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ORIGINAL PAPER

Spinal Cord Injury: Animal Models, Imaging Tools and the Treatment Strategies

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Abstract
Spinal cord injury (SCI) often leads to irreversible neuro-degenerative changes with life-long consequences. While there is still no effective therapy available, the results of past research have led to improved quality of life for patients suffering from partial or permanent paralysis. In this review we focus on the need, importance and the scientific value of experimental animal models simulating SCI in humans. Furthermore, we highlight modern imaging tools determining the location and extent of spinal cord damage and their contribution to early diagnosis and selection of appropriate treatment. Finally, we focus on available cellular and acellular therapies and novel combinatory approaches with exosomes and active biomaterials. Here we discuss the efficacy and limitations of adult mesenchymal stem cells which can be derived from bone marrow, adipose tissue or umbilical cord blood and its Wharton's jelly. Special attention is paid to stem cell-derived exosomes and smart biomaterials due to their special properties as a delivery system for proteins, bioactive molecules or even genetic material.

Keywords Spinal cord injury · Animal models · Imaging · Stem cells · Exosomes · Biomaterials

Abbreviations

ATMSCs	Adipose tissue mesenchymal stem cells
ASIA score	American Spinal Injury Association score
bFGF	Basic fibroblast growth factor
BMSCs	Bone marrow mesenchymal stem cells
CM	Conditioned media
CNS	Central nervous system
DESI imaging	Desorption electrospray ionization imaging technique
DTI	Diffusion tensor imaging
DW-MRI	Diffusion-weighted magnetic resonance imaging
FDG	Fluorodeoxyglucose
GDNF	Glial cell derived neurotrophic factor
LC ACs	Long-chain acylcarnitines
lyso PC	Lysophosphatidylcholines
MALDI	Matrix-assisted laser desorption/ionization
MEPs	Motor-evoked potentials
MRI	Magnetic resonance imaging
MSC	Mesenchymal stem cells
NGF	Nerve growth factor
NT-3	Neurotrophin-3 protein
NT-4	Neurotrophin-4 protein
PAN/PVC	Polyacrylonitrile/polyvinylchloride
PGA	Poly(glycolic acid)
PET	Positron emission tomography
PHEMA	Poly(2-hydroxyethyl methacrylate)
PLA	Poly(lactic acid)
PLCL	Poly(lactic-*co*-caprolactone)
PLGA	Poly(lactic-*co*-glycolic acid)
PNS	Peripheral nervous system
PTFE	Poly(tetrafluoro-ethylene)
PVA	Polyvinylalcohol
ROS	Reactive oxygen species

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SEPs	Somato-sensory evoked potentials
SCI	Spinal cord injury
TBI	Traumatic brain injury
UC	Umbilical cord
UCMSC	Umbilical cord derived mesenchymal stem cells
WJ	Wharton's jelly
WJMSCs	Wharton's jelly derived mesenchymal stem cells

Introduction

Spinal cord injury (SCI) is a devastating neuro-degenerative disorder which still lacks effective treatment and causes a significant financial burden for the families affected and the whole of society [1]. After direct mechanical insult involving initial primary injury, the spinal cord undergoes multiple pathological changes which are associated with secondary damage. This status is represented by the pathophysiological cascade accompanied by progressive haemorrhaging, intravascular thrombosis and vasospasm leading to ischemia and edema. Both events result in production of free radicals, which cause lipid peroxidation, excitotoxicity, apoptotic and necrotic cell death, and finally inflammation and immunological response affecting intact, neighbouring tissue. This secondary damage expands well beyond the original injury site into adjacent spinal cord segments [2].

The Importance of Animal Models for Translation Medicine

In order to understand the pathological basis of SCI and to develop efficient treatment strategies, a number of different animal models have been widely used. Historically, these models included nonhuman primates as well as cats and dogs, prior to using rodents [3, 4]. The first SCI experiment conducted on dogs was described by Allen in 1911. In that study a weight-drop technique was used, and later on it was adapted to rats [5]. Since that time, various surgical approaches to create the most reliable animal models of SCI have been used, such as aneurysm clip compression [6] or forceps compression [7]. Currently the most preferable types of surgery are contusion, compression and transection with various modifications [8]. However, before choosing an appropriate animal model of SCI it is necessary to identify the main aims and purpose of the study. Experimental animals, in contrast to human patients, are injured under controlled conditions with a simple wound to a targeted spinal region and are more uniform and less complex. Generally people suffer from multiple injuries, such as fracture of vertebrae, or even accompanying traumatic injury of the brain [7, 9].

Animal Models Used for SCI Modelling

Currently, rodents seem to be the species most in demand for preliminary SCI experiments for several reasons [9]. Rat models allow us to monitor the physiological parameters and the pathological events that follow SCI. Moreover, some observations from rat studies have led to potential therapy strategies for patients with SCI [10]. Induction of injury in rat models either by spinal contusion, weight-drop or epidural balloon compression can lead to tissue degeneration at the epicenter of the injury, extending to neighbouring segments [11]. Surprisingly, even when severe SCI was induced, after some period progressive spontaneous recovery was observed [12-15]. Spontaneous recovery in rodent models therefore poses an inconvenience that has to be considered when evaluating the final results for therapeutic strategies.

On the other hand, inducing SCI in dogs can be considered as intermediate between rodent models and human clinical trials. Moreover, pre-clinical trials on chondrodystrophic dog's models (with spontaneous acute SCI) can be performed. In that case, dogs are patients and not models. It provides us with an opportunity for screening potential treatments before human application. In dogs the mechanisms of injury are similar to those in human patients and are comparable in terms of pathology, monitoring of function and conclusions [16, 17].

An alternative to already-existing dog and non-human primate models exists in the form of the minipig spinal injury model, which is characterized by consistent injury and stable neurological deficit after surgery [13, 18]. One of the advantages of this model is that the pig spinal cord has some anatomical features similar to the human spinal cord, which allows this model to be used to test the effect of both extradural and intrathecally injected substances [19].

Because of the genetic, biological and physiological similarities, non-human primates may be essential models for the evaluation of potential therapies. The main limitations of using primate models are ethical concerns and high operating and care costs.

Experimental SCI Procedure

Many different studies showed that animal strains, age, sex, injury model, anaesthesia or hypothermia can have an impact upon the obtained data. A considerable variability in locomotor and sensory recovery between rat strains was noted [20, 21]. Thus, the locomotor recovery in Sprague–Dawley is quicker than that of Wistar rats, which is not well understood but seems to be related to genetic factors [20]. Furthermore, both female and male rats enrolled in SCI modelling

have some limitations. In females, which have shorter and straighter urethra, the urinating procedure is easier and much faster onset of an automatic urinary bladder occur in comparison with males. On the other hand, female rats exhibit hormonal instability, which can have an impact on behavioural testing [21, 22]. Other important factors are the conditions during surgery, anaesthesia, body temperature, blood pressure, type and spinal level of injury. In the past, hypothermia has been extensively used in the treatment of traumatic SCI [20-22]. Many studies have shown the effect of hypothermia in optimizing functional outcome of mild and moderate traumatic SCI, but not against severe traumatic injury. Various effects of hypothermia in SCI has been extensively reviewed elsewhere [23].

Assessment of Functional Recovery

An important consequence of SCI is the loss of bladder function. Manual bladder releasing is required during first 7–10 days after injury. In animal studies the assessment of bladder function is rarely used, but some simple procedures are involved to assess bladder dysfunction over time [24, 25].

To evaluate the loss and recovery of sensory and locomotor functions the various behavioral tests are used. The most visible sign of recovery from SCI is recovery of the ability to walk. In order to measure the locomotor outcomes, the BBB scale rating developed by Basso et al.[26] was standardized across laboratories. The BBB score evaluate the functions of hind limbs during free-moving locomotion of animals in open field, on a scale from 0 (complete paralysis) to 21 (normal locomotion). Automatic system for analysing parameters relating to gait is represented by Catwalk. For the monitoring of sensory recovery there are used tests for sensitivity to heat and cold, the von Frey test for mechanical allodynia. In experimental SCI, some reflex response-based tests, such as toe spread reflex, hind limb placing test, righting reflex are used to assess recovery, too. For example, righting reflex test represent the time, which spent an animal to reach a normal position after being placed on its back [27]. For choosing the appropriate behavioural tests it is necessary to consider, which animals we are using and which kind of data we would like to obtain.

Overall, it is essential to understand the different aspects of the pathology of animal models used in SCI research and correlate them with the pathology of human patients, and to find an appropriate interpretation of the results obtained from experimental animal studies [10]. Even though there are some limitations, animal models represent an irreplaceable and crucial tool in research into SCIs. It is important to understand that animal models for human SCI are not able to reproduce all its complexities, thus it is crucial to define a specific question and to ensure that the chosen model is fit-for-purpose. Furthermore, for any translational drug development strategy these models have to be carefully selected and designed.

Advances in Spinal Cord Injury Imaging Techniques

For primary diagnosis of acute SCI to confirm and localize the site and extent of traumatic injury in experimental models or in clinical patients, precise imaging methods are needed (Table 1).

Radiography

One of the first imaging tools was radiography, an imaging technique using X-rays (gamma rays) which could be used for visualization of the spinal cord in three standard projections: antero-posterior, lateral and open-mouth odontoid view. With this imagining we can identify only vertebral fracture, degenerative disorders or tumours, because there is relatively low sensitivity for SCI detection (50.7%) [28].

Myelography

Another imaging method is myelography, a fluoroscopic method based on injection and recording of motion of contrasting solution in the spinal column. Flow of contrast dye is blocked or diverted if there is any abnormality. The benefits of this method lie in obtaining relatively fast results and quick recovery of patients, up to four hours. However, there are many risks such as allergic reactions, nausea, headaches, paralysis or loss of bladder control [29]. Radiography and myelography are nowadays mostly used in small veterinary clinics, especially for their low price. These methods were replaced with traditional computer tomography and magnetic resonance.

Positron Emission Tomography (PET)

The studies on PET use in the spinal cord have been few, no systematic effort to determine the value of PET in the diagnostic and management of SCI was done, comparative to large studies in the brain. The PET imaging of the spinal cord is retrained by the small size of the spinal cord, low resolution of PET, significantly lower ^{18}F-fluorodeoxyglucose (FDG) uptake in the spinal cord than in the brain and potential contamination from vertebral bone marrow. But, acquisition of PET concurrent with MRI can solve the problems related to small size and miserable PET visibility of the spinal cord. Currently technology is sufficient to detect and quantify the abnormalities in the spinal cord, therefore

Table 1 Imaging techniques used for spinal cord injury diagnostic

Methods	Description and outcome of imaging techniques	References
Radiography	– Using X-rays (gamma rays) for visualization of the spinal cord in 3 standard projections – Relatively low sensitivity for SCI detection	[28]
Myelography	– Motion of contrasting solution in the spinal column to evaluate the spinal cord, nerve roots and spinal meninges	[29]
CT	– Series of X-rays in "slices" through the body analysed by a computer – Ideally suited for identifying acute haemorrhage after SCI	[83]
PET	– Use radioactive positrons (positively charged particles) to detect differences in metabolic and chemical activity in the body – Whereas CT and MRI scans look at structures in the body, a PET scan looks at function	[30]
MRI	– Use magnets rather than X-rays to produce the image – Provide information about morphology and anatomy of the spinal cord – Identify bone marrow edema in injured vertebrae that cannot be seen on CT scans – An important role in case of discordance between clinical status and CT imaging – Superior visualization of the soft tissue structures and enabled better recognition of the pathologies involving intervertebral disks, ligaments, and neural tissues including the spinal cord and nerve roots	[84–86]
DTI	– MRI-based technique, used for describing biologic tissues microstructures, that exploit quantification of water diffusion in tissues – To quantify the degree of white matter integrity, to predict recovery, and to monitor the effects of therapeutic interventions – The relationship between nerve tract-specific changes and clinical status	[87]

PET should be routinely correlated with MRI in spinal cord disease to improve patient care [30].

Magnetic Resonance Imaging (MRI) and Different Types of MRI

Computer tomography is another method using X-rays, while MRI uses a magnetic field, magnetic field gradient and radio waves to produce computer cross-sectional images of the internal organs in the body [29]. The mechanism of MRI is based on the fact that the hydrogen atoms in molecules of water in different tissues of the body are excited by the initial signal and then emit a frequency signal which is measured by the receiving coil [31]. Using this method we can distinguish between ligamentous injury, epidural or intramedullary hematoma and disc hernia [32].

A novel non-invasive method using specific MRI sequences is diffusion-weighted MRI (DW-MRI). It analyses the diffusion of water molecules in other tissues of the body to produce contrasts with images of MRI. A specific type of this method is diffusion tensor imaging (DTI), which generates colour images of tissue. Each colour represents some direction of water diffusion in tissue [31]. DTI can be used to diagnose various lesions of the spinal cord, such as inflammation, tumor or ischemia [31]. All these disorders are imaged as deformation, interruption or displacement of fibers as the result of the strength and direction of molecular water movement in the white matter of the spinal cord [33]. According to new findings, DTI can be a useful method for quantification of SCI pathology along the rostro-caudal axis.

In order to better understand the key players in inflammatory processes, a novel form of 3D matrix-assisted laser desorption/ionization (MALDI) MR imaging was recently evolved for a spatio-temporal lipidomic evaluation across the damaged spinal cord tissue. This method demonstrates enhanced distribution of long-chain acylcarnitines (LC ACs) at the lesion site within the first ten days after SCI [34]. Previous studies have clearly documented that LC ACs are pro-inflammatory and their accumulation may induce cytokine and reactive oxygen species (ROS) production, neurotoxicity, mitochondrial damage leading to decreased viability and finally also to myelinated axon degradation in the peripheral nervous system (PNS) [35-37]. Furthermore, identification of the turnip-shaped distribution of LC ACs around the lesion periphery confirms that the immune response along the rostro-caudal axis is heterogeneous [34, 36, 38].

Rostral to the lesion site where the LC AC and lysophosphatidylcholines (lyso PCs) distribution is distinct, the microenvironment is neurotrophic with recruitment of T regulator lymphocytes stimulating neurite outgrowth and axonal repair [36]. Caudal to the lesion site, the LC AC and lyso PC distribution is disrupted, contributing mainly to an inflammatory microenvironment associated with the presence of neutrophils and absence of neurotrophic signals [11]. In another study the authors applied similar MRI analyses to traumatic brain injury (TBI), revealing enhanced brain-specific cerebrosides, but likewise missing the LC AC mass range [39]. In contrast, when desorption electrospray ionization imaging (DESI) imaging was applied in SCI models, important lipid peroxidation products such as AA and DHA in negative mode were detected [40]. Nevertheless, 3D MRI techniques, used for the first time, not only outline accumulation of LC ACs in response to SCI, but also their

spatial regionalization along the rostro-caudal axis, and their potential as candidate targets for SCI therapy.

Role of Adult Stem Cell-Based Therapies

The state-of-the-art interventions for spinal trauma are spinal decompression and stabilization followed by long-term targeted neuro-rehabilitation [41]. However, if the injury is classified as severe, causing complete transection of axons, the standard care and basic treatment are ineffective and patients remain paralyzed for the rest of their lives [42]. Regenerative medicine strategies together with nanotechnologies [43], spinal electric stimulation devices [44, 45] and neuro-rehabilitation [46] are promising in terms of the development of cutting-edge therapies which may reach from bench to bedside for patients with traumatic injuries [47]. Many studies have shown progress in the field of cell-based therapies, with experimental and clinical applications for central nervous system (CNS) trauma [48]. However, the key aspect for successful treatment is the use of proper stem cell sources, their exosomes alone or in combination with smart biomaterials supporting nerve growth within the lesion cavity, by providing trophic factors and bioactive molecules [49]. There are also other aspects which need to be considered, such as appropriate timing for treatment, delivery routes, and of course the age and overall health condition of patients suffering from trauma and other factors.

Bone Marrow Mesenchymal Stem Cells (BMSCs)

BMSCs were one of the first stem cells involved in experimental and clinical trials to treat traumatic injuries [50]. Previous studies showed that after contusion of the thoracic spinal cord and BMSCs transplantation in rats, partial improvements in motor, sensory and autonomic functions may occur which in some cases may also be associated with spared tissue volumes [51]. Further findings showed that intravenous injections of BMSCs are more effective than freshly-prepared mononuclear fractions of bone marrow cells in the treatment of experimental SCI. When considering various delivery routes, similar neuroprotection was observed whether BMSCs were injected locally into the central cavity [52], intrathecally [53] or systemically [54, 55]. These experimental studies launched a series of I/II clinical trials using autologous, allogenic BMSCs or mononuclear fractions in patients with acute, sub-acute or chronic spinal cord lesions. In general, they revealed that the transplantation of BMSCs into patients is safe and without adverse effects [50, 52]. Partial improvement in the American Spinal Injury Association (ASIA) score and partial recovery of somato-sensory evoked potentials (SEPs) and motor-evoked potentials (MEPs) have been observed in some patients in the acute or sub-acute phase. Although promising data have been obtained, larger groups of patients are needed before any conclusions can be drawn [56]. Since bone marrow isolation requires specialist intervention and in some donor cases there are certain limitations, other stem cell sources such as fat cells are presently being taken into account [57]. We can obtain them as waste tissue after liposuction or other surgical operations, and after cultivation a homogeneous population of mesenchymal stem cells (MSCs) is required [57].

Adipose Tissue Mesenchymal Stem Cells (ATMSCs)

ATMSCs seem to share many similar properties with BMSCs, including their morphology and the expression of cell surface antigens, but on the other hand they show different proliferation rates and multilineage capacities [58]. In addition, conflicting results have been reported from the clinical trials. While some indicate that ATMSCs are more effective than or at least as effective as BMSCs, others conclude that BMSCs are superior to ATMSCs [59]. It is very difficult to prefer one type of cell over another, because they differ in their cytokine secretome, chemokine receptor expression and apoptosis [60, 61]. Furthermore, experimental studies have confirmed that ATMSCs are cells with high proliferative capacity, capable of secreting basic fibroblast growth factor (bFGF), interferon-γ, and insulin-like growth factor-1 [60, 61]. On the other hand BMSCs belong among the cells with slower proliferation rates, but with high potency in osteogenic and chondrogenic differentiation, and secreting stem cell-derived factor-1 and hepatocyte growth factor [60, 61]. Thus both stem cell sources show immunomodulatory effects and broad biological activities which should be considered when choosing the MSC source for each specific clinical application [59, 62].

Although the data from studies using adult stem cells are promising, final translations of pre-clinical findings from animal models of SCI to human clinical trials have not met the therapeutic expectations. There were several reasons for this failure, such as variability in SCI experimental models or limited efficacy of adult stem cells, and therefore much effort has been put into finding a more primitive source of stem cells. In this regard the therapeutic potential of umbilical cord (UC) Wharton's jelly (WJ) derived MSCs (WJMSCs) has emerged [63]. These cells are more primitive with high proliferative capacity, low immunogenicity and non-tumorigenicity [64, 65]. Furthermore, they secrete high levels of neurotrophic factors bFGF, nerve growth factor (NGF), NT-3, NT-4, glial cell derived neurotrophic factor (GDNF) and other bioactive molecules related to neuroprotection, neurogenesis and angiogenesis [63]. In the most recent study, repeated intrathecal delivery of WJMSCs into a rat experimental model of spinal cord trauma showed

dose-dependent beneficial effects [66]. There are several clinical trials showing that systemic infusion of UCMSC for patients with multiple sclerosis is safe, but their potential therapeutic benefits need to be further investigated [67]. Overall, it is believed that hWJ-MSCs are the most suitable for potential wide use in clinical trials for neuro-degenerative disorders.

Condition Media and Exosomes as Potential Novel Therapeutic Tools

Even if cell therapies for SCI show promise for tissue regeneration, there are some issues in the utilization of these approaches. The quality and intrinsic differences between cells, the viability of transplanted cells and the immune response after transplantation, may limit stem cell-based treatment for spinal cord regeneration [54, 68].

Condition Media (CM) Derived from Cells

As an alternative to cell therapies, CM may be considered. They have been shown to have beneficial effects on locomotor improvement and tissue repair, including axonal outgrowth, with clear effect on spinal cord regeneration after injuries [69]. Various cells may be sources for CM, such as bone marrow stromal cells [69, 70], dental pulp-derived stem cells [71], endothelial progenitor cells, or UC cells. In general, MSCs release into CM neurotrophic factors which are able to promote locomotor improvements and tissue repair after SCI [68]. The BMSC-CM contain factors with anti-apoptotic, proinflammatory, angiogenic and neuromodulator factors [68, 69]. The CM administered for SCI therapy promoted axonal regrowth and locomotor recovery, reduced the lesion site and increased the blood vessel diameter within the lesion [68, 70]. The composition of CM has limited concentration, so frequent administration over longer periods should be considered. CM may be delivered as intrathecal injections [69, 70], or directly into a transected spinal cord lesion site using an osmotic pump [68].

Exosomes

Besides the positive effects of stem cells and conditioned medium on SCI, very recent studies point out the therapeutic potential of cell-derived exosomes. Exosomes are extracellular vesicles, 50–150 nm in diameter, with a lipid bilayer membrane similar to a cell donor membrane [72]. They are released by multiple types of cells and can be detected in all body fluids [72, 73]. Jeffery D. Kocsisab et al. reported in 2015 that i-v delivery of MSCs exosomes into a rat model of SCI reduced the injury severity and improved functional recovery, although the injected MSCs were not detected at the SCI site [74]. They suggest that MSCs exosomes can mediate transfer of miRNAs or systemic release of trophic factors at lesion level [74]. Recently the same group reported that MSCs exosomes travelled to the injured, but not the uninjured spinal cord, and are associated specifically with M2 macrophages but not with macrophages expressing M1 phenotype or other types of cells [74]. Composition analyses of exosomes showed a complex cargo represented by proteins, lipids, short and long forms of RNA and short fragments of DNA. The miR-133b expressed in MSCs exosomes have potential therapeutic benefit in the CNS, including SCI [75]. The treatment of SCI rats with miR-133b exosomes reduced lesion volume, preserved neuronal cells and promoted neurite outgrowth after SCI [75]. Furthermore, miR-21 and miR-19b detected also in MSCs exosomes have therapeutic effects in the rat model of SCI [76].

Taken together, these data suggest that MSC-derived exosomes have therapeutic effects on SCI through angiogenic properties, promoting axonal regeneration and suppressing glial scar formation. The animals treated with MSC-derived exosomes showed improved recovery of hindlimb locomotor function. These exosomes are more stable and may be stored for a longer time than cells and present no risk of aneuploidy, so have a lower risk of immune rejection [74, 75].

Designing Cellular and Acellular Biomaterial Therapies

After many years of fundamental experimental studies in regenerative medicine, researchers have come to the conclusion that the best therapy approach for SCI lies in the combination of biomaterial scaffolds, stem cell (cellular), CM or exosome (acellular) therapies and molecule delivery [77]. As SCIs result in apoptosis, formation of pseudocysts and scars, which inhibit regeneration in the injured region, there is a need to develop new biomaterials that could be seeded with cells or exosomes [51]. The role of the biomaterial is to stimulate regeneration of cells and bridge disconnected parts of the spinal cord [78]. Biomaterials should include attributes such as biocompatibility, porosity, permeability and mechanical properties to match with neural tissue. They should be able to support the attachment of cells, their growth and differentiation.

Hydrogels and electrospun guidance channels seem to be among the most promising alternatives for SCI regeneration [79]. Besides other characteristics they are able to prevent scar formation and create good conditions for tissue and neural regeneration [79]. Hydrogels are biocompatible, chemically inert and non-toxic. They have many advantages also in clinical practice as they can be administered by injection, so the procedure is non-invasive and easily available

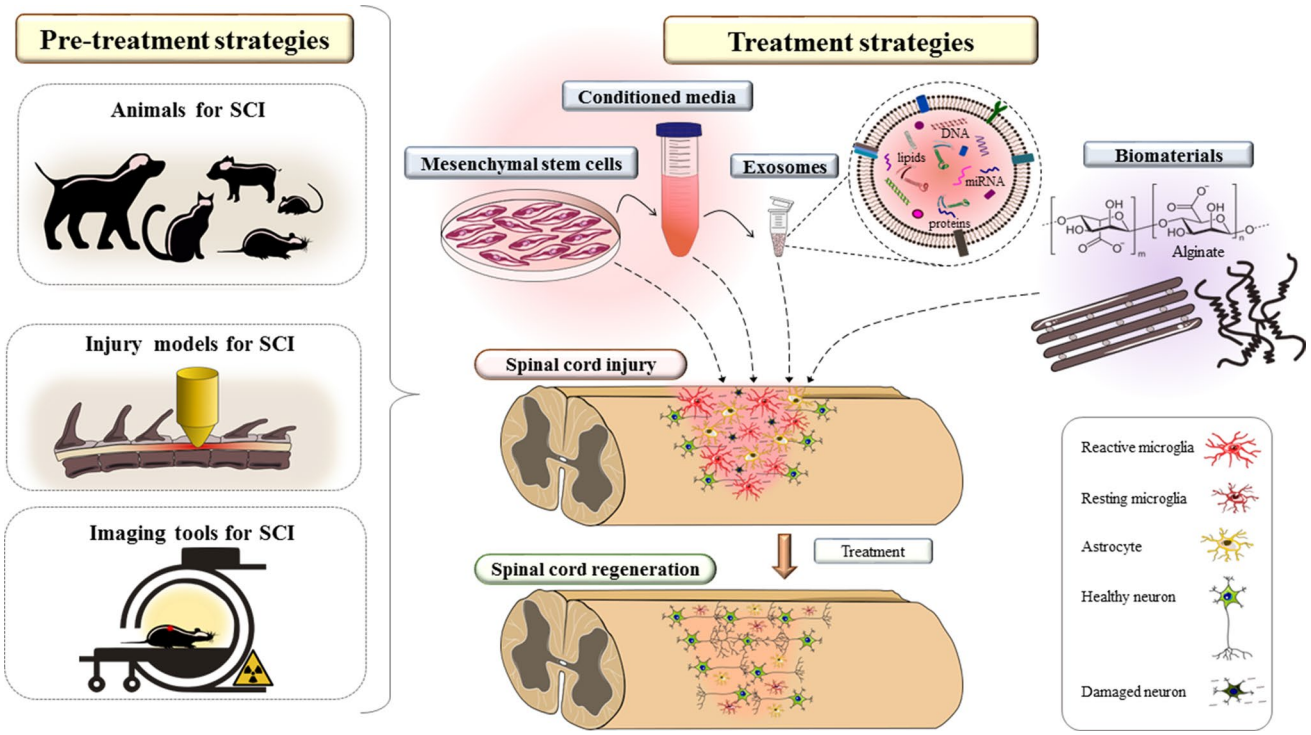

Fig. 1 Schematic design for the preliminary stages leading to experimental treatments of SCI using mesenchymal stem cells, cells derived condition medium, exosomes released by cells or biomaterial scaffolds

for surgeons [80]. Other materials used for SCI treatment include non-degradable and degradable materials.

Non-degradable materials comprise silicone, polyacrylonitrile/polyvinylchloride (PAN/PVC), poly(tetrafluoroethylene) (PTFE), and poly(2-hydroxyethyl methacrylate) (PHEMA) [77]. They are highly biocompatible, but have many disadvantages, stimulate proliferation of inflammatory cells and reveal signs of calcification [81]. On the other hand, there is no need to control the degradation rate and toxicity of degradation products [77]. Degradable materials include synthetic polyvinylalcohol (PVA), Poly(α-hydroxyacids) with poly(glycolic acid) (PGA), poly(lactic acid) (PLA), their co-polymer poly(lactic-*co*-glycolic acid) (PLGA) and poly(lactic-co-caprolactone) (PLCL) [81]. Natural polymers are very accessible but very hard to purify, which can lead to activation of the immune system [77]. They also manifest regular structure caused by highly—controlled synthesis [79].

Alginate sulphate is one of the most interesting injectable biomaterials, capable of binding and then progressively releasing various growth and neurotrophic factors [82]. Providing a complex of bioactive molecules creates an optimal environment for long-term survival and ultimate differentiation of co-cultivated neural progenitors in vitro [82]. Thus, injected alginate-based biomaterial in the central lesion of injured spinal cord tissue stimulated multiple regenerative processes, particularly enhanced preservation of spinal cord tissue with increased numbers of surviving neurons and synaptic vesicles, corticospinal fibers and blood vessels [49]. Inflammation processes mediated by activating microglia were partially suppressed, although the astrocyte response was similar to that in rats after injury but without biomaterial therapy. These results indicate the possibility of therapeutic application of active alginate implants with a complex of bioactive molecules for the treatment of SCI. Thus the ideal biomaterial should be biodegradable, enabling re-growth of the natural environment without activating the immune response. Alginate-based biomaterials appear therefore to fulfil many of these criteria [49]. Of course, there are many other biomaterials accessible and many still in development, so we can expect further progress in the use of biomaterials for SCI regeneration (Fig. 1).

Conclusion

Despite huge scientific efforts being done so far in spinal cord research, the final solution of the mystery of its regeneration is still missing. Fortunately, the care for patients with SCI improved, the new surgical methods together with supporting treatment and rehabilitation can minimize damage to the spinal cord and restore function to varying degree.

It is clear that individual therapy had its particular value, but newer concepts suggest that it is necessary to combine these individual strategies to further enhance the final effect. Progress in research is giving patients hope that SCIs will be eventually repairable.

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Compliance with Ethical Standards

Conflicts of interest The authors declare no conflict of interest.

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