Regenerative medicine for spinal cord injury: focus on stem cells and biomaterials

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ABSTRACT

Introduction: Spinal cord injury (SCI) is a dramatic medical pathology consequence of a trauma (primary injury). However, most of the post-traumatic degeneration of the tissue is caused by the so-called secondary injury, which is known to be a multifactorial process. This, indeed, includes a wide spectrum of events: blood-brain barrier dysfunction, local inflammation, neuronal death, demyelination and disconnection of nerve pathways.

Areas covered: Cell therapy represents a promising cure to target diseases and disorders at the cellular level, by restoring cell population or using cells as carriers of therapeutic cargo. In particular, regenerative medicine with stem cells represents the most appealing category to be used, thanks to their peculiar features.

Expert opinion: Many preclinical research studies demonstrated that cell treatment can improve animal sensory/motor functions and so demonstrated to be very promising for clinical trials. In particular, recent advances have led to the development of biomaterials aiming to promote in situ cell delivery. This review digs into this topic discussing the possibility of cell treatment to improve medical chances in SCI repair.

1. Introduction: spinal cord injury

Spinal Cord Injury (SCI) is a devastating neurological disorder that affects approximately 1.3 million persons worldwide, with 180,000 new cases each year \cite{1}. SCI leads to devastating neurological deficits. Depending on the level of lesion, it results in paraplegia or tetraplegia with partial or total loss of motor/sensory capacity. SCI is aggravated by other frequent dysfunctions, such as cardiac problems, infections, respiratory, bladder, and bowel malfunctions, as well as pain syndromes (nociceptive and/or neuropathic) \cite{2}. All these conditions have an great impact on the lives of SCI patients, carrying heavy psychological issues and representing also a heavy burden for society in terms of health-care costs \cite{1}.

The spinal cord trauma results from a primary injury, mainly caused by: vehicle accidents, violence, accidental falls, and other traumatic events \cite{1}. From primary injury arises a multifactorial 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-spool cord barrier (BSCB), and oxidative stress. Most of the post-traumatic degeneration of the tissue is caused by this multifactorial secondary injury \cite{3}. 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 dose for 48 h in the acute phase \cite{4}. MP is a corticosteroid that inhibits lipid peroxidation, acting as a free radical scavenger. It also limits the inflammatory response and preserves the BSCB, enhancing spinal cord blood flow. However, its efficacy is controversial \cite{5}. Current treatment to ameliorate SCI include also surgery to decompress and stabilize the spinal cord \cite{6}, as well as treatment of spasticity and rehabilitative care \cite{7}. Different strategies have been suggested to promote recovery in preclinical studies, and diverse therapeutic approaches are being tested to counteract the secondary injury and improve regeneration following SCI \cite{3}.

Unfortunately, many of them shown no relevant efficacy when translated to clinical trials (i.e. anti-Nogo antibody and the Rho antagonist Cethrin) \cite{8}. The possible reason could be that most strategies are directed toward a single pathophysiological mechanism; however, SCI is a multifactorial disease, and concomitant and consecutive pathological events occur during the progression of the secondary injury must be treated to achieve a global therapeutic effect \cite{3}. Other reasons are associated with the limited pharmacological treatment by conventional administration, mainly because of the low concentration achieved at the injured site and/or potential unacceptable side effects \cite{9}. To overcome these limitations, a multi-target therapeutic approach administered by alternative route might be promising for SCI patients, also including a highly dedicated neuropsychologic support, still too often neglected, to strengthen rehab protocols \cite{10}. 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...
primary mechanical injury is the starting point for additional adults with underlying degenerative spine disease. This type of injury may be present without flexion, extension, rotation, or dislocation, producing shearing or lowing injury. Another mechanism of primary injury is caused by compression of the SC, fracture-dislocation, or disc rupture following, there are a series of differences in MSCs from different tissues for cell-to-cell contact and/or by transient paracrine interactions and replacement as reported in different models of neurological disease, although the mechanisms have not been directly demonstrated [27].

Different sources of MSCs exist, such as bone marrow, umbilical cord, amniotic liquid and adipose tissue. As discussed in the following, there are a series of differences in MSCs from different sources that can be highlighted for SCI therapy.

Differently from hematopoietic cells, bone marrow (BM)-MSCs are able to adhere to plastic and differentiating into cells of mesodermal origin. Nonetheless, specific markers are necessary to identify such cells [28]. BM-MSCs were originally thought to be pluripotent, differentiating into neurons and glial cells, but recently these findings are being questioned. Undeniably, cell fusions or transdifferentiation in neuron may occur after transplantation [30]. This kind of cells transplanted into mouse and rat

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 additional damaging processes initiated by the primary injury [15,16]. The pathophysiological mechanism of SCI is more than a simple mechanical disruption of nerve transmission following injury.

In addition to the primary lesion of the spinal cord, multi-step cascade results in progressive enlargement of the injury, due to factors that include an inflammatory reaction, ischemia, edema, hemorrhage, and cytotoxicity [17]. Although there is little or no loss of interneurons and motor neurons in several segments beyond the injury, there are significant changes in their biochemical, and consequently physiological, properties [18,19].

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 gray matter. Neurons and their axons become 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 compression. Clinically, there are several types of primary injury. The most common mechanism involves impact with persistent compression [20]. This happens in burst fracture with bone fragment compression of the SC, fracture-dislocation, or disc rupture following injury. Another mechanism of primary injury is caused by flexion, extension, rotation, or dislocation, producing shearing or stretching of the SC. This type of injury may be present without obvious radiological evidence of trauma, but it is common in adults with underlying degenerative spine disease [20]. The primary mechanical injury is the starting point for additional secondary mechanisms of injury extend (Figure 1) [21]. Consequently, the damage can spread from the lesion epicenter to caudal and rostral segments. 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 site of injury. The main cells involved are neurons, astrocytes, oligoden-drocytes, and endothelial cells. The death of the endothelial cells of local blood vessels causes bleeding, which alters the supply of oxygen and nutrients to the tissue, causing additional necrotic cell death [22].

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 initial damage mainly due to the local inflammatory response. In addition, the cell death due to trauma increases the levels of amino acids, such as glutamate, the excitotoxicity contributing extra-cellular fluid, while high levels of calcium activate enzymes that damage cellular structures (phospholipase, protease). The microglial cells are activated and remain in this state for up to 4 months after injury [23]. The chronic phase, which persists throughout life, is characterized by the stabilization of the lesion through the formation of the scar that has the function of confining and separating the lesion from the damaged tissue. However, at the same time this prevents the regeneration of nerves. The scar is surrounded by fibroblasts, activated macrophages and glial cells and often surrounds a cyst or cavity [24]. A progressive expansion of the lesion in more than one segment, a process called syringomyelia, can occur for months or years after the lesion, increasing the severity of the lesion and causing death in some cases.

3. Cell-based approach for SCI repair

Mesenchymal stem cells (MSCs) represent a promising stem type for SCI repair strategies: they do not arouse ethical concerns and are apparently safe inserted into the central nervous system (CNS) [25,26]. MSCs have recently shown a variety of enticing properties promoting their therapeutic use in CNS pathologies. Some examples of the former properties are the anti-inflammatory, immunomodulatory, trophic and anti-apoptotic effects in a variety of animal models of CNS disorder [27–29].

These functions might be mediated by migration to injured tissues for cell-to-cell contact and/or by transient paracrine bystander mechanisms, rather than resulting in cell differentiation and replacement as reported in different models of neurological disease, although the mechanisms have not been directly demonstrated [27].

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SCI models exposed to different types of mechanical stresses of the spinal cord have shown improvements in motor activities [12]. Different routes of administration exist, such as intravenous (IV) infusion [31–34] or grafting directly into damaged spinal cord in preclinical models [12]. BM-MSCs have shown an anti-inflammatory protective role [35] and are able to protect the injured spinal cord from further cellular damage through trophic support and neuroprotective activities. Different trophic factors have been studied: vascular endothelial growth factor (VEGF), nerve growth factor (NGF), glial cell-derived neurotrophic factor (GDNF), and brain-derived neurotrophic factor (BDNF). Furthermore, BM-MSCs can be also used as vectors for therapeutic agent delivery thanks to their ability of migration toward damaged tissues [36]. Following different encouraging results, some treatments have been shifted to clinical practice, showing safe BM-MSCs transplantation in SCI patients and partial efficacy in some cases [37–43].

However, differently from preclinical studies, intrathecal injection has mostly been adopted for SCI patients instead of in situ (within parenchyma) injection.
Umbilical cord (UC)-MSCs are easily obtainable through umbilical cord or cord blood treatments, since they can be stored at cryogenic temperatures until use. UC-MSCs are hypoinmunogenic and cause less graft rejection than other SCs [44].

Different preclinical studies have demonstrated their therapeutic ability [45] with various rats and mouse SCI animal models, showing neurotrophic [45], anti-inflammatory [14,46], anti-apoptotic [47] and angiogenic actions [48]. From preclinical studies, their most frequent route of administration is in situ. Shifting to clinical trials, although numerous promising preclinical studies, only few of them have been published on the safety and efficacy of UC-MSCs [49,50].

Amniotic fetal (AF)-MSCs can be obtained from amniotic fluid or amniotic membrane, elements that are important for fetus sustainability and protection, and organ development during pregnancy [51]. AF-MSCs are a valid alternative source of MSCs for regenerative medicine in SCI treatment [51]: as a matter of fact, they show different advantages, as minimal invasive isolation and no ethical problems. Furthermore, they exhibit multipotency, efficient proliferative activity, non-tumorigenicity, and low immunogenicity. Different studies have shown that these cells could also improve functional recovery in preclinical mouse and rats models [52,53]. Another study also demonstrates that AF-MSCs exhibited an effective treatment even in primates [54].

Adipose Tissue MSCs (A-MSCs) can be obtained through different noninvasive techniques, such as lipoplasty or liposuction, from adipose tissue. A series of features have been attributed to these kinds of MSCs, such as secretion of trophic growth factors [55], modulation of activated immune cells [56], neuroregeneration [57,58], anti-apoptotic action [59] and multilineage differentiation capacity [60].

Different preclinical and clinical studies have demonstrated great regenerative effects of A-MSCs whenever transplanted in situ in SCI [61,62].

Furthermore, some studies have also demonstrated that A-MSCs are also able to improve functional motor recovery, without adverse effects, when injected IV after acute SCI [63]. In particular they demonstrated the ability to reduce glial scar formation and stimulate axonal regrowth together with the promotion of tissue preservation with abundant deposition of laminin. For survival and therapeutic efficacy enhancement of A-MSCs in situ, the coadministration of different compounds (17B-Estradiol and chondroitinase ABC) has been studied [64–66].

All the combinatorial approaches tested have shown a great improvement in efficacy and motor function recovery compared to single treatments. Different clinical trials have also been proposed to confirm safety following intrathecal injection of these MSCs [67–69].

Therapeutic strategies with stem cells have been tested in a series of diseases, as for their ability to repair mechanical damaged nerve tissue in SCI [70,71]. Among them embryonic stem cells (ESCs) are in fact able to generate new cells in human and animal CNS tissue, such as neurons and glial cells [72]. The leading strategies for SCI treatment are ESCs inducement to differentiation into specific phenotypes for desired cells replacement (neurons or glia) or to produce factors able to limiting the damage and sustain the tissue regeneration [73]. Different ESC-based therapeutic approaches have been tested for SCI treatment; some groups have shown improvements in motor and sensory function after transplantation in animal models. A study demonstrated that allogenic neural stem cells (NSCs), derived from ESCs, promoted functional recovery when grafted in the damaged spinal cord of some marmosets. Other studies have shown that stem cells clonally derived from ESCs transplanted into a mouse SCI model allowed preservation of cells and tissue [74]. Furthermore, a combinatorial therapeutic strategy with growth factors has exhibited a survival enhancement and differentiation into neurons [75].

ESCs that have differentiated into oligodendrocytes have also been tested in SCI treatment, achieving motor activity improvements, after reconstitution of a part of the white matter in SCI rat models [76,77]. As far as clinical settings are concerned, Geron Corporation proposed a trial in 2010, recruiting SCI patients to assess ESC-derived oligodendrocytes grafted in the damaged spinal cord, but such a company stopped the trials 1 year after for lack of funds. Recently, these trials have been restarted by Asterias Biotherapeutics and are now on going [78]. Among other cells, NSCs showed to be multipotent, self-renewing progenitor or stem cells isolated from the subventricular zone of the hippocampus and a region of central canal of the spinal cord [79]. NSCs are able to differentiate into specific neuronal or glial phenotypes, thus replacing lost tissue or producing pro-regenerative factors. Studies on rodent SCI models have demonstrated that transplanting NCSs into lesioned spinal cord allowed functional recovery, sustained through neuronal cells replacement able to reconstitute lost neuronal and glial tissue, with trophic support [80], preserving damaged cells and axons. The potential effect of immunomodulation has been shown in other neurodegenerative diseases. Clinical trials proposed this far have demonstrated that NCSs transplantation into the injury site can be effectuated safely, but their effects on recovery have not been documented yet [81]. Interventions that regulate the differentiation, migration, and functional maturation of newly generated cells, without overactivating the NSCs (a speculated tumorigenic risk), should be particularly considered. Moreover, the role of NSCs is also to help the reorganization of neural circuits generated through synaptic formation between graft-derived neurons and host-derived neurons [82,83]. It is indeed well known that one of the mechanisms through which functional improvements occur in SCI is through neural plasticity, so the ability of the central nervous system to reorganize its circuits over time. However its contribution is highly debated and only sophisticated technologies and genetic techniques will hopefully elucidate the contribution of plasticity to recovery from SCI.

Induced pluripotent stem cells (iPSCs) are a valid alternative to ESCs. Such cells are produced by reprogramming somatic cells in the presence of some transcription factors (Yamanaka factors) and by other methods [84]. iPSCs have the advantage of avoiding ethical problems related to the
use of embryos and allow autologous transplantation of pluripotent cells able to reduce rejection risk.

Unfortunately, these cells also share some disadvantages of the ESCs, such as the risk of teratoma formation [84]. Nonetheless, different studies have shown iPSCs safety and therapy efficacy in SCI after in situ injection.

Even if some promising results have been obtained in mouse models for using iPSCs in cell therapy, their potential role in SCI treatment should be demonstrated with further investigations.

Olfactory ensheathing cells (OECs) are glial cell types that play a key role in neural regeneration of olfactory neurons. OECs are obtainable thanks to nasal biopsies from the olfactory mucosa (OM) or from the olfactory bulb (OB) [85].

OECs are promising for SCI treatment because, after their implantation in the damaged zone, they can create a permissive environment for axonal regeneration, able to cross the injured site in several rodent SCI models [86]. Different mechanisms have been proposed to contribute these cells efficacy in axon regeneration support, such as providing several neurotrophic molecules. Different studies have reported that OECs transplanted into the spinal cord are able to facilitate axon regeneration and ameliorate motor function [87,88]. Furthermore, a study has indicated that these cells transplanted into transected spinal cord of rat models promoted tissue regeneration after 3 months, persisting up to 7 months after treatment [89]. Combinatorial therapy has been proposed for pro-regenerative efficacy improvement of OECs in rat SCI models. However, the ability of OECs of promoting tissue regeneration remains controversial [90]. This controversy is probably due to the observation that OECs can comprise a series of subpopulations that are poorly described phenotypically or functionally [91].

In peripheral nerves, Schwann cells support axonal regeneration after damaged, so this has suggested their application in SCI treatment [92]. They could contribute to regeneration after injury sustaining axonal regrowth and myelination. Schwann cells are also able to produce different growth factors, cell adhesion molecules, and extracellular matrix proteins that can enhance recovery after SCI [92]. Their efficacy has been proved in a large number of rat SCI models, showing increased number of myelinated axons, reduced cystic cavities, white matter sparing, and axonal regeneration [93]. Several proposals have also been made in order to increase the therapeutic effect of Schwann cells after in situ transplantation [94].

Some clinical trials have also been effectuated, suggesting their safety and potential efficacy in humans, both as a single transplantation or in combination with BM-MSCs [95] or OECs [96]. Lima et al. [97] found good efficacy rate in ASIA grade improvement: eleven of 20 patients noticed an improvement. In another clinical trial with chronic SCI five out of six patients had neurologic amelioration [98]. Feron et al. [99] have reported clinical trials using cells taken from the olfactory mucosa and olfactory bulb in human SCI patients. In this clinical trial, six patients were treated with autologous OEC transplantation: three patients showed signs of improvement of SCI, and two demonstrated improvement.

Promising results were also obtained using adult endogenous stem cells (AESC), present in adult neural tissue. Indeed, even if transplanted stem cells with their regenerative capacity represent one of the most promising methods it is well known that they also have some drawbacks as previously discussed. It is also well known that, even without any therapeutic intervention, human body has a robust self-healing capability to repair the damaged tissues or organs. In this direction the endogenous stem cells resident in organ and tissues can play critical roles for organogenesis during development and for tissue homeostasis in adulthood. In the spinal cord, ependymal cells show stem cell properties.

After an acute injury, AESCs are able to proliferate and constitute new glial cells in the spinal cord [100]. The regenerative response of AESCs has been shown in different rodent SCI models [100]; such findings have been able to raise hope for future SCI therapies. Lastly, a promising opportunity is represented by the possibility to genetically modify stem cells. In this framework, Kumagai and coworkers [101] genetically modified MSCs to express MNTS1, a multineuropoetin that binds TrkA, TrkB, and TrkC, and p75NTR receptors. In vivo studies showed reduced cystic cavity size and inflammation compared to control SCI animals. Interestingly, transplantation of the MSC-MNTS1 and MSC-MNTS1/p75−, differently from the native MSCs, enhanced axonal growth, promoted angiogenesis, and modified glial scar formation. Further studies are required to ensure the major concern (long-term safety) and then verify the efficacy of manipulated stem cells to improve locomotor function and other quality of life issues after SCI.

4. Biomaterials-based approach for SCI repair

In recent years, different studies have been carried out, aiming to the development of biomaterials for promoting tissue repairing after severe injuries, such as SCI [9]. These materials could enhance the repair in two different ways: they can be used as carriers able to maintain and release their loading, but they can also act as scaffolds for tissue regeneration [13]. In using biomaterials, different characteristics are of pivotal importance: biocompatibility, biodegradability, cytocompatibility, and adaptive mechanical properties [102]. Furthermore, to obtain the desired results for SCI, biomaterials should guarantee stem cell viability and guide axon regrowth across their structure. In this field, hydrogels are recently gaining importance for cell survival in situ: indeed, they are soft matter, able to be injected (intrathecally, reducing surgical risks) or implanted directly and fills lesion cavities [103].

Hydrogels are defined as 3D polymeric networks able to absorb large amount of water and swell, maintaining their three-dimensional structure [104]. 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 hindrance or polymeric entanglement, while on the other hand they are kept together through chemical bonds. Usually, physical hydrogels are also known as reversible hydrogels, due to
their weak crosslinking with respect to the chemical one. Other classification techniques exist, such as based on their polymeric composition or configuration [105]. Hydrogels can be produced in a variety of ways: linking polymers through chemical reactions; using ionic radiation to generate free radicals able to recombine as crosslink junction and/or through physical interactions (e.g. crystalline formation, electrostatics, entanglement) [105]. The hydrogels thus formed need to be purified, usually with washing, to eliminate impurities produced during the preparation. Hydrogels show a variety of properties, such as high flexibility, no toxicity, gas permeability, and good mechanical properties. For SCI treatment, the most important properties that should be guaranteed is the injectability, thus avoiding risks due to surgery; furthermore, also a minimally invasive placement is mandatory for therapy. Hydrogels are so chosen because they can be loaded with cells and/or drugs; their injection is intrathecal and they are able to remain localized wherever they are injected, thus able to deliver the loaded molecules to the spinal cord [103]. Hydrogels also bring some limitations, such as inadequacies to control the delivery (for instance using drug with low steric hindrance that are able to diffuse through the structure), but also the adverse loading of hydrophobic molecules [106,107]. These limitations can be luckily overcome. As far as the first one is concerned, polymer chains of hydrogel can be functionalized with drug or molecules with different post-polymerization strategies, such as the formation of a chemical bond. In this case, the controlled drug delivery is governed by the ability to break that bond.

The link type can be chosen depending on the medical need: in fact, the weaker the bond the quicker the release (Figure 2) [108]. Moreover their functionalization can also increase cell adhesion, improving their performances as carrier [14,109]. Post-polymerization modification is based on the direct polymerization or copolymerization of monomers bearing chemo-selective handles that are inert toward the polymerization conditions but can be quantitatively converted in a subsequent step into a broad range of other functional groups. The success of this method is based on the excellent conversions achievable under mild conditions, the excellent functional-group tolerance, and the orthogonality of the post-polymerization modification reactions [110].

For the hydrophobic drugs, their problem could be overcome loading them into polymeric nanoparticles that will be later loaded into the hydrogel [111].

Different studies have suggested the use of biopolymers for cell delivery [112], due to the presence of stem cells at the damaged site, in order to maximize the treatment efficacy. Loaded hydrogels are able to release factors secreted by stem cells or sustaining them to fill the gap at the damaged site [14]. For BM-MSCs, in order to maximize their efficacy after in vivo transplantation, the use of 3D supports have been proposed for mimicking the stem cell niche [113–118]. The possibility of scaffolds to help axon regrowth is visible in Figure 3.

Different studies have also been carried out to increase the survival and the efficacy of UC-MSCs thanks to polymeric scaffolds able to mimic a physiological niche in situ, able to preserve them from hostile environment and permitting paracrine release of factors [14,119].

Hydrogels also for A-MSCs have been studied, in particular scaffolds for facilitating the connection of the damaged spinal cord segment; such studies have been carried out in rat and dog SCI models [120].

In hydrogel-based approaches, extracellular matrix showed to be extremely important for stem cells viability [14,121]. An other possibility is represented by biological scaffolds composed of native extracellular matrix. They represent structures similar to those of the uninjured host tissue with advantages like natural 3D network, biodegradability and ability to guarantee proper cell adhesion and growth [122]. In SCI repair the optimization of the host response, hydrogel degradation rate, and ability to restore neural function are necessary to consider the potential of these hydrogels for clinical translation. Not only injectable hydrogels but also implantable polymeric scaffolds, with proper fiber design, demonstrated to be extremely promising for SCI treatment. They are able to sustain stem cells viability, thus offering different advantages for cell survival after transplantation [75,123–125]. The fiber diameter can be easily controlled by modulating the operating parameters and physical properties of the solution and the chemical composition can be easily tailored. Electrospun nanofibers can also be functionalized either by blending, encapsulation, or immobilization of bioactive materials to work on specific

Figure 2. Representation of hydrogels modified with drug-binding cleavable linker. Transient association between drug and polymeric chains modulates release of the drug mimetic from the matrix. Reprinted from Materials Science and Engineering: C, Volume 61, E Mauri, F Rossi, A Sacchetti, Tunable drug delivery using chemoselective functionalization of hydrogels, pp. 851–857, Copyright (2016), with permission from Elsevier [108].
biological responses. Furthermore, electrospun nanofibers can be aligned uniaxially with anisotropic properties and they can be utilized to construct microstructured units such as sheets, disks, and tubes able to support oriented axonal regeneration.

In parallel with research conducted with biomaterials loaded with stem cells, in the last years a lot of interest was devoted to the ability of stem cells to produce many cytokines, growth factors, and cell adhesion factors that play important roles in improving the microenvironment and promoting tissue regeneration \cite{3,11,126–128}.

Papa and coworkers \cite{129} discovered that CCL2 chemokine secreted from human MSCs can be delivered efficaciously in the lesioned spinal cord acting not only on recruitment of macrophages, but driving also their conversion to an M2 neuroprotective phenotype. Surprisingly, human CCL2 delivered also plays a key role in preventing motor neuron degeneration in vitro and after spinal cord trauma in vivo, with a significant improvement of the motor performance of the rodent SCI models. In addition in the last years, stem cells demonstrated the ability to release extracellular vesicles (EVs): microvesicles and exosomes. They are considered mediators in communication between cells and able to mimic the action of stem cells carrying active molecules to the damaged cells from stem cells \cite{130,131}. The big advantage resides in the possibility to have cell-free treatments that can be properly designed for controlled release therapies. The big challenge is represented by the extreme small amount of factors secreted and the issues related to the minimum amount needed to obtain a functional outcome.

5. Conclusions

SCI is a very complex and dynamic pathology with different mechanisms involved starting from the lesion time. Indeed after the primary injury many other damages take place with consequent patient clinical worsening. Some promising results have been collected using stem cells transplantation in preclinical models but are not enough to be promoted to clinical trials. Moreover, many ethical and practical (cell source and reproducibility) issues are connected to this research area together with the big question related to the fate of cells once injected into the body patient. In this direction the possibility to load cells within injectable polymeric systems that can support and sustain the release of bio compounds from stem cells, maintaining them in the target site, seems to be a promising strategy to improve medical chances in SCI repair.

6. Expert opinion

A realistic therapeutic strategy for SCI should consider to rescue sufficient nervous tissue and connections that, together with proper rehabilitation, can improve clinical outcome. In the wide scenario of SCI recovery stem cells represent a very promising possibility due to their multitherapeutic abilities to act as multiple release systems of different beneficial factors. Multitherapeutic possibility related to the use of stem cells considers trophic support, immunomodulation, anti-inflammation, and anti-apoptosis-based effects together with the possibility to reduce scar formation and neutralize inhibitory compounds \cite{132}. The main restoring possibility
associated with stem cells is represented by the replacement of neurons and glial cells taking advantage of the their multipotency. However clinical applications of stem cells are limited due to many problems that are still present [133].

For example, endogenous stem cells are surrounded by ethical debate and their transplantation limited by the possibility to cause teratomas following uncontrollable cell proliferation [134]. In order to overcome this drawback in the last year strong attention was devoted to iPSCs with good results collected but some concerns on their safety and efficacy in the CNS [135]. Other cells collected from adult, so free from ethical issues, are MSCs, olfactory ensheathing cells, and Schwann cells. MSCs can be used for several medical options: in SCI they can prevent inflammation, support tissue regrowth, and bloody supply [136]. Immune rejection can be eliminated with MSCs because they can be collected by bone marrow, amniotic fluid or adipose tissue of patients representing so an autologous transplantation.

Among MSCs, BM-MSCs are the most widely studied and investigated, showing promising results in SCI recovery (in rat models) [12]. BM-MSCs, in general, are the most widely used MSCs with many preclinical studies and clinical trials still ongoing [137]. Studies conducted on cervical SCI patients demonstrated improvement in motor and sensory performances [137–139]. Another parameter that should be taken into account is represented by time of intervention. Indeed therapeutic success depends on the phases of SCI injury: chronic, subacute, or acute.

Particularly, clinical trials demonstrated efficacy of cell transplantation after 1 year from accident (chronic phase) [37,38]. These results should be confirmed and validated by larger studies to confirm the proper therapeutic window. In summary promising results in spinal cord injury recovery were collected using stem cells, but in order to guarantee reproducibility high attention should be given to the standardization of cell sources, protocols, and the numerosity of transplanted cells (extremely high, hence representing a big issue). The use of polymeric scaffolds can reduce the number of cells needed ensuring proper cell release, preventing the dispersion in cerebrospinal fluid, and maintaining them in situ in the target site [140,141]. This approach demonstrated to be essential because without a proper support stem cells can easily leave the zone of injection and get into the circulatory torrent.

In the last year, not only scientific literature but also regulatory directives (EU668/2009, 47/2007/EC, and EU2017/745) are considering this combined approach as mandatory. In the wide field of biomaterials injectable hydrogels and implantable scaffolds proved to be extremely promising strategies able to carry stem cells. Although progress in the application of biomaterials has been successful, several main challenges remain in this field before it reaches clinical applications [142]. Among them safety issues that involve not only polymeric devices but also their degradation products. Moreover manufacture and scale-up might be optimized before reaching patients together with the proper evaluation of economic and financial barriers. So, even if many questions and challenges remain unsolved, the success of several novel cell transplantation protocols based on biomaterials offers a promising foothold for future treatments in humans, hopefully optimized to achieve positive clinical outcomes.

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Declaration of Interest
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Papers of special note have been highlighted as either of interest (●) or of considerable interest (•) to readers.

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