Chapter 18

Injectable Smart Hydrogels for Spinal Cord Regeneration

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Abstract:

Spinal cord injury (SCI) is a dramatic condition that begins with immediate physical damage of the spinal cord and associated regions during an acute traumatic event. The damage of the tissue expands both in intensity and volume in the next subacute phase. At this stage multiple events complicate the pathologic condition and herein lies the main cause of post-traumatic neural degeneration that also ends with the so called chronic phase. In recent times different medical treatments, addressing different neurodegenerative mechanisms, have been proposed but were met with limited success when restated into clinical settings. The main reasons for this are that the pathogenesis of SCI is a continued multifactorial complaint and the treatment of only one factor is not sufficient to check neural degeneration and performing paralysis. Recent advances have led to the development of injectable hydrogels aiming to promote in situ delivery strategies of cells and/or drug to maximize the medical outcome. This chapter aims to provide an overview of hydrogels regenerative treatments that can be used to ameliorate SCI patients condition.

Keywords: drug delivery, hydrogels, polymer, regenerative medicine, tissue engineering.

18.1 Introduction

Spinal Cord Injury (SCI) is a ruinous neurological complaint that affects roughly 1.3 million persons worldwide, with 180,000 new cases each time [1]. SCI leads to ruinous neurological consequences. Depending on the position of the lesion, it results in paraplegia or tetraplegia with partial or total loss of motor/sensitive capacity. SCI is exacerbated by other frequent dysfunctions, similar as cardiac problems, infections, respiratory, bladder and bowel malfunctions, as well as pain runs (nociceptive and/ or neuropathic). All these conditions have a great impact on the lives of SCI patients, carrying heavy cerebral issues and representing also a heavy burden for society in terms of healthcare costs [1]. The spinal cord trauma results from a primary injury, substantially caused by vehicle accidents, violence, accidental cascade, and other traumatic events [2]. From primary injury arises a multi-factorial secondary injury, involving complex pathological mechanisms that start after primary SCI and can last months. These events include neuronal injury and death, neuroinflammation, breakdown of the blood-spinal cord barrier (BSCB) and oxidative stress. Utmost of the post-traumatic degeneration of the tissue is caused by this multifactorial secondary injury. Current treatment for SCI is represented by a drug accepted by both European Medicine Agencies and the FDA, methylprednisolone (MP), which is administered at a high cure for 48h in the acute phase [3]. MP is a corticosteroid that inhibits lipid peroxidation, acting as a free radical scavenger. It also limits the seditious response and preserves the BSCB, enhancing spinal cord blood inflow. However, its efficacity is controversial [4]. Current treatment to ameliorate SCI include also surgery to stabilize the spinal cord, as well as treatment of spasticity and rehabilitative care. Different strategies have been suggested to promote recovery in preclinical studies, and different remedial approaches are being tested to offset the secondary injury and ameliorate regeneration following SCI. Unfortunately, many of them shown no efficacity when restated to clinical trials (i.e. anti-Nogo antibody and the Rho antagonist Cethrin) [5]. The possible reason could be that utmost strategies are directed towards a single pathophysiological target while SCI is a multifactorial disease. So attendant and successive pathological events that take place during the progression of the secondary injury must be treated to achieve a global remedial effect [6].

Other reasons are associated with the limited pharmacological treatment by conventional administration, substantially because of the low attention achieved at the injured point and/ or implicit inferior side goods [7]. To overcome these limitations, a multitarget approach administered by indispensable route might be promising for SCI cases, also including a largely devoted neuropsychologic support, still too frequently neglected, to strengthen recovery protocols [8]. In this scenario, regenerative medicine using stem cell-based therapies is potentially protective in view of its own broad-spectrum efficacy, and has been extensively investigated preclinically in different SCI models. In this direction, thanks to recent advances, hydrogels that aim to promote functional tissue form following SCI are representing a promising strategy to work against SCI condition [7]. In particular injectable hydrogels are the most promising due to the possibility to pass through a needle and place at the target site avoiding surgical implantation and all the problems connected. The main issue that should be considered is that the mechanical stress of passing through the needle does not provoke damage to the hydrogel cargo on one side and, on material reorganization and biological tissues on the other. This approach can ameliorate spinal cord form in two ways: from a structural point of view, acting as supporting materials for tissue regeneration and, at the same time, acting as carriers able to maintain and release their cargo (e.g., stem cells and/or drugs) [9].

18.2 Pathophysiology of SCI

The pathological events following acute SCI are divided in two broad chronological events: the primary injury and the secondary injury, due to the fresh dangerous processes initiated by the primary injury [10]. The pathophysiological mechanism of SCI is further than a simple mechanical dislocation of nerve transmission following injury. In addition to the primary lesion of the spinal cord, a multi-step cascade events results in progressive exacerbation of the injury, due to factors that include ischaemia, oedema, haemorrhage and cytotoxicity [10, 11]. Although there is little or no loss of interneurons and motor neurons in several parts beyond the injury, there are significant changes in their biochemical, and accordingly physiological, properties [12]. The primary injury is represented by an immediate mechanical damage to the SC (contusion, compression, or laceration) that leads to a hemorrhagic zone of necrosis in the slate matter. Neurons and their axons come permeabilized

acutely following injury due to compressive and shear forces. Animal studies have demonstrated that neurological impairment increases relative to the force of trauma and the duration of contraction. Clinically, there are several types of primary injury. The most common medium involves impact with patient contraction. This happens in burst fracture with bone scrap contraction of the SC, fracture-disturbance or slice rupture following injury. Another mechanism of primary injury is caused by flexion, extension, gyration or disturbance, producing shearing or stretching of the SC. As said the primary mechanical injury is the starting point for fresh secondary mechanisms of injury extend (Figure 1) [13]. Accordingly, the damage can spread from the lesion center to caudal and rostral parts. The acute phase represents the first 48 hours after injury and is a direct result of physical trauma to the nervous tissue that causes death of cells near the point of injury. The main cells involved are neurons, astrocytes, oligodendrocytes and endothelial cells. The death of the endothelial cells of original blood vessels causes bleeding, which alters the force of oxygen and nutrients to the tissue, causing necrotic cell death. The subacute phase develops within minutes and can last several weeks following injury, up to 6 months in humans. During this phase we are witnessing a worsening of the original damage substantially due to the original seditious response. In addition, the cell death due to trauma increases the levels of amino acids, similar as glutamate, the excitotoxicity contributing extra-cellular fluid, while high situations of calcium activate enzymes that damage cellular structures (phospholipase, protease). The microglial cells are actuated and remain in this state for over to four months after injury [14]. The chronic phase, which persists throughout life, is characterized by the stabilization of the lesion through the conformation of the scar that has the function of confining and separating the lesion from the damaged tissue. Still, at the same time this work against the regeneration of nerves. The scar is surrounded by fibroblasts, activated macrophages and glial cells and frequently surrounds a cyst or a cavity [15]. A progressive expansion of the lesion in further than one segment, a process called syringomyelia, can take place for months or years after the lesion, adding the inflexibility of the lesion and causing death in some cases.

Figure 18.1 Pathophysiology and concomitant gene expression changes after spinal cord injury (SCI). (a) During the acute early phase after injury, immediate damage to the axonal tracts occurring at the moment of the insult are followed by general metabolic disturbances involving Na and K channels, as reported by gene expression analysis data. Dysfunction of ATPases (Ca and Na/K ATPases) also contributes to the general failure of the spinal cord to function appropriately. Early attempts to regrow are illustrated by the overexpression of Janus-activated kinase 1 (JAK1) and signal transducer and activation of transcription 3 (STAT3) molecules, and high expression of interleukins IL-1b and IL-6 illustrate the strong inflammatory response in the cord with recruitment of immune cells in the phase of secondary damage. (b) The secondary phase is characterized by accumulation of immune-system molecules within the injury site and the release of free radicals into the spinal cord. Accordingly, gene expression data highlight the upregulation of inducible nitric-oxide synthase (iNOS), IL-6, and IL-1b. The downregulation of myelin proteins such as myelin-oligodendrocyte glycoprotein (MOG) can be interpreted as an indication of demyelination, which is concomitant with the loss of cytoskeletal proteins inherent to cell death [e.g., tau, neurofilament proteins (NFs) and microtubule-associated protein 2 (MAP-2)]. Attempts of axonal regrowth are shown by the overexpression of metallothioneins and growth factors. (c) In the chronic phase following the injury, demyelination accompanies Wallerian degeneration of the distal axons (MOG, NFs and MAP-2 are downregulated). At the same time, upregulation of growth-promoting factors [e.g. brain-derived neurotrophic factor (BDNF) and the neurotrophin receptor trkB] and growth-associated molecules [e.g. vimentin and growth-associated protein 43 (GAP-43)] indicates a sustained effort of axonal regrowth. Increased glial fibrillary acid protein (GFAP) expression highlights the formation of scar tissue around the lesion site. Cells shown are: oligodendrocytes (light green), in part degenerating after the lesion; Schwann cells (dark green), potentially remyelinating the axons in the chronic phase; macrophages (blue) invading the lesion; neutrophils (red) invading the lesion; astrocytes (yellow) forming the glial scar in (c); and neurons (black) in the gray matter (GM), degenerating following the insult. Black lines represent axonal tracts running in the spinal cord and degenerating distal to the lesion through Wallerian degeneration in (b) and (c), and attempting to regrow in (c). Abbreviations: LAMP, limbic-system-associated membrane protein; MCP, monocyte-chemoattractant protein; NGFI-A, nerve-growth-factor-induced A; PDGF, platelet-derived growth factor; SNAP-25, 25 kDa synaptosomal-associated protein; VCAM, vascular cell adhesion molecule; VGF, inducible nerve growth factor; WM, white matter. Reprinted with permission from Elsevier [13].

18.3 Hydrogels

Hydrogel are three-dimensional networks of hydrophilic cross-linked polymers, natural or synthetic, held together by covalent bonds or other cohesive forces such as hydrogen or ionic bonds. They are glassy in the dry state and then, in presence of solvents, able to swell while preserving their original shape to form elastic gels. In particular, hydrogels are able to retain a large amount of water (up to 95% of the total weight) making the simulation of a tissue microenvironment possible in term of elasticity. Hydrogels are widely used in drug delivery and in tissue engineering thanks to their ability to carry drugs, growth factors and cells.

Hydrogel can be classified using different criteria:

- *Classification based on source*

1) Natural hydrogels, made of naturally derived polymers;
2) Synthetic hydrogels, made of synthetic polymers;
3) Hybrid hydrogels, made of both natural and synthetic polymers.

On one hand, synthetic polymers can be tuned in terms of composition, rate of degradation, and mechanical and chemical properties. On the other hand, natural hydrogels provide structures that are very similar to living tissues such as stimulating a specific cellular response.

- *Classification based on preparation method*

1) Homopolymer hydrogels, cross-linked networks of one type of hydrophilic monomer unit;
2) Copolymeric hydrogels, produced by cross-linking of many comonomer units;
3) Interpenetrating polymer hydrogels (IPN), consisting in two independent cross-linked polymers intermeshing to form a network.

- *Classification based on ionic charge*

1) Neutral;
2) Anionic;
3) Cationic;
4) Ampholytic;

5) Zwitterionic.

- *Classification based on physical structural features of the system*
  1) Amorphous hydrogels, in which macromolecular chains are randomly arranged;
  2) Semicrystalline hydrogels, characterized by dense regions of ordered macromolecular chains;
  3) Hydrogen-bonded, in which hydrogen bonds are responsible for the three-dimensional structure.

18.3.1 Structure and characteristic parameters

Variables that most characterize a hydrogel are: mesh size, cross-linkage density, average molecular weight between two following cross-link points and volume, both in dry and swelling state (Figure 2) [16]. Flory-Rehner theory defines the most important parameters as follows:

Figure 18. Errore. Nel documento non esiste testo dello stile specificato.**.** Network structure and characteristic parameters. Reprinted with permission from [16].

- polymer volume fraction in the swollen state (u_s) is the ratio between the polymer volume (V_p) and the swollen gel volume (V_g). It is also the reciprocal of volumetric swelling ratio (Q_v):

Equation 1

$$v_s = \frac{V_p}{V_g} = \frac{1}{Q_V}$$

Where Q_v can also be defined as:

Equation 2

$$Q_v = 1 + \frac{\rho_p}{\rho_s}(Q_m - 1)$$

ρ_p is the density of the dry polymer and ρ_s the density of the solvent; Q_m is the ratio between the weights of swollen polymer ($W_{swollen}$) and dry polymer (W_{dry}):

Equation 3

$$Q_m = \frac{W_{swollen}}{W_{dry}}$$

- effective molecular weight of polymer chain between two following cross-linking points (M_c) is related to the degree of gel cross-linking (X) and the molecular weight of repeating monomer unit (M_0):

Equation 4

$$M_c = \frac{M_0}{2X}$$

- distance between sequential points of crosslink (ξ) represents an estimate of space between macromolecular chains accessible for drug or cell diffusion. It can be calculated as:

Equation 5

$$\xi = \upsilon_s{}^{-1/3} C \left(\frac{M_c}{M_0}\right)^{1/2}$$

where C is a constant for a given polymer-solvent system.

- cross-linkage density (υ_e) is the ratio between polymer density (ρ_p) and M_c:

Equation 6

$$\upsilon_e = \frac{\rho_p}{M_c}$$

18.3.2 Swelling behavior

The peculiar property of hydrogels is their ability to swell, when put in contact with a thermodynamically compatible solvent (Figure 3) [17]. When a hydrogel in its initial state is in contact with solvent molecules, the latter attacks the hydrogel surface and penetrates into the polymeric network. The meshes of the network in the rubbery phase will start expanding, allowing other solvent molecules to penetrate within the hydrogel network.

Figure 18.3. Schematic of relevant state changes in hydrogel swelling. In the uncrosslinked state, chains are not connected and therefore cannot exert force on each other. Crosslinking results in a relaxed state as long as the polymer volume fraction remains unchanged. The addition of excess solvent drives swelling to free energy equilibrium. For calculation of polymer volume fraction, a final drying step enables accurate measurement of the polymer network's mass and volume. Reprinted with permission from [17].

The polar hydrophilic groups are the first to be hydrated upon contact with polar solvent (in general aqueous solutions) which leads to the formation of primary bound water, and then, the hydrophobic groups so exposed are able to interact with the water molecules. This leads to the formation of hydrophobically-bound water, also called secondary bound water. The network will absorb additional water, due to the osmotic driving force of the network chains towards infinite dilution. Swelling is not a continuous process: network absorbs water due to the osmotic force but there is an opposition due to the covalent or physical cross-links leading to an elastic force, which balances the stretching of the network and prevents its deformation. An equilibrium swelling level is reached: the elasticity and osmotic forces are balanced and there is no additional swelling. The additional absorbed water is called "free water" or "bulk water" and assumed to fill the space between the network chains, or the centres of macro-pores, or voids. Depending on the nature and composition of the hydrogel the next step is the disintegration of dissolution, if the network chain or cross-links are degradable. A very important feature of hydrogels is the rate of swelling which is determined by several physicochemical parameters, the most remarkable are the extent of porosity and the structure of pores. On this basis hydrogel can be classified in four classes:

- non-porous hydrogels;
- micro-porous hydrogels;
- macro-porous hydrogels;
- super-porous hydrogels.

18.3.3 Design Features

Any different application requires hydrogels to meet some biological and physical design criteria. Some key aspects are the following:

- **Biocompatibility**: it is defined as the ability of a biomaterial to perform its desired function without eliciting any undesirable local or systemic side effects. Such as inflammation and toxicity.
- **Crosslinking in presence of cells**: the ability to form hydrogels in the presence of cells and cargo molecules is critical for creating three-dimensional, controlled microenvironments and offers the ability to mold the gel to the shape of the defect site and delivery in a minimally invasive way. The chemical transformations involved in hydrogel formation, however, can be damaging to cells. Moreover, sudden localized changes in temperature, pH, and free radicals during gelation also can affect the activity of cargo molecules or cell function or viability.
- **Mechanical properties**: the success of cell-compatible hydrogels in a given bioengineering application is usually coupled with achieving appropriate mechanical properties. For example, load bearing capability is fundamental for therapeutic efficiency. Polymer concentration, the stoichiometry of reactive groups, and crosslinking density are all commonly used to tune the mechanical properties of cell-compatible hydrogels and accordingly to control the cellular microenvironment.
- **Degradation**: hydrogels must be designed to degrade in the right way: ester hydrolysis, enzymatic hydrolysis, photolytic cleavage or a combination of these mechanisms are some of the ways a hydrogel can be degraded. Even release kinetics plays a major role in degradation: it is dictated primarily by surface erosion or bulk degradation. An optimum balance between degradability and mechanical properties, such as elastic modulus and matrix integrity, is crucial to ensure the proper functionality of the hydrogel within the desired timespan. In addition, it is important to generate biocompatible byproducts and with no side effects, such as cytotoxicity, inflammation or immunological or foreign body responses.
- **Mass transport**: since in tissue engineering and cell encapsulation, continuous exchange of nutrients, proteins, gases (i.e., O_2 and CO_2) and waste products into, out of, or within the

hydrogel is essential for survival and proliferation of encapsulated cells, hydrogel matrix permeability is an important design parameter, given that mass transport in these materials is controlled primarily by diffusion. The permeability of the scaffold is also correlated with the mechanical properties of the hydrogel network and its swelling properties, and as expected, variation in the permeability is a widely employed strategy for controlling cargo release.

- **Microenvironment**: a great challenge in designing cell-compatible hydrogels is the ability to mimic the dynamic nature of the extracellular matrix (ECM). Spatiotemporal control over biological interactions at the material–cell interface, whether that material is native ECM or an engineered hydrogel, mediate cell proliferation, adhesion, migration, and receptor–ligand binding events.

18.3.4 Hydrogel production

Hydrogels can be prepared from either synthetic polymers or natural polymers. The synthetic polymers are chemically stronger compared to natural polymers. Their mechanical strength results in slow degradation rate, but on the other hand, mechanical strength provides the durability as well. These properties can be balanced in order to achieve a specific feature. Copolymerization/cross-linking free-radical polymerizations are commonly used to produce hydrogels by reacting hydrophilic monomers with multifunctional cross-linkers. Water-soluble linear polymers of both natural and synthetic origin are cross-linked to form hydrogels in a number of ways:

- linking polymer chains via chemical reaction;
- using ionizing radiation to generate main-chain free radicals which can recombine as cross-link junctions;
- physical interactions such as entanglements, electrostatics, and crystallite formation;
- Any of the various polymerization techniques can be used to form gels, including bulk, solution, and suspension polymerization.

In general, hydrogels are produced starting from monomer, initiator, and cross-linker. To control the heat of polymerization and the final hydrogels properties, diluents can be added, such as water or other aqueous solutions. The resulting hydrogel needs to be washed to remove impurities that may include non-reacted monomer, initiators, cross-linkers, and by-products form side reactions. The polymerization techniques are:

- **Bulk polymerization**: it involves only monomer and monomer-soluble initiators. High rate of polymerization and degree of polymerization occur because of the high concentration of monomer. However, the viscosity of reaction increases markedly with the conversion which generates the heat during polymerization. These problems can be avoided by controlling the reaction at low conversions.
- **Solution polymerization/cross-linking**: the presence of solvent as a heat sink is the major advantage of the solution polymerization over the bulk polymerization. The prepared hydrogels need to be washed to remove the monomers, oligomers, cross-linking agent, the initiator, the soluble and extractable polymer, and other impurities. Polymerization is brought on in monomer droplets.
- **Polymerization by irradiation**: ionizing high energy radiation has been used as an initiator to prepare hydrogels of unsaturated compounds. The irradiation of aqueous polymer solution results in the formation of radicals on the polymer chains. The major advantage of the radiation initiation over the chemical initiation is the production of relatively pure and initiator-free hydrogels.

18.3.5 Hydrogel technical features

Functional features of an ideal hydrogel material can be listed as follows:

- the highest absorption capacity in saline;
- desired rate of absorption depending on the application requirement;
- the highest absorbency under load;

- the lowest soluble content and residual monomer;
- the lowest price;
- the highest durability and stability in the swelling environment and during the storage;
- the highest biodegradability without formation of toxic species following the degradation;
- pH-neutrality after swelling in water;
- it has to be colourless, odourless, and absolute non-toxic;
- photo stability;
- re-wetting capability (if required).

Obviously, it is impossible that a hydrogel sample would simultaneously fulfil all the above mentioned required features.

18.3.6 Degradation

Degradation is one of the key properties of hydrogels, especially those ones employed in drug delivery and tissue engineering. It can be considered the opposite of polymerization and usually it is due to enzymatic, hydrolytic, or environmental (*e.g.* pH, temperature, or electric field) processes.
In our study only hydrolytic degradation is considered: it can be described as the insertion of a water molecule in an ester bond. There are two types of degradation processes of hydrogel networks: surface erosion and bulk erosion. In the process of surface erosion, the water will be adsorbed on the surface before it diffuses into the bulk of the sample, for the rate of water diffusion into a sample is slower than the degraded reaction. Bulk erosion, on the other hand, occurs when the rate of water diffusion into the sample is much faster than the hydrolysis reaction.

18.3.7 Hydrogel injectability

An injectable hydrogel is generally grounded on the idea that it can be fitted as liquid into human body, forming solid hydrogel in situ. One of the most important factors to be considered is the viscosity of the polymer solution, as this point is profitable in minimally invasive surgical procedures.

Another important factor is the hydrogel porosity, where largely inter-connected networks are preferred, as they grease better diffusion of nutrients and adaption to the surrounding biological tissues. This also relates to the proper mechanical properties as tensile strength and modulus, compressive stress and modulus, shear stress, stiffness as the hydrogel should repel the distortion that occurs in the mechanically dynamic environment in the body. Injectable hydrogels need also a good control of the gelation kinetics on the process procedure, which requires transporting the sol or the pre-gel to a targeting point through an injection device. This means that the sol – gel transition of an injectable hydrogel should be within a defined time interval to get the right injectability. In addition, the injection procedure for injectable hydrogels impacts on the structure and the properties of the final bulk gel, reaching a poorer performance against distortion than the corresponding in situ formed gels. As in other hydrogels, the mechanical properties and continuity can be tuned by varying the portion of the monomers or oligomers, the molecular weights, and the crosslinking density of the hydrogel. The typical crosslinking strategies applicable for hydrogels are also applied for the development of injectable hydrogels. So far, injectable hydrogels can be crosslinked by different mechanisms as physical and chemical interactions that can be set up.

18.4 Hydrogels in SCI repair

18.4.1 Hydrogels as cell carriers in SCI repair

In recent times, different studies have been carried out, aiming to the development of biomaterials for promoting tissue repairing after severe injuries, similar as SCI [7]. These accoutrements could enhance the restitution in two different ways they can be used as carriers suitable to maintain and release their content, but they can also act as scaffolds for tissue engineering [9].

In using biomaterials, different characteristics are of vital significance like biocompatibility, biodegradability, cytocompatibility and adaptive mechanical properties [18]. To gain the asked results for SCI, biomaterials should guarantee stem cell viability and companion axon regrowth across their structure. In this field, hydrogels are lately gaining significance for cell survival in situ indeed, they are soft matter, suitable to be injected (intrathecally, reducing surgical pitfalls) or implanted directly

and fills lesion depressions. Hydrogels are defined as 3D polymeric networks suitable to absorb large quantum of water and swell, maintaining their three-dimensional structure. They can be classified depending on the type of crosslinking that held together their networks, in general physical or chemical. As far as physical hydrogels are concerned, their structure is guaranteed thanks to electrostatic forces, steric interference or polymeric trap, while on the other hand they are kept together through chemical bonds. Generally physical hydrogels are also known as reversible hydrogels, due to their weak crosslinking with respect to the chemical one. As said hydrogels can be produced in a variety of ways linking polymers through chemical responses; using ionic radiation to induce free revolutionaries suitable to recombine as crosslink junction and/ or through physical relations (e.g. crystalline conformation, electrostatics, trap) [19]. The hydrogels therefore formed need to be purified, generally with washing, to exclude contaminations produced during the medication. Hydrogels show a variety of properties, similar as high inflexibility, no toxicity, gas permeability and good mechanical performances that make them ideal candidates for SCI treatment. Moreover injectability, therefore avoiding pitfalls due to surgery, is extremely important for a minimally invasive placement. Hydrogels are so chosen because they can be loaded with cells and/ or drugs; their injection is intrathecally and they are suitable to remain localized wherever they are fitted, therefore suitable to deliver the loaded solutes to the spinal cord [2, 20]. Hydrogels also bring some limitations, similar as difficulty to control the delivery (for case of using drugs with low steric hindrances that can easily escape from the structure), but also the problematic loading of hydrophobic molecules within their 3D network [21, 22]. These limitations can be luckily overcome. As far as the first one is concerned, polymer chains of hydrogel can be functionalized with different post-polymerization strategies, similar as the conformation of a chemical bond.

In this case, the controlled drug delivery is governed by the capability to break that bond (reactive controlled drug delivery). The link type can be chosen depending the medical need in fact, the weaker the bond the hastily the release [23]. Their proper functionalization can also increase cell adhesion, perfecting their performances as carrier [24]. Post-polymerization functionalization is grounded on the direct polymerization or copolymerization of monomers bearing chemo-picky handles that are inert toward the polymerization conditions but can be quantitatively converted in a posterior step into a

broad range of other functional groups. The success of this system is grounded on the excellent transformations attainable under mild conditions, the excellent functional-group forbearance, and the orthogonality of the post-polymerization revision responses [25]. For the hydrophobic solutes, their problem could be overcome loading them into polymeric nanoparticles that will be latterly loaded into the hydrogel [26]. Different studies have suggested the use of biopolymers for cell delivery [27], due to the presence of stem cells at the damaged point, in order to maximize the treatment efficacity. Loaded hydrogels are suitable to release factors buried by stem cells or sustaining them to fill the gap at the damaged point [28]. For bone marrow mesenchymal stem cells, in order to maximize their efficacity after in vivo transplantation, the use of 3D supports have been proposed for mimicking the stem cell niche [29]. The possibility of scaffolds to help axon regrowth is visible in Figure 4. Different studies have also been carried out to increase the survival and the efficacity of umbilical cord mesenchymal stem cells thanks to polymeric chains suitable to mimic a physiological niche in situ, suitable to save them from hostile environment and permitting paracrine release of factors [30]. Hydrogels structures were used also with adipose mesenchymal stem cells, in particular scaffolds for grease the connection of the damaged spinal cord member. Similar studies have been carried out in rat and canine SCI models [31].

Figure 18.4. Axonal growth in scaffolds seeded with bone marrow stromal cells. Only few bIII-tubulin-labeled axons are found in channels filled with (A) BMSCs expressing GFP, whereas many more axons are visible in (B) channels filled with BMSCs secreting BDNF. Higher magnifications of insets in (A, B) are shown in (A') and (B'). (C) Quantification of bIII-tubulin-labeled axons crossing a virtual plane vertical to the rostrocaudal orientation of channels, 100 lm and 500 lm from the edge of the gel. **p < 0.01. (D) Axons are able to extend throughout the entire length of the channels with a maximum distance to the host tissue of about 1 mm. Scale bars: 100 mm in (A, B, D); 50 mm in (A', B'). Reprinted with permission from Elsevier [29].

In hydrogel-grounded approaches extracellular matrix showed to be extremely important for stem cells viability [32]. An other possibility is represented by natural materials composed of native extracellular matrix. They represent structures analogous to those of the uninjured host tissue with advantages like natural 3D network, biodegradability and capability to guarantee proper cell adhesion and growth. In SCI repair the optimization of the host response, hydrogel degradation rate and capability to restore neural function are necessary to consider the eventuality of these hydrogels for clinical trials. Not only injectable hydrogels but also implantable polymeric devices, with proper fiber design, demonstrated to be extremely promising for SCI treatment. They are suitable to sustain stem cells viability, therefore offering different advantages for cell survival after transplantation. The fiber dimension can be controlled by modulating the operating parameters and physical properties of the solution and the chemical composition can be fluently acclimatized. Electrospun nanofibers can also be functionalized either by blending, encapsulation, or immobilization of bioactive molecules to work on specific natural responses. In addition, electrospun nanofibers can also be aligned uniaxially with anisotropic characteristics and they can be employed to construct microstructured units similar as sheets, disks, and tubes suitable to support axonal regrowth. In resembling with exploration conducted with biomaterials loaded with stem cells, in the last years a lot of interest was devoted to the capability of stem cells to produce numerous cytokines, growth factors, and cell adhesion factors that play important roles in perfecting the medium and promoting regeneration.

Papa and coworkers discovered that CCL2 chemokine from human MSCs can be delivered efficaciously in the lesioned spinal cord acting not only on macrophages, but driving also their conversion to an M2 neuroprotective phenotype [33]. Unexpectedly, CCL2 delivered also plays a crucial part in precluding motor neuron degeneration in vitro and after spinal cord trauma in vivo, with a significant enhancement of the motor performance of the rodent SCI models. In addition in the last years stem cells demonstrated the capability to release extracellular vesicles (EVs) microvesicles and exosomes. They are considered intercessors in communication between cells and suitable to mimic the action of stem cells carrying active molecules to the damaged cells from stem

cells. The big advantage resides in the possibility to have cell-free treatments that can be duly designed for therapies. The big challenge is represented by the extreme small quantity of factors buried and the issues related to the minimal amount demanded to gain a functional outcome.

18.4.2 Hydrogels as drug carriers in SCI repair

A said due to their elastic nature, hydrogels can be implanted or injected at the injury point, filling the SCI depression where they can release active agents and cells. Indeed a promising property of hydrogels is the possibility of direct in situ gelation. Its advantages are related to the reduction of several downsides of classical surgery that can complicate the cases conditions. Moreover their swelling capability, degradation rates and mechanical characteristics make hydrogels ideal tools, not only for the delivery of factors and small molecules, but also for hosting cells which in turn serve as drug delivery units. In the last years great attention was devoted to hydrogels in SCI especially for drug delivery. They can indeed be loaded with drugs and sustain their release during time. The release of small molecules have the problem that the associated kinetics can be uncontrolled (burst release) and so different strategies should be considered, with respect to the physical loading within the 3D network. As an example curcumin can ameliorate SCI condition once it is released constantly over time from a dynamic reversible hydrogel made of fluorenylmethoxycarbonyl protecting group (Fmoc)-grafted chitosan and Fmoc peptide. In this case the relations between the matrix and the drug molecules can sustain the release during time.

About biopharmaceutical release, different in vivo studies showed that hydrogels can be designed for a sustained release of neurotrophins into the SCI lesion. Recent studies have indeed demonstrated that the administration of neurotrophins (exogenous) like NT- 3, NT-4/4, NGF, BNDF and glial cell line-deduced neurotrophic factor (GDNF) promotes regeneration in SCI. In the last times, different styles were used to administer them using systemic administration route, direct injection or intrathecal infusion pump. As formerly refocused out, all these systems show numerous disadvantages like the impossibility to cross the blood spinal cord barrier (BSCB), no control of the release and problems due to surgery as the placement of a catheter, creation of a poke for a pump etc. In order to solve these problems, hydrogels were chosen as promising biomaterials that can

sustain the release of growth factors directly at the injury point, a winning strategy also considering their short bioavailability [34, 35]. Indeed, hydrogel networks demonstrated good capability to save bioactivity of GDNF, NT- 3, BDNF and fibroblast growth factor- 2 (FGF- 2). Growth factor can be also loaded in different gels (as in silk protein nanofiber hydrogels [36]) with hierarchical anisotropic microstructures to give multiple physical and natural cues. The maintained bioactivity of the growth factors inside the hydrogels can regulate the neuronal/astroglial isolation of neural stem cells. The aligned microstructures can help cell migration and exposure, that also stimulated neuroregeneration. The release of growth factors can also be dragged over time using soft thermo-sensitive electroactive hydrogels combined with functional electrical stimulation. An indispensable system suitable to guarantee great distribution of trophic factors at the injury point is represented by the use of scattered cells loaded within hydrogels, the so called " medicinal cells approach. Indeed one thesis validated by numerous studies is that stem cells can regulate the delivery of trophic factors. In addition a crucial advantage of loading cells within hydrogels is that they are confined prostrating the problems of unbridled isolation after transplantation and adverse impunity response. Different kinds of hydrogels were used for this purpose like a system grounded on thiol-functionalized hyaluronic acid and thiol-functionalized gelatin that can produce a neuroregenerative scaffold for oligodendrocyte stem cells [37]. In addition hydrogels from 2- hydroxyethyl methacrylate or 2-hydroxypropyl methacrylamide can reduce the lesion after being loaded with bone mesenchymal stem cells [38].

Some studies devoted a lot of attention on the specific types of molecules delivered from stem cells, like human chemokine ligand 2 from human mesenchymal stem cells. Its release from hydrogels can regulate macrophage inflammation and convert them to neuroprotective phenotype M2 showing a good enhancement of the motor performance of the rodent SCI models [33, 39, 40]. Indeed if the strategy to load only factors and not cells is veritably intriguing, the multitude of molecules released from cells cannot be fluently dissembled. Moreover an another crucial aspect that lately showed promising results is represented by extracellular vesicles, microvesicles and exosomes delivered from cells [41]. They can be considered as intercessors in cells communication that can mimic the action of stem cells carrying active molecules to the damaged cells [42, 43]. The use of stem cells to give extracellular

vesicles is a good strategy but the uncontrolled release and problems in their preservation are big issues that should be solved [44]. A possible result could be represented by the fabrication of an injectable tenaciousanti-inflammatory F127 - polycitrate- polyethyleneimine hydrogel (FE) with sustainable and long-term extracellular vesicle delivery (FE@EVs) that can ameliorate motor functional recovery after SCI. This delivery can suppress scar formation, reduce inflammation and promote neuroregeneration and remyelination.

18.4.3 Combinatorial strategies

Indeed even if theoretical studies on secondary injury are well supported by experimental substantiation, the results of clinical trials on SCI still present disappointing results. One of the reasons could be that the treatments proposed are directed only to specific mechanisms, not considering that SCI is a dynamic complaint where the different physiopathological medium be not contemporaneously and so it is reasonable to suppose that different targets should be addressed contemporaneously at different times. Following this direction, several studies that use combinatorial treatments, can be set up in preclinical models. Indeed recent studies are devoted to multitherapeutic composites that can efficiently target different mechanisms of the secondary injury [7, 11, 12]. Many of them propose the use of biomaterials that can release combinatorial curatives at the target point.

To more explain the possibilities, the SCI combinatorial curatives can be divided in four orders i) different growth factors directed to neuronal survival, axonal regrowth and creation of malleability; ii) different drugs; iii) scattered stem cells with different neurotrophic factors or iv) cells with trophic factors and biomaterial pulpits. In this environment, biomaterials can work as substrates for cell transplantation, drive axonal regrowth, fill the depression at the injury point and act as force that can be released with a controlled and sustained kinetics. Biomaterials can also be used against scar formation releasing chondroitinase ABC and also helping tissue regeneration [45]. Different studies demonstrated that this combination can help to restore the tissue in transected mouse models [46].
Musieko and coworkers described the possibility to combine pharmacological treatment with epidural stimulation to restore locomotion in mouse models [47]. Indeed if recent studies demonstrated that this

combination is salutary, the mechanisms behind locomotor advancements are still batted [48]. Some further considerations regards the fact that in numerous studies the active composites (growth factors, trophic factors or drugs) are administered systemically with consequent limited biodistribution. Another crucial point is that with systemic administration the treatments cannot be picky and so, for illustration, the use of different trophic/ growth factors may affect different cells contemporaneously and this may affect in some adverse responses like the revision of responsiveness in spinal circuitry. If used as single boluses, neurotrophic factors cannot maintain constant natural efficacity with consequent limited issues that so should bear multiple administrations [49]. All these findings suggest that indeed if the combinatorial curatives are promising they need to be bettered and biomaterials can play a vital role. Indeed, Hwang and coworkers used a biomaterial made of PCL loaded with stem cells and NT- 3 to ground the depression in a hemisected SCI model [50]. An analogous strategy is represented by the combination of human neural stem cells loaded within a polymeric matrix together with serotonin [51]. This could lead to the reduction of towel atrophy and astrocytic exertion adding axons ingrowth after biomaterial implantation. Another combinatorial approach [52] regards the use of adult brain-induced neural stemcells together with recombinant rat platelet-induced growth factor-A. In order to ensure a proper release kinetic, the growth factor isn't simply loaded but covalently linked to a hyaluronan-grounded hydrogel.

In addition also agarose hydrogels embedded with lipid microtubes were used to sustain the contemporaneous release of both Rho GTPases and BDNF [53]. This study demonstrates that the contemporaneous revision of multiple axonal responses can represent a promising approach to sustain spinal cord regeneration. Hydrogels can also be used to release contemporaneously drug and different growth factors with or without cells bedded in the 3D network [54]. Following this strategy the synergistical release of methylprednisolone sodium succinate and growth factors can cover axons and tissue from secondary injury, promote scar boundary- and depression-free crack mending, performing in permissive islands for axonal regrowth. Recent examinations have reported that docetaxel (DTX) can ameliorate axonal regeneration while FGF can regulate malleability and neuronal survival after SCI. They can be loaded in a liposome (LIP) with a silk fibroin (SF) hydrogel core for their contemporaneous release [55] (Figure 5). This combination showed to have the capability

to ameliorate different crucial pathological mechanisms. Indeed, docetaxel is suitable to promote microtubule stabilization and stimulate axonal growth while FGF can reduce the depression area and neuronal loss creating a good substrate for neuroregeneration. To guarantee proper release of hydrophobic and hydrophilic drugs a good strategy is represented by the use of NPs together with hydrogels. In particular hydrophobic active molecules can be loaded within NPs within a 3D polymeric matrix that can also produce a compound hydrogel after gelation. In this framework, the use of paclitaxel and minocycline showed to reduce inflammation and drop scar tissue.

Figure 18.5. Schematic diagram of the injectable liposome-silk fibroin composite hydrogel as an in situ multiple drug delivery system for the treatment of SCI. Reprinted with permission from [55].

18.5 Emerging trends and future directions

Despite the promising results obtained some issues should be answered before reaching clinical practice [56, 57]. First of all toxicity detailed studies are necessary to be sure about the degradation of hydrogels in non-toxic by-products. Other challenges should be overcome during the restatement process, similar as hydrogel fabrication and production, cost and nonsupervisory complexity. Indeed their high water content makes sterilization extremely delicate and sterility should be assured for all manufacturing processes and raw materials. However, treatment used must guarantee that both its structure and drug bioactivity are unaltered, if stored in dry state. On the contrary, if maintained in wet state, the manufacturing and transport conditions should minimize water evaporation and unwanted drug loss. So the high costs together with limited patent protection can be an issue for their marketable viability.

18.6 Conclusions

SCI physiopathology is an extremely disabling condition that heavily affect the life of the patients. As preliminarily described, it is the result of a primary injury also followed by a secondary one, generally known as the main cause of post traumatic neural degeneration. Secondary injury involves different

mechanisms, and everyone plays a part in the progressive loss of locomotor performances and tissue degeneration. Unluckily, different treatments produced only modest results when restated to clinical trials. A possible reason could be represented by the limitation of systemic drug administration due to BSCB restrictions and uncontrolled release rates of the active agents. To overcome these critical issues, experimenters are pointing toward the use of biomaterials-grounded delivery tools (hydrogels and nanoparticles) to optimize SCI treatments. The main advantage in using this kind of devices is related on the localization of the curatives in the target point. As bandied, the use of nano-systems can insure targeted release directed to specific cell lines taking advantage on the selectivity of proper formulated systems. Also, hydrogels can be confined in the injury point, filling the SCI depression and release in situ active agents and cells. So, gels, thanks to their properties, are ideal tools not only for the delivery of factors or active molecules, but also for hosting cells and serving as drug delivery units and because of this are numerous times combined with nanoparticles to increase their efficacity and confine their action. The use of these devices can ensure different advantages like the localization in the target point prostrating the problems related to BSCB as well as the release of active composites within a asked range, reducing side goods of conventional treatments.

The lack of satisfactory results in SCI treatment presumably is due to the fact that they are directed only to single mechanisms losing the complexity and the multitude of mechanisms involved in SCI. Following this direction combinatorial treatments represent a new challenge in SCI treatment and so the possibility to have contemporaneous releases from the same device can be a crucial point in synergizing the efficacity of multitarget treatments against a multifactorial complaint like SCI.

References

1. A. Singh, L. Tetreault, S. Kalsi-Ryan, A. Nouri and M. G. Fehlings, *Clin. Epidemiol.*, 2014, **6**, 309–331.
2. G. Perale, F. Rossi, M. Santoro, M. Peviani, S. Papa, D. Llupi, P. Torriani, E. Micotti, S. Previdi, L. Cervo, E. Sundstrom, A. R. Boccaccini, M. Masi, G. Forloni and P. Veglianese, *J. Control. Release*, 2012, **159**, 271-280.
3. K. Breslin and D. Agrawal, *Pediatr. Emerg. Care.*, 2012, **28**, 1238–1245.

4. R. J. Hurlbert, M. N. W. Hadley, B. C. Walters, B. Aarabi, S. S. Dhall, D. E. Gelb, C. J. Rozzelle, T. C. Ryken and N. Theodore, *Neurosurgery*, 2013, **72**, 93–105.
5. B. K. Kwon, E. B. Okon, W. Plunet, D. Baptiste, K. Fouad, J. Hillyer, L. C. Weaver, M. G. Fehlings and W. Tetzlaff, *J. Neurotrauma.*, 2011, **28**, 1589–1610.
6. N. A. Silva, N. Sousa, R. L. Reis and A. J. Salgado, *Prog. Neurobiol.*, 2014, **114**, 25-57.
7. F. Rossi, G. Perale, S. Papa, G. Forloni and P. Veglianese, *Expert Opin. Drug Deliv.*, 2013, **10**, 385–396.
8. S. Taylor-Schroeder, J. LaBarbera, S. McDowell, J. M. Zanca, A. Natale, S. Mumma, J. Gassaway and D. Backus, *J. Spinal Cord Med.*, 2011, **34**, 196–204.
9. M. S. Shoichet, *Macromolecules*, 2010, **43**, 581–591.
10. R. J. Dumont, S. Verma, D. O. Okonkwo, R. J. Hurlbert, P. T. Boulos, D. B. Ellegala and A. S. Dumont, *Clin. Neuropharmacol.*, 2001, **24**, 265-279.
11. M. W. Ronsyn, Z. N. Berneman, V. F. I. Van Tendeloo, P. G. Jorens and P. Ponsaerts, *Spinal Cord*, 2008, **46**, 532-539.
12. R. R. Roy, S. J. Harkema, V. R. Edgerton and *Arch. Phys. Med. Rehabil.*, 2012, **93**, 1487–1497.
13. F. M. Bareyre and M. E. Schwab, *Trends Neurosci.*, 2003, **26**, 555–563.
14. J. R. Bethea and W. D. Dietrich, *Curr. Opin. Neurol.*, 2002, **15**, 355-360.
15. J. H. Bruce, M. D. Norenberg, S. Kraydieh, W. Puckett, A. Marcillo and D. Dietrich, *J. Neurotrauma*, 2000, **17**, 781-788.
16. J. M. Uruena, A. A. Pitenis, R. M. Nixon, K. D. Schulze, T. E. Angelini and W. G. Sawyer, *Biotribology*, 2015, **1**, 24-29.
17. N. R. Richbourg and N. A. Peppas, *Prog. Polym. Sci.*, 2021, **105**, 101243.
18. A. Nolan Wilson and A. Guiseppi-Elie, *Adv. Healthc. Mater.*, 2013, **2**, 520-532.
19. E. M. Ahmed, *J. Adv. Res.*, 2015, **6**, 105–121.
20. F. Pinelli, O. F. Ortola, P. Makvandi, G. Perale and F. Rossi, *Nanomedicine*, 2020, **15**, 2707-2727.
21. H. R. Culver, J. R. Clegg and N. A. Peppas, *Acc. Chem. Res.*, 2017, **50**, 170–178.
22. B. Sharma, R. Bharti and R. Sharma, *Mater. Today Proc.*, 2022, **65**, 3657-3664.
23. E. Mauri, F. Rossi and A. Sacchetti, *Mater. Sci. Eng. C.*, 2016, **61** 851–857.
24. S. B. Anderson, C. C. Lin, D. V. Kuntzler and K. S. Anseth, *Biomaterials*, 2011, **32**, 3564–3574.
25. M. A. Gauthier, M. I. Gibson and H. A. Klok, *Angew. Chemie - Int. Ed.*, 2009, **48**, 48-58.
26. F. Rossi, R. Ferrari, F. Castiglione, A. Mele, G. Perale and D. Moscatelli, *Nanotechnology*, 2015, **26**, 015602.
27. A. Agbay, J. M. Edgar, M. Robinson, T. Styan, K. Wilson, J. Schroll, J. Ko, N. K. Mohtaram, M. B. G. Jun and S. M. Willerth, *Cells Tissues Organs*, 2016, **202**, 42-51.

28. I. Caron, F. Rossi, S. Papa, R. Aloe, M. Sculco, E. Mauri, A. Sacchetti, E. Erba, N. Panini, V. Parazzi, M. Barilani, G. Forloni, G. Perale, L. Lazzari and P. Veglianese, *Biomaterials*, 2016, **75**, 135-147.
29. M. I. Günther, N. Weidner, R. Müller and A. Blesch, *Acta Biomater.*, 2015, **27**, 140–150.
30. J. Liu, J. Chen, B. Liu, C. Yang, D. Xie, X. Zheng, S. Xu, T. Chen, L. Wang, Z. Zhang, X. Bai and D. Jin, *J. Neurol. Sci.*, 2013, **325**, 127–136
31. S. S. Park, Y. J. Lee, S. H. Lee, D. Lee, K. Choi, W. H. Kim, O. K. Kweon and H. J. Han, *Cytotherapy*, 2012, **14**, 584-597.
32. M. C. Prewitz, F. P. Seib, M. von Bonin, J. Friedrichs, A. Stibel, C. Niehage, K. Muller, K. Anastassiadis, C. Waskow, B. Hoflack, M. Bornhauser and C. Werner, *Nat. Methods.*, 2013, **10**, 788-794.
33. S. Papa, I. Vismara, A. Mariani, M. Barilani, S. Rimondo, M. De Paola, N. Panini, E. Erba, E. Mauri, F. Rossi, G. Forloni, L. Lazzari and P. Veglianese, *J. Control. Release*, 2018, **278**, 49-56.
34. J. S. Katz and J. A. Burdick, *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.*, 2009, **1**, 128-139.
35. S. Jacob, A. B. Nair, J. Shah, N. Sreeharsha, S. Gupta and P. Shinu, *Pharmaceutics*, 2021, **13**, 357.
36. X. Gao, W. Cheng, X. Zhang, Z. Zhou, Z. Ding, X. Zhou, Q. Lu and D. L. Kaplan, *ACS Appl. Mater. Interfaces*, 2022, **14**, 3701–3715.
37. X. Li, X. Liu, L. Cui, C. Brunson, W. Zhao, N. R. Bhat, N. Zhang and X. Wen, *FASEB J.*, 2013, **27**, 1127–1136.
38. E. Syková, P. Jendelová, L. Urdzíková, P. Lesný and A. Hejcl, *Cell. Mol. Neurobiol.*, 2006, **26**, 1113–1129.
39. S. Papa, F. Pizzetti, G. Perale, P. Veglianese and F. Rossi, *Expert Opin. Biol. Ther.*, 2020, **20**, 1203–1213.
40. K. Park, *J. Control. Release*, 2014, **190**, 3-8.
41. J. Feng, Y. Zhang, Z. Zhu, C. Gu, A. Waqas and L. Chen, *Front. Cell Dev. Biol.*, 2021, **9**, 703989.
42. K. A. Ruppert, T. T. Nguyen, K. S. Prabhakara, N. E. Toledano Furman, A. K. Srivastava, M. T. Harting, C. S. J. Cox and S. D. Olson, *Sci. Rep.*, 2018, **8**, 480.
43. M. Gimona, K. Pachler, S. Laner-Plamberger, K. Schallmoser and E. Rohde, *Int. J. Mol. Sci.*, 2017, **18**, 1190.
44. C. Wang, M. Wang, K. Xia, J. Wang, F. Cheng, K. Shi, L. Ying, C. Yu, H. Xu, S. Xiao, C. Liang, F. Li, B. Lei and Q. Chen, *Bioact. Mater.*, 2021, **6**, 2523–2534.
45. E. J. Bradbury, L. D. F. Moon, R. J. Popat, V. R. King, G. S. Bennett, P. N. Patel, J. W. Fawcett and S. B. McMahon, *Nature*, 2002, **416**, 636–640.

46. K. Loy and F. M. Bareyre, *Neural Regen. Res.*, 2019, **14**, 405–412.
47. P. Musienko, R. van den Brand, O. Märzendorfer, R. R. Roy, Y. Gerasimenko, V. R. Edgerton and G. Courtine, *J. Neurosci.*, 2011, **31**, 9264–9278.
48. J. Donovan and S. Kirshblum, *J. Am. Soc. Exp. Neurother.*, 2018, **15**, 654–668.
49. J. Peleshok and H. U. Saragovi, *Biochem. Soc. Trans.*, 2006, **34**, 612–617.
50. D. H. Hwang, H. M. Kim, Y. M. Kang, I. S. Joo, C. S. Cho, B. W. Yoon, S. U. Kim and B. G. Kim, *Cell Transplant.*, 2011, **20**, 1361–1379.
51. J. Růžička, N. Romanyuk, A. Hejčl, M. Vetrík, M. Hrubý, G. Cocks, J. Cihlár, M. Přádný, J. Price, E. Syková and P. Jendelova, *Acta Neurobiol. Exp.*, 2013, **73**, 102–115.
52. A. J. Mothe, R. Y. Tam, T. Zahir, C. H. Tator and M. S. Shoichet, *Biomaterials*, 2013, **34**, 3775–3783.
53. A. Jain, R. J. McKeon, S. M. Brady-Kalnay and R. V. Bellamkonda, *PLoS One*, 2011, **6**, e16135.
54. S. Zhu, Y. Ying, Q. Wu, Z. Ni, Z. Huang, P. Cai, Y. Tu, W. Ying, J. Ye, R. Zhang, Y. Zhang, M. Chen, Z. Xiang, H. Dou, Q. Huang, X. Li, H. He, J. Xiao, Q. Ye and Z. Wang, *Chem. Eng. J.*, 2021, **426**, 130827.
55. Q. Wang, X. Dong, H. Zhang, P. Li, X. Lu, M. Wu, W. Zhang, X. Lin, Y. Zheng, Y. Mao, J. Zhang, Y. Lin, X. Chen, D. Chen, J. Wang and J. Xiao, *Chem. Eng. J.*, 2021, **415**, 128964.
56. J. Li and D. J. Mooney, *Nat. Rev. Mater.*, 2016, **1**, 16071.
57. S. Bernhard and M. W. Tibbitt, *Adv. Drug Deliv. Rev.*, 2021, **171**, 240–256.