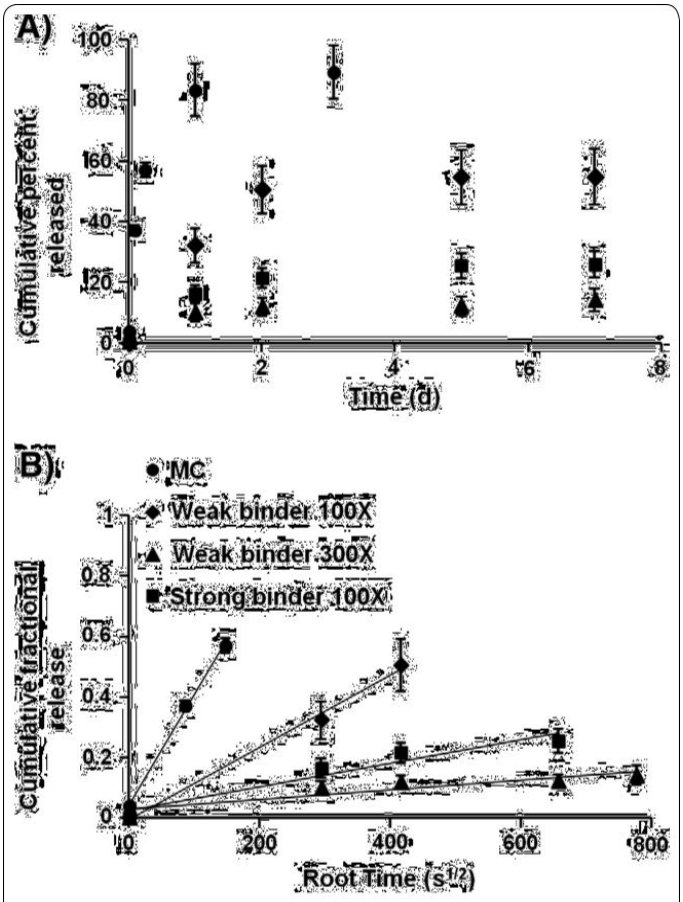


**Figure 2.** Release of ChABC–SH3 from modified methylcellulose hydrogels over time. A) Cumulative release profiles of ChABC–SH3 from a series of gels: MC alone vs. MC-weak binding peptide 100X vs. MC-weak binding peptide 300X vs. MC-strong binding peptide 100X. 100X and 300X indicate 100-fold and 300-fold molar excess of peptide to protein within the gel, respectively. Weak binder Kd = 2.7 × 10−7 M; strong binder Kd = 2.7 × 10−5 M. B) Release profiles of (A) fit to a short time approximation for unidirectional diffusion from a plane sheet. The slopes are proportional to apparent diffusivity of the protein through the gel. Release can be controlled by changing the ratio of peptide to protein (weak binder 100X vs. weak binder 300X vs. no peptide, MC alone) or changing the peptide–protein binding strength (weak binder 100X vs. strong binder 100X). (n = 3 independent studies for each condition, mean ± cumulative standard deviation are plotted) (40).

hancing neural growth at and around the lesion site. Anne dex Rieux et al. demonstrated that local delivery of VEGF from injectable alginate: fibrinogen-based hydrogel sup-ported angiogenesis and neural growth. Although no func-tional recovery was observed, fibrinogen-based hydrogel is well tolerated by spinal cord tissue (42).

Glial cell-line-derived neurotrophic factor (GDNF) is a promising strategy because GDNF promotes survival and growth of dopaminergic, motor, peripheral sensory, and neurons. It is demonstrated that GDNF has neuroprotec-tive effects which lead to tissue and axonal regeneration. Eduardo et, engineered a delivery system by encapsulating GDNF in microspheres and releasing it from an injectable alginate hydrogel. This kind of delivery system showed support to spinal cord plasticity and functional recovery (43). Deniece Fon et al. demonstrated that GDNF-loaded injectable Gelatin-based hydrogel can attract NPCs mi-gration from the subventricular zone (SVZ) and support NPCs survival while reducing reactive gliosis (44).

Nogo-A is a myelin-associated inhibitor that can re-

duce neurite growth by creating an inhospitable regene-ration environment at the lesion site. Nogo-A can also bind to Nogo-66 receptors (NgR) to mediate the inhi-bition of axonal regeneration (45). Yue-Teng Wei et al. combined hyaluronic acid (HA) based hydrogel modified with poly-L-lysine (PLL) and Nogo-66 receptor antibody (antiNgR) and then delivered the HA PLL/antiNgR to the hemisection spinal cord model of rats. Eight weeks after transplantation, the method showed significant support in angiogenesis and helped the reduction of glial formation (46). Anti-NogoA has been shown to improve functional recovery and it is an IgG that can not cross BBB and BCS-FB. Jason C et al. engineered a delivery system composed of anti-NogoA-loaded poly(lactic-co-glycolic acid) nano-particles dispersed in a hydrogel of hyaluronan (compo-site HAMC). Co-capsulated with MgCO3 and CaCO3, this achieved a long-term release of anti-NogoA and improved the bioactivity of anti-NogoA (47).

Dafin F et al. demonstrated that delivery of growth hormones protected neurons after SCI (48). Furthermore, DrewL Sellers et al. explored thrombin delivery through neural progenitor proliferation (49).

Blood–spinal cord barrier is the current limitation of SCI in clinical treatment. B Chen et al.combined hydroxyl ethyl methacrylate [2-(methacryloyloxy)ethyl] trimethy-lammonium chloride (HEMA-MOETACL) hydrogel with basic fibroblast growth factor (bFGF) into complete spinal cord transaction model of Sprague–Dawley rats.The ex-periments demonstrated that HEMA-MOETACL hydro-gel is a promising delivery system for sustained and local release of bFGF to the injured spinal cord and this study provides a potential therapy for SCI (50).

In conclusion, remarkble improvements have been made combining hydrogel-based delivery system with trophic factors and systemic administration of these mo-lecules is limited due to BCSFB. Hydrogel-based local delivery can bypass this barrier to increase the efficiency of therapy without damaging bioactivity of these mole-cules. Sustained release can be achieved by encapsulation of molecules into nanoparticles or microspheres.

## Conclusion

SCI is a complex disease that has challenging obstacles yet to be overcome. Systemic administration is hampered by BBB and BCSFB, which lead to therapeutic failure. However, great progress has been made in the field of tis-sue engineering and regenerative medicine due to the ap-plication of hydrogels which not only provide support for grafted cells, but also achieve a sustained and local release of trophic molecules which modulate cells' processes and inflammation, promote angiogenesis, and inhibit the formation of glial scars Unfortunately, there still remain challenges in cell-based and molecule-based delivery via hydrogels treatment strategies we need to face. It is de-monstrated that the survival of grafted cells needs to be improved. Further modification of hydrogels are required to achieve long-term support of diffusion of implanted cells (51). Cerebrospinal fluid flux is proven to play a crucial role in molecule delivery and influences hydrogel placing site (52). In the future, combined strategies based on cells and molecules will be needed to improve outco-mes of SCI regeneration, and more modifications will be required to achieve various demands of delivery system according to different conditions.

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