

Hydrogels in Spinal Cord Injury Repair Strategies

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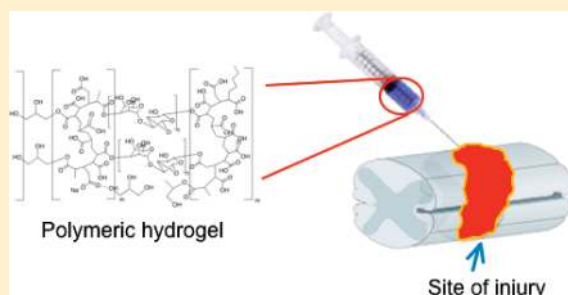
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ABSTRACT: Nowadays there are at present no efficient therapies for spinal cord injury (SCI), and new approaches have to be proposed. Recently, a new regenerative medicine strategy has been suggested using smart biomaterials able to carry and deliver cells and/or drugs in the damaged spinal cord. Among the wide field of emerging materials, research has been focused on hydrogels, three-dimensional polymeric networks able to swell and absorb a large amount of water. The present paper intends to give an overview of a wide range of natural, synthetic, and composite hydrogels with particular efforts for the ones studied in the last five years. Here, different hydrogel applications are underlined, together with their different nature, in order to have a clearer view of what is happening in one of the most sparkling fields of regenerative medicine.

KEYWORDS: Hydrogels, polymers, scaffold, regenerative medicine, spinal cord injury, tissue engineering



Traumatic spinal cord injury (SCI) is an irreversible dramatic event that can incapacitate victims for life.^{1–4} Although the incidence is relatively low, the often severe disability that follows and the fact that the victims are often young people, the consequences for the patient is severe and the impact on societal costs is significant. The injury is the result of a primary event due to contusive, compressive, or stretch injury,^{1,2,5} followed by the so-called “secondary injury”, commonly considered the main cause of the post-traumatic neural degeneration of the cord itself.^{6–8} Functional deficits of SCI are caused by different temporal events: spinal cord compression and/or contusion lead to ischemic events that limit both oxygen and glucose contribution to the tissue, with concomitant neuronal cell death, axon damage, and demyelination.⁵ Subsequently, glial activation, release of inflammatory factors and cytokines, and scar formation that impedes axons to regrow^{8,9} aggravate the progression of the damage.

SCI research is following two principal paths.^{6,9–11} The first one, already applied in human cases, is based on systemic pharmacological treatments in order to contain side effects (ischemia, free radical release, and inflammation) using neuroprotective drugs (such as corticosteroids)^{12–14} and to promote self-regeneration using stimulating factors.¹⁵ The second one relies on tissue engineering^{16–18} approaches such as the direct injection of stem cells^{19–21} and active agents (drugs, antibodies, and peptides) into the affected area with the aim to bridge the lesion, possibly after removal of the glial scar or reducing endogenous neurite-inhibitory molecules.^{22,23} Direct injection of in vitro cultured cells or drugs is the most common choice, but keeping transplanted cells in the lesion area is often desired as transplanted

cells readily leave the zone of injection if not confined by any support. To achieve this, a new potential approach is to combine material science with tissue engineering as has been proposed and developed.^{16,24–26} In Figure 1 are presented classic tissue engineering approaches as the combination of scaffolds with cells and active agents in order to replace damaged parts of biological tissues.^{17,18}

In the wide field of biomaterials, increased attention is given to polymers, not only to fabricate three-dimensional scaffolds but also to develop injectable systems for tissue engineering.^{26–34} One of the most suitable classes of compounds for these purposes is surely represented by hydrogels.^{16,28,31,35–39} These polymers are typically soft and elastic due to their thermodynamic compatibility with water.^{16,33,36,40} They can be designed as temporary structures having desired geometry and physical, chemical, and mechanical properties adequate for implantation into chosen target tissue.^{6,41–43}

The aim of this Review is to show the different types of hydrogels used as scaffolds for SCI repair strategies. We on purpose decided to focus our attention only on the past few years, in order to show the most promising and recent perspectives in this field. Indeed, the rapid expansion of nanotechnology during the last years has led to new perspectives and advances in biomedical research as well as in clinical practice.⁷

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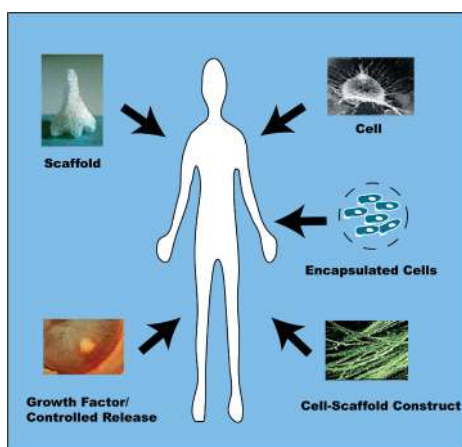


Figure 1. Tissue engineering approaches: the smart combination of cells and materials to replace damaged or missing parts of living tissues. Reproduced with permission from ref 16. Copyright Wiley-VCH Verlag GmbH & Co. KGaA.

■ HYDROGELS

The physical aspects of scaffold design, as with polymer choice, depend largely on the application. The scaffold is meant to provide the appropriate chemical, physical, and mechanical properties required for cell survival and tissue formation.^{35,36,40} Essentially, the polymeric scaffold is designed to define the cellular microenvironment required for optimal function. Indeed, in the wide field of biopolymers,^{20,44,45} one of the most suitable classes for these purposes is represented by hydrogels. They are three-dimensional (3D) networks of hydrophilic polymers held together by covalent bonds or other cohesive forces such as hydrogen or ionic bonds.^{36,46–48}

They are glassy in the dry state and then, in the presence of solvents, able to swell while preserving their original shape to form elastic gels. Capable to retain a large amount of water in their structure (up to 95% of the total weight), they can either degrade in it by polymer chain degradation reactions (e.g., hydrolysis or proteolysis into smaller molecules) and are then called resorbable hydrogels, or they cannot and are then called stable hydrogels.^{35,36} These scaffolds slowly degrade in the physiological environment, leading the growing tissue to replace the former filled site.⁴⁶ An important advantage is the possibility to minimize the risks of surgical procedures due to their injectability and ability to create a 3D network in situ, in the target tissue.^{26,35}

In general, hydrogels may be classified as either synthetic or natural in origin. On one hand, synthetic polymers can be tuned in terms of composition, rate of degradation, and mechanical and chemical properties.^{49,50} On the other hand, naturally derived polymers provide structures extremely similar to living tissues such as stimulating a specific cellular response, which sometimes supersedes the advantages of synthetic polymers. Moreover, owing to their similarity with the extracellular matrix (ECM), natural polymers may also reduce the stimulation of chronic inflammation or immunological reactions and toxicity, often detected with synthetic polymers.^{51,52} However, this is not true for every natural-derived polymer; the ones from nonmammalian sources (e.g., seaweed and crustaceans) can induce immune reactions. Moreover, even mammalian hydrogels (e.g., those collagen-based), if raw materials are improperly harvested from some species, might induce immune reactions in humans.

Thus, different reasons make the above-mentioned biomaterials very attractive for improving tissue regeneration and central nervous system (CNS) repair:⁴⁵ (i) tissuelike mechanical abilities, conformable to the CNS tissue;^{43,53} (ii) porous structure allowing cell infiltration, transplantation, and axon outgrowth;⁵³ (iii) ability to incorporate adhesion and/or growth-promoting molecules in the hydrogel to enhance cell attachment and tissue growth;⁵⁴ (iv) capacity of drug/gene vector incorporation and precise in situ delivery.^{38,42,55,56}

In order to make a comprehensive overview of their use in spinal cord injury repair strategies we decided to classify hydrogels on the basis of the following.

Nature: natural, synthetic, or a combination of the two.^{49,50}

Function: drug or cell carriers or a combination of the two.^{24,39,57}

1. Natural Derived Hydrogels. In order to follow the similarities between the implanted materials and the living tissue, researchers studied the possibility to synthesize hydrogels starting from molecules present in living tissues. In particular, the most suitable are collagen, hyaluronic acid (HA), and polysaccharides (agarose, alginate, cellulose, gellan gum, scleroglucan, and xyloglucan). Although regenerative axonal growth occurs in a liquified spinal cord lesion cavity without obvious physical support,⁵⁸ regeneration is facilitated by a supporting scaffold equivalent to the endoneurium and perineurium in a peripheral nerve, that can act as a bridge in order to approximate the disconnected axonal groups for the damaged area.^{43,53} The aim of using hydrogel is to replace the damaged area with a structural matrix.^{24,51}

As explained before, naturally derived macromers and their use have increased in the past few years due to inherent biocompatibility and enzymatic degradation.⁴⁹ They are macroporous, soft materials able to allow cell adhesion and migration.⁵⁰ Moreover, they can be manipulated in order to obtain channels for nerve guidance or sustained drug delivery. Table 1 presents in detail the main natural polymers and highlights examples of their SCI application. For the sake of clarity, it is useful to briefly comment on Table 1. Following most promising regenerative medicine approaches toward other pathologies, also several recent studies in SCI repair are combining hydrogels with stem cells in order to provide in situ cell delivery. In these applications, hydrogels are used as 3D cell growth matrices and cell reservoirs. Hence, it has to be underlined that not only materials but also stem cell choice are key points in the regeneration strategies: indeed, researchers are mostly focusing their attention on pluripotent stem cells (embryonic)^{59,60} or multipotent ones (mesenchymal or neural).^{38,61–69} Natural hydrogels used for this purpose are either synthesized starting from polysaccharides such as alginate^{59,66,69} and hyaluronic acid as homopolymer^{62,65} or copolymerized with methylcellulose,⁶¹ cellulose,⁶³ and xyloglucan.^{60,67} Other materials are also being investigated to support cell therapies: the commercial Matrigel,^{38,69} fibrin,⁶⁸ and gelatin.⁶⁴ A dedicated mention should be addressed to in vivo studies that already showed functional improvement in animal models after hydrogel implantation. In these studies, hydrogels, such as agarose^{70,71} or alginate,⁷² were used as scaffolds able to support oriented axonal regeneration. Moreover, with hydrogels being able to provide controlled drug delivery to improve axonal regrowth, they can be loaded with active substances such as chondroitinase ABC,⁷³ methylprednisolone,⁷⁴ or brain-derived neurotrophic factor (BDNF).^{62,75}

Table 1. Naturally Derived Hydrogels Used for SCI Repair

material	description	acronym	application in SCI
agarose	polysaccharide		cell growth matrix ⁷⁴ encapsulation and delivery of neurotrophic factors ¹⁴ controlled chondroitinase delivery ⁷³ support for nanoparticle delivery ^{14,74} brain-derived neurotrophic factor (BDNF) controlled delivery ^{75,76} linear guidance (freeze-dried) ^{70,71} cell encapsulation for growthmatrix ³⁷
alginate	co-methylcellulose polysaccharide	agarose/MC	nerve guidance ⁷⁷ anisotropic scaffold for axonal regrowth ⁷² neural stem cell growth matrix ^{66,69,78} embryonic stem cell growth matrix ⁵⁹
cellulose	polysaccharide		mesenchymal stem cell growth matrix ⁶³
chitosan	polysaccharide		scaffold for cell adhesion and growth with polylysine ⁷⁹ scaffold for neurite regrowth with hyaluronic acid ⁸⁰
collagen	polypeptide		polymeric channels ⁸¹ filament bridges as growth substances ⁸² cell growth matrix ^{83,84}
fibrin	linked proteins		neural stem cell growth matrix ⁶⁸
gelatin	hydrolyzed collagen		mesenchymal stem cell growth matrix ⁶⁴
gellan gum	polysaccharide		tubular, porous scaffold for axonal regrowth ⁸⁵
hyaluronic acid	polysaccharide	HA	controlled delivery of neurotrophic factors ⁶² scaffold for neurite regrowth ^{65,80} controlled peptide delivery ^{26,86,87}
	co-polylysine		Nogo 66 receptor antibody delivery system ^{88,89}
	co-methylcellulose	HAMC	intrathecal drug and growth factor delivery ^{90–95} neural stem cell carrier for cell therapies ⁶¹
	co-collagen		cell growth matrix ⁹⁶
Matrigel	laminin, collagen IV, heparin		scaffold supporting cell adhesion and growth ³⁸ neural stem cell carrier for cell therapies ⁶⁹
scleroglucan	polysaccharide		controlled drug delivery ⁹⁷
xyloglucan	polysaccharide		scaffold supporting cell adhesion and growth ^{60,67}

2. Synthetic Hydrogels. Synthetic hydrogels, such as those based on poly(hydroxyethyl methacrylate) (PHEMA), were some of the earliest biomaterials used as tissue engineering scaffolds.^{43,98} This class of materials shows very important advantages in this field: easier large-scale production and highly tunable properties.⁴⁹ Both of them contributed to the large number of formulations. In contraposition with the advantages of the naturally derived hydrogels, synthetic polymers offer wider scope to design and control the characteristics of the material. Moreover, the possibility to reduce the allergenic risks using a completely artificial biocompatible material devoid of animal proteins is evident.^{98,99}

The more recent use of hydrogels as cell carriers offers the possibility to provide precise temporal control of the donor and host cell interactions. The ability to carry cells in a matrix, initially impermeable to cells, could afford donor cells protection from potentially harmful substances such as cytotoxic cytokines immediately after transplantation, being a barrier for their diffusion as in the case of hydrogel based microcapsules.^{100–102} At later time points, as the gel degrades and the overall mesh size of the gel increases, donor cells will be delivered. Additionally, the gel network can serve as a scaffold to support regeneration within the host environment until the material is ultimately resorbed by the tissue. The surface of the hydrogel could also be easily modified

or charged in order to favor cell attachment, or differentiation. They can also be cross-linked with other polymers in a classic block copolymerization in order to design smart delivery systems. Emblematic is the case of cyclodextrin, able to carry insoluble drugs into water based systems. With respect to synthetic formulations, care must be taken to ensure that contaminant and unreacted reagents present during synthesis are completely removed due to their possible toxicity. Details of synthetic polymers used in SCI are presented in Table 2.

Briefly commenting on this second table, being that regenerative medicine is considered the future in life sciences, several studies were performed to develop synthetic polymeric gels showing full compatibility with stem cells. Stem cells are mainly chosen between multipotent cell lines (mesenchymal and neural)^{99,103–107} and pluripotent ones (embryonic).¹⁰⁸ Synthetic materials that seem to be extremely suitable as 3D growth matrices are polymethacrylates, such as pHPMA and pHEMA, which were tested with mesenchymal stem cells¹⁰³ and also showed relevant improvement in chronic spinal cord injury.^{103,109,110} In addition some studies, involving polymethacrylates, underlined relevant functional improvements on animals after hydrogel implantation: pHEMA and pHPMA favor axonal ingrowth,¹¹¹ showing also good outcome in chronic cases as said before,¹⁰³ while pHEMA-MMA influences axonal regrowth.^{112–114} Stem

Table 2. Synthetic Based Hydrogels Studied in SCI Research

material	description	acronym	application in SCI
Carbopol	branched poly(acrylic acid)		controlled drug delivery with cyclodextrin ^{115,116}
lysine-leucine	co-polypeptide	DCH	tunable vehicles for factor delivery ¹¹⁷
polyacrylamide			scaffold for neurite outgrowth ¹¹⁸
polyalkylimide	acrylates		scaffold supporting cell adhesion and growth ¹¹⁹
poly- ϵ -caprolactone	polyester	PCL	nanofiber for axonal growth orientation ⁹⁹
poly(ethylene glycol)	polyether	PEG	3D cell growth matrix ^{31,104,120–122}
			microcapsules for cell growth ¹²³
			controlled drug delivery with cyclodextrin ¹²⁴
			microvascular networks for cell growth with PLGA ^{105,107,108}
			controlled delivery of methylprednisolone ¹²⁵
		PLA- <i>b</i> -PEG- <i>b</i> -PLA	delivery of neurotrophins ^{126–129}
		PNIPAA-PEG	cell adhesion and neurotrophins release ¹⁰⁶
polyethylene oxide		PEO	injectable scaffold for drug delivery with cyclodextrin ¹³⁰
poly(hydroxethyl methacrylate)	polyester	PHEMA	charged modified scaffold as bridges for axonal growth ^{98,111}
			guidance channels ^{111,131}
			fiber templated scaffold ¹³²
			bone marrow stem cell carrier for cell therapies ^{109,110}
	co-methylmethacrylate	PHEMA-MMA	reinforced guidance channels for nerve regrowth ^{112–114}
			controlled drug delivery ¹³³
poly(hydroxypropyl methacrylate)	polyester	PHPMA	mesenchymal stem cell growth matrix ¹⁰³
poly(<i>N</i> -isopropylacrylamide)- <i>co</i> -polyvinylpyrrolidone	copolymer	PNIPAA-PVP	scaffold for controlled drug delivery ¹³⁴
Pluronic	polypropylene oxide + ethylene oxide	PF127	scaffold supporting cell adhesion and growth ^{38,135}
PuraMatrix	oligopeptides		scaffold supporting cell adhesion and growth ³⁸
polyvinylalcohol	acetate	PVA	scaffold for controlled drug delivery ¹³⁶

cell studies were conducted also using poly(ethylene glycol) (PEG) scaffold with neural,^{106,107} embryonic,¹⁰⁸ or mesenchymal stem cells^{104,105} and poly- ϵ -caprolactone with neural stem cells.⁹⁹

3. Synthetic–Natural Composite Hydrogels. The idea of using natural macromers such as fibronectin, laminin, or agarose in order to coat synthetic polymers to favor cell attachment and viability has been suggested in tissue engineering concepts from its very first description.¹⁷ However, in order to overcome matters of only natural or only synthetic hydrogels, this suggestion was dismissed, but now it has come back on the scene as one of the novelties of the last three years where great importance has been given to composite (synthetic–natural) hydrogels for spinal cord injury repair strategies.²⁸ They could be the result of a block copolymerization between synthetic and natural macromers, or just an interpolymer complex bonded by physical interactions. The goal of this approach should be to combine the biocompatibility of natural gels with the possibility to tune mechanical and physical properties by the inclusion of synthetic ones.¹³⁷ For example, the adhesive properties could be increased by adding polylysine to PEG channels, or chitosan to methacrylamide. This strategy was also studied in order to overcome the disadvantages of the “classic” 3D growth matrices, increasing cell viability and biocompatibility as in the case of agarose–Carbopol or hyaluronan–PEG. In these studies, multipotent stem cell lines appear to be promising great therapeutical advantages. Agarose–Carbopol hydrogels were tested with mesenchymal stem cells,¹³⁸ while

chitosan–methacrylamide and PEG–polylysine hydrogels were tested with neural ones.^{139–141} The complete list of the composite hydrogels used is presented in Table 3.

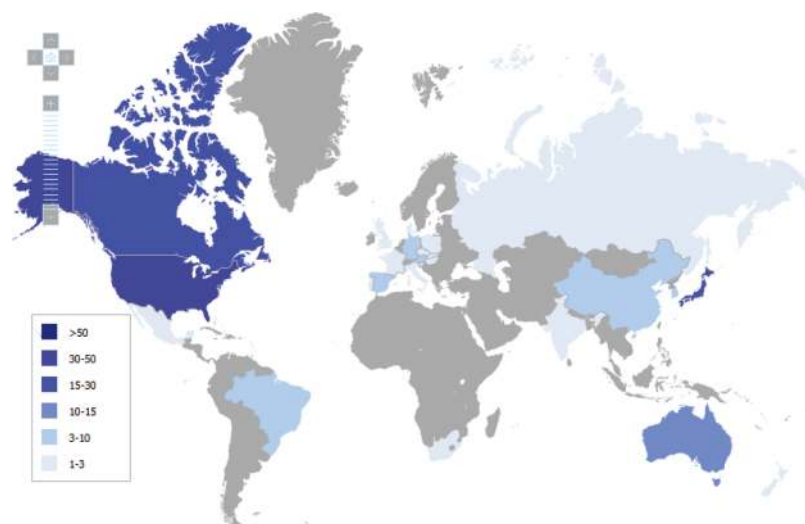
4. Patented Hydrogels. The field of materials for supporting SCI repair strategies is not only scientifically very rich but also very promising from an industrial point of view. Social and economical impacts of impaired SCI patients are unluckily very well-known, and the possibility to develop therapies toward repair is definitely appealing for the industry. Indeed, patent trends can be used as good indicators reflecting the increasing business interests in a specific technological area. This is illustrated by the fact that about 80% of technical and scientific knowledge generated worldwide is only published as patents and not elsewhere.¹⁵¹

Looking into biomaterials for SCI repair strategies, the first patent claiming the use of a hydrogel in SCI repair strategies dates back no further than 1984,¹⁵² while the oldest patent application on a hydrogel was filed on 1966.¹⁵³ An ever since cumulated snapshot, taken today, accounts for just 44 patents on hydrogels for SCI repair, out of about 4000 patents relating to hydrogels in general (search performed with QPat). Nevertheless, looking at the filing trend, the tremendous interest displayed in the scientific literature in the last 5 years is also reflected onto intellectual property rights: the same number of patent applications filed from 1984 to 2005 was filed only from 2006 up to today.

The same criteria applied in scientific literature was also used to categorize claims on hydrogels based on their main components: natural, synthetic, or both. As expected, the vast majority (71.5%)

Table 3. Synthetic–Natural Composite Hydrogels Studied in SCI Research

material	description	acronym	application in SCI
Carbopol + agarose	copolymer	AC	3D mesenchymal stem cell growth matrix ¹³⁸ scaffold for controlled drug delivery ¹⁴²
Carbopol + chitosan	interpolymer complex	IPC	multiple drug delivery ¹⁴³
methacrylamide + chitosan	cross-linked polymer	MC	cell adhesion and neurite penetration ^{140,144} neural stem cell growth matrix ¹³⁹
polyglycolic acid + chitosan	interpolymer complex	chitosan/PGA	bridge for neurite regrowth ¹⁴⁵
poly(ethylene glycol) + hyaluronan	interpolymer complex	HA-DTPH-PEGDA	3D growth matrix ¹⁴⁶
poly(ethylene glycol)/polyacrylic acid/agarose	layer	PEG/PAA/agarose	multilayer scaffold for BDNF controlled drug delivery ¹⁴⁷
poly(ethylene glycol) + polylysine	copolymer	PEG/PLL	cell growth matrix ¹⁴¹
poly(ethylene glycol) + polypeptides	copolymer	PEG/peptide	3D growth matrix ¹⁴⁸
polylactide-co-glycolic acid + dex-lactate	interpolymer complex	DP,DS	controlled protein release ¹⁴⁹
tetronic + lactide + heparin	copolymer	TL	bridge, with antiinflammatory agents, for axonal regeneration ¹⁵⁰

**Figure 2.** Geographical areas of intellectual property rights protection on hydrogels for SCI repair: darker colors illustrate a higher number of applications.

of applications claims the use of combined natural–synthetic hydrogels,^{154–168} while natural^{169–172} or synthetic^{173,174} hydrogels only accounted for 19% and 9.5% of applications, respectively. The industrial attitude is indeed generally pointing toward the wider possible intellectual property protection, which in this specific field is represented by combined solutions.

Lastly, a statistical study was performed on all applications filed since 1984, to show the main geographic areas of protection. These data are shown in Figure 2 where the darker colors illustrate a higher number of applications.

CONCLUSIONS

It is increasingly recognized that cell or drug therapies alone will not be sufficient for successful tissue engineering in many CNS disorders and insults. For this reason, engineered scaffolds have gained greater interest in the last years. In particular, spinal cord injury for its neuropathological features (loss of neuronal tissue and presence of cavity) represents a good candidate to develop an engineered scaffold able to carry substances (drugs, antibodies, peptides, or other proteins) and/or

cells. In this Review, we have given an overview of hydrogels used for experimental SCI repair, since this is an expanding field and most probably will be a useful applicable therapeutic tool in the near future. In this way, medicine and engineering work together to better define the promising therapies using this hybrid knowledge to design and engineer better tissue scaffolds.

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Dr. G. Perale, Dr. F. Rossi, and Dr. P. Veglianesi equally wrote the paper. Dr. S. Bacchiega performed patent research. Prof. Dr. E. Sundstrom, Prof. Dr. M. Masi, and Dr. G. Forloni coordinated the research.

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