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Animal models for treating spinal cord injury using biomaterials-based tissue engineering strategies

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ABSTRACT

Objective: To provide an up-to-date review of studies that used preclinical animal models for the evaluation of tissue engineering treatments for spinal cord injury (SCI), which involved the use of biomaterials with or without the addition of cells or biomolecules.

Methods: Electronic search of the PubMed, Web of Science and Embase databases was performed for relevant studies published between January 2009 and December 2019.

Results: 1579 articles were retrieved, of which 58 studies were included for analysis. Among the included studies, rats were the most common species used for animal models of SCI, while complete transection was the most commonly used injury pattern. Immediate intervention after injury was conducted in the majority of studies, and 8 weeks was the most common final time point of outcome assessment. A wide range of natural and synthetic biomaterials with different morphologies were used as a part of tissue engineering treatments for SCI, including scaffolds, hydrogels and particles.

Conclusion: Experimental parameters in studies using SCI animal models to evaluate tissue engineering treatments should be carefully considered to match the purpose of the study. Biomaterials that have functional modifications or are applied in combination with cells and biomolecules can be effective in creating a permissive environment for SCI repair in preclinical animal models.

IMPACT STATEMENT

This review provides an up-to-date summary of the preclinical landscape where tissue engineering treatments involving biomaterials were tested in animal models of SCI. Using studies published within the last 10 years, novel perspectives were presented on the animal species used, injury pattern, timing of intervention and outcome measurement, and biomaterials selection, as well as a summary of the individual findings of each study. This review gives unique insight into biomaterials-based

tissue engineering strategies that have progressed to testing in animal models of SCI, which will help shape future research in the field and propel the clinical translation of discoveries.

KEYWORDS: animal model; biomaterial; scaffold; spinal cord injury; tissue engineering

INTRODUCTION

Spinal cord injury (SCI), most often caused by traffic accidents, is one of the most serious diseases of the central nervous system (CNS), leading to devastating neurological deficits and disabilities in the patient. The incidence of SCI is estimated to be between 10.4 and 83 cases per million people per year[1]. Less than 1% of SCI patients can achieve complete recovery of neurological function, with most cases resulting in partial or complete paralysis, and the cost of lifetime care for each SCI patient is in the range of 0.7-3 million USD[2]. SCI therefore imposes a significant socioeconomic burden, particularly since the majority of patients are younger than 30 years old at the time of injury[3]. The management of SCI patients is challenging, since the loss of sensory, motor and autonomic functions distal to the point of injury often leads to multiple health problems including recurrent kidney stones, urinary tract infection, pressure sores, and cardiac and respiratory dysfunction[4], as well as major impacts on quality of life due to complications such as neuropathic pain[5], spasticity[6], heterotopic ossification[7], and syringomyelia[8].

Current clinical approaches for treating SCI include early surgical decompression, drugs, and cell therapy. Early surgical decompression has been found to have positive effects on improving behavioural and pathological outcomes in preclinical SCI models[9]. However, satisfactory clinical outcomes are difficult to achieve, and there is little consensus regarding the role and timing of

decompression in SCI[10]. Anti-inflammatory drugs, such as a high dose of methylprednisolone, can be administered for acute SCI to reduce swelling and secondary injury[11]. However, the common methods for drug delivery such as intraperitoneal injection using a syringe or intrathecal infusion using an osmotic mini-pump can lead to scar formation and infection[12], and tissue penetration by the drug is also limited by the blood-spinal cord barrier[13]. Cell therapy, such as stem cells, Schwann cells or olfactory ensheathing cells (OECs) have been used for reducing secondary injury and boosting axonal and neuronal regeneration following SCI. Nevertheless, these therapies are associated with risks of immunological rejection, tumorigenicity, low survival rate of transplanted cells, and potential dangers in genetic manipulation of the host tissue[14]. The current clinical treatments are not ideal for the safe and effective restoration of neural function following SCI. After swelling from the injury subsides, the patient begins a long period of rehabilitation, which may allow some lost spinal function to be compensated by the remaining nerve fibres.

Tissue engineering approaches have been recently explored as new therapeutic strategies for the treatment of SCI. Tissue engineering has been used across many applications in tissue regeneration to construct biological substitutes that can replace, restore or enhance tissue function[15]. For SCI, tissue engineering strategies such as cell delivery using a biomaterial system have been shown to preserve spared neural tissue and bridge the injury site with local tissue[16]. In this review, tissue engineering is defined as the insertion of biocompatible or functional scaffolds at the injury site that may or may not be combined with living cells, biomolecules or other therapeutic agents. Current tissue engineering strategies aiming to achieve functional recovery in SCI are focused on reproducing the native architecture of the extracellular matrix surrounding the injury site[17, 18], and tuning the differentiation of transplanted cells to re-establish communication through new neural relay circuits[19]. The overall

aim is to create a permissive environment for the interactions among cells, scaffolds and bioactive molecules that can limit inflammation and promote the restoration of sensory and motor function[20].

The safety and feasibility of using bioresorbable polymer scaffolds for the clinical treatment of SCI has been reported in a small number of patients[21, 22]. These studies have shown some evidence of functional recovery, as reflected through the results of magnetic resonance imaging, neuronal electrophysiology, and scores for sensory, motor and autonomic neural function. However, due to the limited sample size, it was not possible to obtain reliable, high-quality evidence from follow-up. In addition, retrieving spinal cord specimens from patients to observe pathological changes is ethically prohibited. Therefore, animal models with disease aetiology that have a degree of similarity to humans are being widely used in preclinical studies to assess tissue engineering strategies for treating SCI. A holistic view of the current findings in animal models will help shape future research directions in the field and propel the clinical translation of discoveries.

The use of animal models for the preclinical assessment of tissue engineering strategies for SCI treatment has been reviewed in selected studies[4, 23, 24]. However, the last comprehensive review on this topic was published more than 15 years ago. More recent reviews have not specifically focused on tissue engineering strategies, or focused on specific aspects within tissue engineering. They have also mainly focused on the effects of animal species selection and the injury pattern, but other important parameters such as the timing of intervention and outcome assessment, and experimental variables such as biomaterials selection have not been addressed. Biomaterials selection can play an important role in the outcome of SCI repair. Collagen[25], chitosan[26], and polyethylene glycol (PEG)[27, 28] are some of the most commonly chosen biomaterials, but their effects in treating SCI may differ

depending on the animal species, injury pattern and timing of intervention, among other variables. In this systematic review, we provide an up-to-date analysis of studies involving animal models to assess SCI treatment using biomaterials-based tissue engineering approaches, and report on the trends observed in the selection of animal species, injury pattern, and timing of intervention and outcome assessment.

MATERIALS AND METHODS

Literature search strategy

A comprehensive systematic search was conducted in PubMed, Web of Science and Embase for studies published in the last 10 years (between 1 January 2009 and 31 December 2019), on using tissue engineering approaches to treat SCI that involved animal models. The following search terms were used: "spinal cord injury" AND "animal model" AND ("polymer" OR "hydrogel" OR "biomaterial" OR "scaffold" OR "tissue engineering"). Specific search strategies used for each database have been included in the supplementary information.

Study selection

The records of retrieved studies were imported into Endnote. After the removal of duplicate records, two reviewers independently screened all studies for inclusion in this systematic review. Any disagreement was adjudicated by a third reviewer. The inclusion criteria were: (1) studies focused on the treatment of SCI; (2) studies that used a tissue engineering approach involving biomaterial(s); (3) studies that used an animal model; and (4) studies published in English. The exclusion criteria were: (1) non-original studies, such as reviews, editorials and opinion pieces; (2) absence of essential information, including the injury pattern, timing of intervention and outcome assessment, and type of

intervention used; (3) conference abstracts and studies where the full text was unavailable.

Data extraction

For each included study, two reviewers independently extracted all relevant information for the review: (1) study characteristics (authors, journal, year of publication); (2) study design (animal species, injury pattern, timing of intervention and outcome assessment, type of intervention used); (3) outcomes and findings.

RESULTS

The search strategy identified 1579 potential studies. After the removal of duplicate records, 1400 studies were screened by title and abstract, through which 769 unrelated studies, 368 reviews and 130 studies on other aspects of tissue engineering were excluded. The full text of 133 articles were screened according to the inclusion and exclusion criteria, which gave rise to 62 eligible studies. Four of these were excluded for using an uncommon injury model, and presenting unclear outcomes, respectively. Finally, 58 articles were included for analysis in this systematic review. The study selection process is depicted in Figure 1, and a summary of the included studies is presented in Table 1.

Animal species

SCI animal models for evaluating tissue engineering treatments included rat (77.4%), mouse (6.5%), dog (7.8%), non-human primate (3.2%), pig (1.9%), and other rodents (guinea pig and rabbit, 3.2%). Rodents were the most common species used. Larger animals such as dogs and pigs, and non-human primates which have the greatest resemblance to humans, are gradually being adopted in preclinical

experiments of SCI treatment. The advantages and disadvantages of each species are shown in Figure 2.

Injury pattern

Transection and contusion (or compression) injuries were induced in SCI animal models used to evaluate tissue engineering treatments, as shown in Figure 3. For transection models, the biomaterial can be transplanted directly into the injury site, while contusion models have an intact dural structure and the biomaterial can be injected to fill the gaps. The most common injury pattern was complete transection (42.4%), followed by hemisection (33.9%). Contusion models (23.7%) were often used to test soluble or microparticle scaffolds.

Timing of intervention and outcome assessment

For the timing of intervention (Figure 4A), the biomaterial was immediately implanted into the host after SCI in the majority of studies (74.1%). Implantation at 1-2 weeks after injury (19.1%), together with other shorter times of intervention were adopted in the remaining studies. For the timing of outcome assessment (Figure 4B), 8 weeks was most commonly chosen as the final time point, followed by comparable numbers of studies that chose 4 and 12 weeks. In addition, studies involving small (Figure 4C) and large (Figure 4D) animals differed in the most common final time point chosen for outcome assessment. The majority of small animal studies were terminated at 8 weeks, while longer time points were generally chosen for large animal studies.

Biomaterials selection

A wide range of biomaterials were used as a part of tissue engineering approaches to treat SCI in

animal models, including both natural and synthetic polymers. The studies could be broadly classified by the composition of the biomaterials-based intervention: biomaterials alone, biomaterials with cells, biomaterials with drugs, or biomaterials with a combination of additional factors (Table 1). Collagen and chitosan were the most commonly used natural biomaterials, while PLGA (poly lactic-co-glycolic acid) and PEG (polyethylene glycol) were the most commonly used synthetic materials. In most studies, the biomaterials were either implanted into the injury site as scaffolds or hydrogels, or injected in the form of particles, solutions or hydrogels. The majority of studies loaded cells and/or bioactive molecules into the biomaterial(s) before implantation.

DISCUSSION

The complicated pathophysiology of SCI poses significant barriers to functional recovery, and the pace of advances in therapeutic interventions has been slow for many years. Rapid progress in tissue engineering over the last two decades has opened up the potential for new therapeutic strategies, which have already demonstrated some promising results in animal models of SCI. From the included studies, it is apparent that tissue engineering strategies consisting of a biomaterial coupled with the delivery of permissive cell types and growth factors could promote repair in SCI. Nevertheless, full recovery has been rarely achieved in animal models, and the treatment effects may be related to a variety of factors in the study design. Our review of studies published over the last 10 years, on the assessment of tissue engineering strategies to treat SCI in animal models, has indicated that the rat is the most commonly used species and complete spinal cord transection is the most commonly adopted injury pattern. The tissue engineering construct is usually implanted immediately after injury, and 8 weeks is the most frequently used final time point for outcome assessment. A wide variety of natural and synthetic polymers have been used in the form of scaffolds, hydrogels or other forms for

implantation. Gaining an understanding of the preclinical landscape for treating SCI using tissue engineering is important in the planning of future studies, and for ultimately translating the application of these therapeutic strategies to humans.

Selection of animal species

Our study showed that rats were the most commonly used species for evaluating tissue engineering treatments of SCI. Rats have the beneficial characteristics of low cost, abundance source, ease of care and operation, well-understood anatomy, and ability to test a range of injury patterns. Most types of SCI encountered in humans can be replicated in adult rats, and several established behavioural tests are available to assess the loss and recovery of sensory and motor functions[29, 30]. Pathological changes in rats due to SCI have partial similarity to humans. For instance, the early formation of fibrotic tissue at the core of the lesion site in rats and humans are both typically associated with a breach of the three meninges, allowing fibroblasts to invade the injury site[31]. Rats also often develop large cystic cavities at the site of injury, a pathological feature which is seen in human SCI[24]. Some methods of SCI treatment analogous to human therapy are well-established in rats, such as neuroprotective drugs and autologous cell transplantation. For example, the efficacy of riluzole in functional recovery and inhibition of damage extension[32], and the effect of autologous OEC transplantation in increasing axonal growth across the injury site and promoting recovery of neural circuits[33] have been confirmed in rat SCI models. However, whether the results of tissue engineering treatments in rats can be extrapolated to human SCI still needs further exploration[34], for several reasons. First, the efficacy of interventions in rats is over-predicted by their high rate of spontaneous recovery[35], which is rarely seen in humans. Second, the design of the tissue engineering construct, including the size, elasticity and morphology of the biomaterial implant, depends greatly on the structure of the spinal cord[36],

which is vastly different between rats and humans. Third, the evaluation of functional recovery may be influenced by the different functions of key spinal tracts between rats and humans. For instance, the corticospinal tract is thought to be critical for fine motor control in humans and non-human primates, but less so in rats[37].

It is important to assess the substantial risk of moving tissue engineering treatments to human clinical trials from rodents without testing in an intermediate large animal model, such as dog, pig or non-human primate. The spinal cord anatomy and physiology of these larger animals have a greater degree of similarity to humans compared to rodents, particularly in the position and function of the spinal tracts[35]. The spinal circuitry of non-human primates has a high degree of similarity to humans, and the activation of motor-related circuitry depends more on supraspinal input than in non-primates[37]. Other animal models may provide species-specific benefits compared to rats, such as an adequate arterial blood supply to the spinal cord in rabbits[38], and similar mRNA sequence in pigs compared to humans[39]. Large animal models of SCI may therefore allow a more physiologically-relevant evaluation of outcomes, but are limited by higher cost and more stringent ethical requirements, particularly for large vertebrates and non-human primates. We believe that the use of large animals in SCI research should only be considered when models in less developed species are inadequate for addressing important mechanistic or translational questions.

Selection of injury pattern

Transection (complete or partial) is the most commonly adopted injury pattern in animal models of SCI to test the effects of tissue engineering treatments, even though the pathology of this model is far from human SCI and causes higher complications and mortality rate compared with other models[40].

This is in large part due to the convenience of this model in providing space for easy implantation of biomaterials and evidence of repair. Complete transection results in no sparing of axons or neural tissue in the lesion site, which is useful for demonstrating genuine axonal regeneration[41] and neuroplasticity[42] when evaluating the effects of exogenous interventions. However, completely severed spinal cord stumps may form new circuits, particularly in animals where there is a high ability for spontaneous healing, leading to difficulties in evaluating the true efficacy of implanted biomaterials[43]. For instance, self-regenerative neural circuits formed at the stumps after complete transection may play a functional role in initial locomotor improvement, independent of the biomaterial[44]. Complete transection is also associated with a risk of retraction of the rostral and caudal stumps, which may cause unexpected damage if no other treatments were used to bridge the gap[45]. As an alternative, partial transection or hemisection can be used to evaluate tissue engineering implants. These injury patterns lead to less severe functional deficits compared to complete transection, and can help avoid excessive animal loss[46]. Nevertheless, secondary injury may be associated with hemisection that have detrimental effects on the contralateral spinal cord around the surgery site, such as the appearance of post-operative oedema and severance of midline blood vessels.

Contusion or compression, while being the least commonly used injury pattern in SCI animal models, allow for minimally-invasive implantation and are useful for injecting biomaterials in the form of particles or solutions[47-49]. The main advantage of this injury model is that the integrity of the spinal dura mater is preserved, avoiding the need for bigger surgical incision[46]. This leads to a low fatality rate, low cost, and easy handling. However, the precision of contusion injury is difficult to maintain[50], and the presence of uninjured nerve fibres and axons in the injury site may allow compensatory proliferation and establishment of new neural connections, making it difficult to accurately evaluate

neural regeneration[51].

Selection of timing of intervention

In a clinical setting, the progression of SCI should ideally be controlled during the acute or subacute phase of injury[52]. However, immediate or early intervention is difficult and largely depends on the availability of medical services, patient condition, and complexity of complications. Nevertheless, we found that the majority of studies testing tissue engineering treatments in SCI animal models implanted the biomaterial immediately following spinal operation. While this is not necessarily reflective of the clinical reality, immediate intervention has the advantage of minimising differences in individual responses to injury, particularly for small animals. A small number of studies conducted intervention within a few hours or days after injury, which may better mimic the clinical situation, but also introduces additional risks as a second injury is performed soon after the first one[53, 54]. During acute injury, the trauma site is dominated by inflammation and cell necrosis. Previous studies in murine models indicated that homologous neural grafts and dissociated cell grafts survive poorly in acute lesion sites[55, 56]. A biomaterial implant is beneficial in offering immediate protection to grafted cells and nutrients from inflammatory mediators and reactive oxygen species during acute injury. For instance, neural stem cells transplanted within a scaffold were found to survive in all grafted animals in a rat complete transection model, and completely filled the scaffold channels one month after injury [28]. However, cells transplanted without a scaffold showed poor survival and invariably failed to fill the gaps at the injury site.

A substantial portion of studies have evaluated the effects of delayed intervention during the subacute phase of injury (1-2 weeks after the initial injury). From a clinical standpoint, the pathology of the

lesion has stabilised to some extent at this stage, and the lesion site may have a more permissive environment for supporting regeneration. In a rat model, delaying hydrogel implantation to 1 week after SCI led to greater anatomical improvements than immediate implantation, as evidenced by a greater decrease in cavity volume[57]. Other studies in rats suggested that SCI might be better stabilised at 2 weeks after injury, based on the injury size and glial scar formation[58]. The transplantation of foetal spinal cord tissue with neurotrophins also improved axonal growth and functional recovery to a greater extent at 2 weeks compared to when applied acutely[59]. The benefits associated with delayed intervention in tissue engineering studies are possibly due to avoiding the impact of environmental fluctuations and influx of inflammatory cells in the acute phase, which are thought to negatively affect the bioactivity of implanted biomaterials[58].

Selection of timing of outcome assessment

The timing of outcome assessment for different animal models of SCI needs to be adjusted according to their time course of pathological changes and potential for spontaneous recovery. The majority of included studies used 8 weeks as the final time point, which was largely tailored for the rat model. Studies conducted using larger SCI models such as dogs and non-human primates reported proportionally longer observation times typically extending beyond 12 weeks. For the rat model, histopathological changes typically stabilise and plateau at 8 weeks after injury[60], and the size of the lesion epicentre also remains constant during this time[61]. Additionally, 8 weeks usually allows the test group to show significant increases in the repair of neural circuits, density of fibrous tissues, and infiltration of host cells compared to the control[12, 62]. It is also a suitable time point for assessing behavioural improvements[42, 63], where motor function has been reported to increase gradually in rats within 8 weeks, after which functional scores tend to stabilise[64].

The need to have longer observation times for large animal models of SCI is related to the increasing complexity of their central nervous system compared to small animals, and the greater similarity of their neural circuitry as well as injury and repair processes compared to humans. The vertebrate motor system has undergone pronounced evolutionary changes that have resulted in significant variations between rodents and non-human primates[37]. One example is the motor cortex and the corticospinal tract as its descending output, which are highly similar between humans and non-human primates but less so in rodents. In higher vertebrates including humans which have a complex and sophisticated central nervous system, it becomes more difficult to recover neural function following injury compared to smaller animals. For this reason, the time frame for recovery in large animal models of SCI is generally longer than in rodents. Some large animal models of SCI have been used as parallel groups alongside human patients to verify their clinical relevance. For example, a canine model of complete SCI at 3 months after injury was thought to be comparable to American Spinal Injury Association (ASIA)-A patients (complete loss of motor and sensory function) at 12 months, and dogs with some retention of motor function were thought to be fit models for intervention efficacy in human ASIA-C patients (incomplete loss of function below the lesion)[65]. A study which tested a combination of materials and stem cell therapy saw significant improvements in motor function in a canine complete SCI model at 3 months post-surgery, and it was speculated that a similar recovery pattern might be applicable to humans[41]. When choosing an SCI model to test for the clinical relevance of interventions, the timing of outcome assessment should be considered together with the animal species.

It is worth noting that the included studies typically used a series of time points for behavioural and

histological assessments, although we only included the final time point in our analysis. Behavioural assessments were usually performed weekly or at even shorter time periods, while the time points of histological assessments were carefully selected to cover the major phases of change following SCI (acute, subacute and chronic). In rodents, the transition between acute and subacute phases often occurs within a few hours to 1day post injury (DPI), while 7 DPI is considered the point of transition to the chronic phase. In one study, a lesion core of dense fibrotic tissue was observed to form at 7 DPI, which maintained a constant morphology and showed no major changes after this time[61]. The transition between phases in SCI can also be identified through sequential phenotypic changes in astrocytes (naïve, reactive and scar-forming) at corresponding time points[66].

Selection of biomaterials

A wide range of biomaterials have been applied as a part of tissue engineering treatments in SCI animal models. The choice of biomaterial was influenced by the animal species and injury pattern in the majority of studies, which could impose limitations on the morphology of the biomaterial. For instance, scaffolds with pores, channels or bundles of fibres were often used for transection injuries, while particles and solutions were typically used for contusion injuries. Hydrogels were used for all injury types. Although none of the studies specifically compared the effects of different scaffold morphologies, it is expected that these would impart some effects on repair outcomes, for instance between collagen bundles[67] and linear ordered scaffolds[68], and between PLGA nanoparticles[53] and channelled scaffolds[69].

Synthetic polymers including PLGA and PEG were frequently chosen for SCI, which have the advantages of allowing versatile surface modification, mechanical tuning, and chemical

functionalisation. All of these properties are useful in producing constructs that can protect transplanted cells from intrinsic secondary injury in SCI, while promoting their attachment, proliferation and differentiation[70]. Synthetic polymers can also be modified with extracellular matrix (ECM) components, such as collagen, laminin or synthetic peptides, which can help generate a permissive microenvironment for recovery[71]. Some synthetic materials, such as peptide amphiphile hydrogels, can undergo self-assembly and are useful for binding and releasing growth factors and other bioactive substances[72, 73]. Natural polymers such as collagen, chitosan, alginate and hyaluronic acid have structures that mimic native ECM, which can help maintain the normal function of host cells without introducing cytotoxic effects[70].They often contain sites for cell adhesion and can intrinsically promote cellular infiltration. However, due to their natural origin, batch-to-batch variation is an issue for these materials, and their applications in SCI repair may be further limited by weak mechanical properties and a high degradation rate *in vivo*.

It should be mentioned that the focus in the included studies was on comparing the effects of loading cells, biomolecules, and/or bioactive motifs in the biomaterials to unmodified or partially modified biomaterials. Systematic studies using well-established SCI animal models will need to be performed in the future to compare the effects of different biomaterial morphologies or compositions to advance tissue engineering solutions towards applications in the clinical treatment of SCI. A few selected biomaterials that have proceeded beyond preclinical testing to being used in clinical trials for treating complete SCI patients are presented in Table 2.

The most common form of treating clinical cases of SCI using biomaterials is to remove the damaged spinal cord and replace it with the biomaterial, with or without the combination of cells. The

biomaterials selected for testing in clinical studies to date have been confirmed to have low antigenicity, suitable mechanical strength and biodegradability, as well as significant therapeutic effects in SCI animal models. A scaffold that has currently undergone the most testing in clinical studies is NeuroRegen, a collagen scaffold made from bovine aponeurosis. This scaffold has an ordered collagen filament structure to provide nerve guidance, as well as sufficient space for cell adhesion and growth without causing significant immune responses[74]. When combined with cells, either autologous bone marrow mononuclear cells (BMMCs) or allogeneic mesenchymal stem cells (MSCs), the NeuroRegen scaffold achieved a significant recovery effect in patients with acute or chronic SCI[21, 75]. In two acute SCI patients, sensory functions began to recover at 2 months post-surgery, and one patient showed the ability to raise their lower legs against gravity when sitting on a wheelchair at 6 months[75]. In five chronic SCI patients, the erection reflex was improved in two cases at 2 months post-surgery, and the recovery of somatosensory evoked potentials were detected in the lower limbs of two cases at 6 months[21]. Although these positive results have only been demonstrated in a very limited number of patients, the use of biomaterials to achieve clinical repair of SCI shows significant promise. It is anticipated that the testing of new biomaterials using physiologically-relevant animal models of SCI will expedite the process of clinical translation.

Animal models of SCI can help to achieve rigorous evaluation of biomaterials before they are considered for use in clinical treatment. Although a wide range of biomaterials are being developed with improved physical and chemical properties for SCI repair, many issues can surface during preclinical testing relating to the complexity of preparation, ease of handling, biocompatibility, biodegradability, and ability to integrate with the host tissue. For instance, some biomaterials may be non-biodegradable, while others may lack good biocompatibility to neuronal cells or induce an

immune response following implantation[76]. While scaffolds with an oriented inner structure may be beneficial for guiding axonal regrowth, these scaffolds are typically stiffer and may not integrate well with the host spinal cord, sometimes requiring a surgical opening to be made which increases the invasiveness of the implantation process[46]. On the other hand, injectable scaffolds are soft and can conform to the injury site to integrate with host tissue, but cannot be used to achieve targeted neural growth[73]. A balanced consideration of such factors, based on the information derived from testing in preclinical SCI models, is essential for advancing new biomaterials to clinical studies.

Perspectives and outlook

The satisfactory treatment of SCI to this day remains a significant challenge, with most cases resulting in irreversible damage to neurological functions. Although a small number of tissue engineering strategies involving biomaterials have been tested in clinical trials for SCI repair, these have failed to achieve the desired prognosis despite early improvements. For example, the implantation of NeuroRegen scaffolds together with autologous BMMCs were found to recover or improve sensory and autonomic nervous function in some SCI patients, such as defecation sensation, physiological erection, sweating, and superficial or deep sensations[77]. However, no motor function recovery was observed in this 3-year clinical study. A good explanation is still lacking for these findings. The difficulty of producing long-term improvements using tissue engineering or other strategies is possibly related to the complex progression of SCI following the initial injury. SCI proceeds according to a sustained injury cascade, which can be divided into several phases: acute (<48 hours), subacute (48 hours to 14 days), intermediate (14 days to 6 months), and chronic (>6 months)[78]. During the acute phase, injury processes including cell death, blood vessel injury, expression of pro-inflammatory cytokines, and infiltration of inflammatory cells can trigger secondary injury[79]. This

leads to the subacute phase, where ischaemia and excitotoxicity result in ongoing necrosis of neurons and glia, as well as the release of excessive harmful factors that contribute to a loss of ionic homeostasis[80]. Finally, cystic cavitation and glial scar formation occur when the injury has entered the intermediate–chronic phase. It is due to this cascade of dynamic changes extending to several months after SCI that an inhibitory microenvironment is formed at the injury site or even systemically, creating significant difficulties for repair processes by limiting nerve regeneration. The rational use of animal models can help overcome the hurdles of studying SCI progression in humans and evaluating the efficacy of possible treatment strategies, for several reasons. First, animal models provide a convenient and repeatable means of observing and studying injury processes in SCI under artificially designed and controlled experimental conditions. Second, the ideal animal model can mimic human SCI anatomically and functionally, which can help researchers understand the pathophysiology of SCI and be used for preclinical validation of new therapies. For instance, large animal models such as dogs and non-human primates have an injury response similar to that observed in human SCI[37, 65]. Third, animal models allow further investigations into the mechanisms of healing following SCI treatment, which cannot be performed in humans. Using spinal cord specimens harvested from animals that have undergone SCI treatment, a range of outcomes can be evaluated including the morphology of tissue, expression of inflammatory factors, and condition of nerve fibre regeneration.

There are some limitations in our analysis. First, we were not able to perform a meta-analysis on the included studies. The purpose of our study was to observe the selection of animal species, injury model, timing of intervention and outcome measurement, and biomaterials in preclinical studies of SCI involving biomaterials-based tissue engineering treatments. Among the included studies, there

lacked a systematic reporting process for these selection parameters, leading to huge variations in study design and significant difficulties in performing quality assessment of studies. The quality of preclinical studies could be improved by well-designed author submission checklists and analogous journal initiatives[81], such as the Stroke journal's Basic Science Checklist[82]. However, such checklists are currently field-specific and similar standards are not established in preclinical studies of SCI. It would hence be challenging to conduct an accurate meta-analysis for the included studies due to greatly variable sample numbers and surgical methods. Another significant challenge arises from the non-standardised evaluation of 'effective' repair in SCI animal models. For example, some studies used histological staining to observe the morphology of the spinal cord or neurons as the primary outcome, such as haematoxylin and eosin, Nissl, and Luxol fast blue staining[63, 73]. Meanwhile, other studies have used functional outcome measures, such as the Basso–Beattie–Bresnahan (BBB) locomotor rating scale and swimming test[76]. Still others have chosen neuroelectrophysiology[83] or imaging examination[84] to observe spinal cord recovery in animals. These substantial variations limit the ability to conduct meta-analyses, since evaluation and interpretation of the results from animal studies of SCI need to be made in the context of the types of tests performed and relevance of the model to human pathophysiology. To enable more comprehensive analyses to be performed in the future, such as Bayesian meta-analysis[85], the quality of preclinical SCI studies needs to be improved by standardising the consideration of study design elements, such as randomisation, blinding, sample size estimation, and sex bias.

Second, among the included studies on SCI repair in animal models, neural regeneration has been used as a primary indicator for outcomes measurement. However, other important factors may contribute to injury repair that have not been explicitly evaluated in these studies. For instance, white

matter injury is a potential cause of function loss after SCI[86]. Some studies suggest that white matter recovery is closely correlated with functional restoration of paralysed hind limbs, and may hold the key to motor recovery[87]. Interestingly, some studies have shown locomotor recovery in rodents despite not having any corticospinal fibres pass through the lesion area[14], implying that other tracts may have played an important role. Indeed, it has been confirmed by electrophysiological evaluation that 10–25 % of rubrospinal tracts were linked in the rat model[83], which may provide additional mechanisms for locomotor recovery. Future studies may provide a better understanding of the role of white matter in the regulation and recovery of motor function in SCI by studying some of the less accessible tracts in animal models, such as the rubrospinal tract.

CONCLUSION

This review provides an up-to-date summary of the application of animal models in evaluating tissue engineering strategies for treating SCI. The animal species and injury pattern, as well as timing of intervention and outcome assessment are all important parts of the experimental protocol for gaining a practical understanding of the effects of tissue engineering treatment. To maintain translational relevance, biomaterials selection should be carefully considered to be applicable both to the animal model and for future human use. Until more advanced screening technologies can be developed, preclinical animal models remain an essential step in the testing of tissue engineering products intended for the clinical treatment of SCI.

AUTHORSHIP CONFIRMATION STATEMENT

All named authors have made substantial contributions to the conception of the review, and drafting and/or revising it critically for important intellectual content. All authors have approved the final

version of the manuscript, and agree to be accountable for all aspects of the submitted work.

AUTHOR DISCLOSURE STATEMENT

The authors have no competing interests to declare.

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REFERENCES

1. Wyndaele M, Wyndaele JJ. Incidence, prevalence and epidemiology of spinal cord injury: what learns a worldwide literature survey? *Spinal Cord*. 2006;**44**(9):523-9.
2. Willerth SM, Sakiyama-Elbert SE. Cell therapy for spinal cord regeneration. *Adv Drug Deliv Rev*. 2008;**60**(2):263-76.
3. Singh A, Tetreault L, Kalsi-Ryan S, Nouri A, Fehlings MG. Global prevalence and incidence of traumatic spinal cord injury. *Clin Epidemiol*. 2014;**6**:309-31.
4. Talac R, Friedman JA, Moore MJ, Lu L, Jabbari E, Windebank AJ, et al. Animal models of spinal cord injury for evaluation of tissue engineering treatment strategies. *Biomaterials*. 2004;**25**(9):1505-10.
5. Finnerup NB. Neuropathic pain and spasticity: intricate consequences of spinal cord injury. *Spinal Cord*. 2017;**55**(12):1046-50.
6. Lin Y, Chen Z, Tang J, Cao P, Shi R. Acrolein Contributes to the Neuropathic Pain and Neuron Damage after Ischemic‐Reperfusion Spinal Cord Injury. *Neuroscience*. 2018;**384**:120-30.
7. Ranganathan K, Loder S, Agarwal S, Wong VW, Forsberg J, Davis TA, et al. Heterotopic Ossification: Basic-Science Principles and Clinical Correlates. *J Bone Joint Surg Am*. 2015;**97**(13):1101-11.
8. Brodbelt AR, Stoodley MA. Post-traumatic syringomyelia: a review. *J Clin Neurosci*. 2003;**10**(4):401-8.
9. Batchelor PE, Wills TE, Skeers P, Battistuzzo CR, Macleod MR, Howells DW, et al. Meta-analysis of pre-clinical studies of early decompression in acute spinal cord injury: a battle of time and

pressure. *PLoS One*. 2013;**8**(8):e72659.
10. Wilson JR, Voth J, Singh A, Middleton J, Jaglal SB, Singh JM, et al. Defining the Pathway to Definitive Care and Surgical Decompression after Traumatic Spinal Cord Injury: Results of a Canadian Population-Based Cohort Study. *J Neurotrauma*. 2016;**33**(10):963-71.
11. Bracken MB. Steroids for acute spinal cord injury. *Cochrane Database Syst Rev*. 2012;**1**:CD001046.
12. Elliott Donaghue I, Tator CH, Shoichet MS. Local Delivery of Neurotrophin-3 and Anti-NogoA Promotes Repair After Spinal Cord Injury. *Tissue Eng Part A*. 2016;**22**(9-10):733-41.
13. Thorne RG, Frey WH, 2nd. Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations. *Clin Pharmacokinet*. 2001;**40**(12):907-46.
14. Wang N, Zhang S, Zhang AF, Yang ZY, Li XG. Sodium hyaluronate-CNTF gelatinous particles promote axonal growth, neurogenesis and functional recovery after spinal cord injury. *Spinal Cord*. 2014;**52**(7):517-23.
15. Berthiaume F, Maguire TJ, Yarmush ML. Tissue engineering and regenerative medicine: history, progress, and challenges. *Annu Rev Chem Biomol Eng*. 2011;**2**:403-30.
16. Mothe AJ, Tam RY, Zahir T, Tator CH, Shoichet MS. Repair of the injured spinal cord by transplantation of neural stem cells in a hyaluronan-based hydrogel. *Biomaterials*. 2013;**34**(15):3775-83.
17. Vismara I, Papa S, Rossi F, Forloni G, Veglianese P. Current Options for Cell Therapy in Spinal Cord Injury. *Trends Mol Med*. 2017;**23**(9):831-49.
18. Li X, Han J, Zhao Y, Ding W, Wei J, Li J, et al. Functionalized collagen scaffold implantation and cAMP administration collectively facilitate spinal cord regeneration. *Acta Biomater*. 2016;**30**:233-45.
19. Centeno EGZ, Cimarosti H, Bithell A. 2D versus 3D human induced pluripotent stem cell-derived cultures for neurodegenerative disease modelling. *Mol Neurodegener*. 2018;**13**(1):27.
20. Samadikuchaksaraei A. An overview of tissue engineering approaches for management of spinal cord injuries. *J Neuroeng Rehabil*. 2007;**4**:15.
21. Xiao Z, Tang F, Tang J, Yang H, Zhao Y, Chen B, et al. One-year clinical study of NeuroRegen scaffold implantation following scar resection in complete chronic spinal cord injury patients. *Sci China Life Sci*. 2016;**59**(7):647-55.
22. Theodore N, Hlubek R, Danielson J, Neff K, Vaickus L, Ulich TR, et al. First Human Implantation of a Bioresorbable Polymer Scaffold for Acute Traumatic Spinal Cord Injury: A Clinical Pilot Study for Safety and Feasibility. *Neurosurgery*. 2016;**79**(2):E305-12.
23. Yousefifard M, Maleki SN, Askarian-Amiri S, Vaccaro AR, Chapman JR, Fehlings MG, et al. A combination of mesenchymal stem cells and scaffolds promotes motor functional recovery in spinal cord injury: a systematic review and meta-analysis. *Journal of Neurosurgery-Spine*. 2020;**32**(2):269-84.
24. Sharif-Alhoseini M, Khormali M, Rezaei M, Safdarian M, Hajighadery A, Khalatbari MM, et al. Animal models of spinal cord injury: a systematic review. *Spinal Cord*. 2017;**55**(8):714-21.
25. Han S, Yin W, Li X, Wu S, Cao Y, Tan J, et al. Pre-Clinical Evaluation of CBD-NT3 Modified Collagen Scaffolds in Completely Spinal Cord Transected Non-Human Primates. *J Neurotrauma*. 2019;**36**(15):2316-24.
26. Kim H, Tator CH, Shoichet MS. Chitosan implants in the rat spinal cord: biocompatibility and biodegradation. *J Biomed Mater Res A*. 2011;**97**(4):395-404.
27. Tom B, Witko J, Lemay M, Singh A. Effects of bioengineered scaffold loaded with neurotrophins and locomotor training in restoring H-reflex responses after spinal cord injury. *Exp Brain Res*. 2018;**236**(11):3077-84.

28. Koffler J, Zhu W, Qu X, Platoshyn O, Dulin JN, Brock J, et al. Biomimetic 3D-printed scaffolds for spinal cord injury repair. *Nat Med*. 2019;**25**(2):263-9.
29. Roman JA, Niedzielko TL, Haddon RC, Parpura V, Floyd CL. Single-walled carbon nanotubes chemically functionalized with polyethylene glycol promote tissue repair in a rat model of spinal cord injury. *J Neurotrauma*. 2011;**28**(11):2349-62.
30. Imani S, Zagari Z, Rezaei Zarchi S, Jorjani M, Nasri S. Functional Recovery of Carbon Nanotube/Nafion Nanocomposite in Rat Model of Spinal Cord Injury. *Artif Cells Nanomed Biotechnol*. 2016;**44**(1):144-9.
31. Josephson A, Greitz D, Klason T, Olson L, Spenger C. A spinal thecal sac constriction model supports the theory that induced pressure gradients in the cord cause edema and cyst formation. *Neurosurgery*. 2001;**48**(3):636-45; discussion 45-6.
32. Zhou LY, Tian ZR, Yao M, Chen XQ, Song YJ, Ye J, et al. Riluzole promotes neurological function recovery and inhibits damage extension in rats following spinal cord injury: a meta-analysis and systematic review. *J Neurochem*. 2019;**150**(1):6-27.
33. Thornton MA, Mehta MD, Morad TT, Ingraham KL, Khankan RR, Griffis KG, et al. Evidence of axon connectivity across a spinal cord transection in rats treated with epidural stimulation and motor training combined with olfactory ensheathing cell transplantation. *Exp Neurol*. 2018;**309**:119-33.
34. Nout YS, Rosenzweig ES, Brock JH, Strand SC, Moseanko R, Hawbecker S, et al. Animal models of neurologic disorders: a nonhuman primate model of spinal cord injury. *Neurotherapeutics*. 2012;**9**(2):380-92.
35. Nardone R, Florea C, Holler Y, Brigo F, Versace V, Lochner P, et al. Rodent, large animal and non-human primate models of spinal cord injury. *Zoology (Jena)*. 2017;**123**:101-14.
36. Pritchard CD, Slotkin JR, Yu D, Dai H, Lawrence MS, Bronson RT, et al. Establishing a model spinal cord injury in the African green monkey for the preclinical evaluation of biodegradable polymer scaffolds seeded with human neural stem cells. *J Neurosci Methods*. 2010;**188**(2):258-69.
37. Courtine G, Bunge MB, Fawcett JW, Grossman RG, Kaas JH, Lemon R, et al. Can experiments in nonhuman primates expedite the translation of treatments for spinal cord injury in humans? *Nat Med*. 2007;**13**(5):561-6.
38. Mazensky D, Flesarova S, Sulla I. Arterial Blood Supply to the Spinal Cord in Animal Models of Spinal Cord Injury. A Review. *Anat Rec (Hoboken)*. 2017;**300**(12):2091-106.
39. Tigchelaar S, Streijger F, Sinha S, Flibotte S, Manouchehri N, So K, et al. Serum MicroRNAs Reflect Injury Severity in a Large Animal Model of Thoracic Spinal Cord Injury. *Sci Rep*. 2017;**7**(1):1376.
40. Li X, Dai J. Bridging the gap with functional collagen scaffolds: tuning endogenous neural stem cells for severe spinal cord injury repair. *Biomater Sci*. 2018;**6**(2):265-71.
41. Han S, Xiao Z, Li X, Zhao H, Wang B, Qiu Z, et al. Human placenta-derived mesenchymal stem cells loaded on linear ordered collagen scaffold improves functional recovery after completely transected spinal cord injury in canine. *Sci China Life Sci*. 2018;**61**(1):2-13.
42. Marchini A, Raspa A, Pugliese R, El Malek MA, Pastori V, Lecchi M, et al. Multifunctionalized hydrogels foster hNSC maturation in 3D cultures and neural regeneration in spinal cord injuries. *Proc Natl Acad Sci U S A*. 2019;**116**(15):7483-92.
43. Kim CY. PEG-assisted reconstruction of the cervical spinal cord in rats: effects on motor conduction at 1 h. *Spinal Cord*. 2016;**54**(10):910-2.
44. Estrada V, Brazda N, Schmitz C, Heller S, Blazyca H, Martini R, et al. Long-lasting significant functional improvement in chronic severe spinal cord injury following scar resection and polyethylene glycol implantation. *Neurobiol Dis*. 2014;**67**:165-79.

45. Yoshida Y, Kataoka H, Kanchiku T, Suzuki H, Imajyo Y, Kato H, et al. Transection method for shortening the rat spine and spinal cord. *Exp Ther Med*. 2013;**5**(2):384-8.
46. Hejčl A, Růžička J, Kekulová K, Svobodová B, Proks V, Macková H, et al. Modified methacrylate hydrogels improve tissue repair after spinal cord injury. *International Journal of Molecular Sciences*. 2018;**19**(9).
47. Jeong SJ, Cooper JG, Ifergan I, McGuire TL, Xu D, Hunter Z, et al. Intravenous immune-modifying nanoparticles as a therapy for spinal cord injury in mice. *Neurobiol Dis*. 2017;**108**:73-82.
48. Wang Q, Zhang H, Xu H, Zhao Y, Li Z, Li J, et al. Novel multi-drug delivery hydrogel using scar-homing liposomes improves spinal cord injury repair. *Theranostics*. 2018;**8**(16):4429-46.
49. Papa S, Caron I, Erba E, Panini N, De Paola M, Mariani A, et al. Early modulation of pro-inflammatory microglia by minocycline loaded nanoparticles confers long lasting protection after spinal cord injury. *Biomaterials*. 2016;**75**:13-24.
50. Vijayaprakash KM, Sridharan N. An experimental spinal cord injury rat model using customized impact device: A cost-effective approach. *J Pharmacol Pharmacother*. 2013;**4**(3):211-3.
51. Tuszynski MH, Steward O. Concepts and methods for the study of axonal regeneration in the CNS. *Neuron*. 2012;**74**(5):777-91.
52. Bramlett HM, Dietrich WD. Progressive damage after brain and spinal cord injury: pathomechanisms and treatment strategies. *Prog Brain Res*. 2007;**161**:125-41.
53. Gao Y, Vijayaraghavalu S, Stees M, Kwon BK, Labhasetwar V. Evaluating accessibility of intravenously administered nanoparticles at the lesion site in rat and pig contusion models of spinal cord injury. *J Control Release*. 2019;**302**:160-8.
54. Tysseling VM, Sahni V, Pashuck ET, Birch D, Hebert A, Czeisler C, et al. Self-assembling peptide amphiphile promotes plasticity of serotonergic fibers following spinal cord injury. *J Neurosci Res*. 2010;**88**(14):3161-70.
55. Kadoya K, Lu P, Nguyen K, Lee-Kubli C, Kumamaru H, Yao L, et al. Spinal cord reconstitution with homologous neural grafts enables robust corticospinal regeneration. *Nat Med*. 2016;**22**(5):479-87.
56. Lu P, Wang Y, Graham L, McHale K, Gao M, Wu D, et al. Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. *Cell*. 2012;**150**(6):1264-73.
57. Hejcl A, Urdzikova L, Sedy J, Lesny P, Pradny M, Michalek J, et al. Acute and delayed implantation of positively charged 2-hydroxyethyl methacrylate scaffolds in spinal cord injury in the rat. *J Neurosurg Spine*. 2008;**8**(1):67-73.
58. Johnson PJ, Parker SR, Sakiyama-Elbert SE. Fibrin-based tissue engineering scaffolds enhance neural fiber sprouting and delay the accumulation of reactive astrocytes at the lesion in a subacute model of spinal cord injury. *J Biomed Mater Res A*. 2010;**92**(1):152-63.
59. Coumans JV, Lin TT, Dai HN, MacArthur L, McAtee M, Nash C, et al. Axonal regeneration and functional recovery after complete spinal cord transection in rats by delayed treatment with transplants and neurotrophins. *J Neurosci*. 2001;**21**(23):9334-44.
60. Kjell J, Olson L. Rat models of spinal cord injury: from pathology to potential therapies. *Dis Model Mech*. 2016;**9**(10):1125-37.
61. Forgione N, Chamankhah M, Fehlings MG. A Mouse Model of Bilateral Cervical Contusion-Compression Spinal Cord Injury. *J Neurotrauma*. 2017;**34**(6):1227-39.
62. Shi Q, Gao W, Han X, Zhu X, Sun J, Xie F, et al. Collagen scaffolds modified with collagen-binding bFGF promotes the neural regeneration in a rat hemisected spinal cord injury model. *Science China Life sciences*. 2014;**57**(2):232-40.
63. Wang C, Sun C, Hu Z, Huo X, Yang Y, Liu X, et al. Improved Neural Regeneration with Olfactory

Ensheathing Cell Inoculated PLGA Scaffolds in Spinal Cord Injury Adult Rats. *Neurosignals*. 2017;**25**(1):1-14.
64. Bonnet M, Trimaille T, Brezun JM, Feron F, Gigmes D, Marqueste T, et al. Motor and sensitive recovery after injection of a physically cross-linked PNIPAAm-g-PEG hydrogel in rat hemisectioned spinal cord. *Mater Sci Eng C Mater Biol Appl*. 2020;**107**:110354.
65. Jeffery ND, Hamilton L, Granger N. Designing clinical trials in canine spinal cord injury as a model to translate successful laboratory interventions into clinical practice. *Vet Rec*. 2011;**168**(4):102-7.
66. Hara M, Kobayakawa K, Ohkawa Y, Kumamaru H, Yokota K, Saito T, et al. Interaction of reactive astrocytes with type I collagen induces astrocytic scar formation through the integrin-N-cadherin pathway after spinal cord injury. *Nat Med*. 2017;**23**(7):818-28.
67. Suzuki H, Kanchiku T, Imajo Y, Yoshida Y, Nishida N, Gondo T, et al. Artificial collagen-filament scaffold promotes axon regeneration and long tract reconstruction in a rat model of spinal cord transection. *Medical molecular morphology*. 2015;**48**(4):214-24.
68. Fan C, Li X, Xiao Z, Zhao Y, Liang H, Wang B, et al. A modified collagen scaffold facilitates endogenous neurogenesis for acute spinal cord injury repair. *Acta biomaterialia*. 2017;**51**:304-16.
69. McCreedy DA, Margul DJ, Seidlits SK, Antane JT, Thomas RJ, Sissman GM, et al. Semi-automated counting of axon regeneration in poly(lactide co-glycolide) spinal cord bridges. *J Neurosci Methods*. 2016;**263**:15-22.
70. Willerth SM, Sakiyama-Elbert SE. Approaches to neural tissue engineering using scaffolds for drug delivery. *Adv Drug Deliv Rev*. 2007;**59**(4-5):325-38.
71. Li X, Han J, Zhao Y, Ding W, Wei J, Li J, et al. Functionalized collagen scaffold implantation and cAMP administration collectively facilitate spinal cord regeneration. *Acta biomaterialia*. 2016;**30**:233-45.
72. Hassannejad Z, Zadegan SA, Vaccaro AR, Rahimi-Movaghar V, Sabzevari O. Biofunctionalized peptide-based hydrogel as an injectable scaffold for BDNF delivery can improve regeneration after spinal cord injury. *Injury*. 2019;**50**(2):278-85.
73. Tavakol S, Saber R, Hoveizi E, Aligholi H, Ai J, Rezayat SM. Chimeric Self-assembling Nanofiber Containing Bone Marrow Homing Peptide's Motif Induces Motor Neuron Recovery in Animal Model of Chronic Spinal Cord Injury; an In Vitro and In Vivo Investigation. *Mol Neurobiol*. 2016;**53**(5):3298-308.
74. Lin H, Chen B, Wang B, Zhao Y, Sun W, Dai J. Novel nerve guidance material prepared from bovine aponeurosis. *J Biomed Mater Res A*. 2006;**79**(3):591-8.
75. Xiao Z, Tang F, Zhao Y, Han G, Yin N, Li X, et al. Significant Improvement of Acute Complete Spinal Cord Injury Patients Diagnosed by a Combined Criteria Implanted with NeuroRegen Scaffolds and Mesenchymal Stem Cells. *Cell Transplant*. 2018;**27**(6):907-15.
76. Sitoci-Ficici KH, Matyash M, Uckermann O, Galli R, Leipnitz E, Later R, et al. Non-functionalized soft alginate hydrogel promotes locomotor recovery after spinal cord injury in a rat hemimyelonectomy model. *Acta Neurochir (Wien)*. 2018;**160**(3):449-57.
77. Chen W, Zhang Y, Yang S, Sun J, Qiu H, Hu X, et al. NeuroRegen Scaffolds Combined with Autologous Bone Marrow Mononuclear Cells for the Repair of Acute Complete Spinal Cord Injury: A 3-Year Clinical Study. *Cell Transplant*. 2020;**29**:963689720950637.
78. Ahuja CS, Wilson JR, Nori S, Kotter MRN, Druschel C, Curt A, et al. Traumatic spinal cord injury. *Nat Rev Dis Primers*. 2017;**3**:17018.
79. LaPlaca MC, Simon CM, Prado GR, Cullen DK. CNS injury biomechanics and experimental models. *Prog Brain Res*. 2007;**161**:13-26.

80. Kwon BK, Tetzlaff W, Grauer JN, Beiner J, Vaccaro AR. Pathophysiology and pharmacologic treatment of acute spinal cord injury. *Spine J*. 2004;**4**(4):451-64.
81. Ramirez FD, Jung RG, Motazedian P, Perry-Nguyen D, Di Santo P, MacDonald Z, et al. Journal Initiatives to Enhance Preclinical Research: Analyses of Stroke, Nature Medicine, Science Translational Medicine. *Stroke*. 2020;**51**(1):291-9.
82. Minnerup J, Zentsch V, Schmidt A, Fisher M, Schabitz WR. Methodological Quality of Experimental Stroke Studies Published in the Stroke Journal: Time Trends and Effect of the Basic Science Checklist. *Stroke*. 2016;**47**(1):267-72.
83. Suzuki H, Kanchiku T, Imajo Y, Yoshida Y, Nishida N, Gondo T, et al. Artificial collagen-filament scaffold promotes axon regeneration and long tract reconstruction in a rat model of spinal cord transection. *Med Mol Morphol*. 2015;**48**(4):214-24.
84. Luo L, Albashari AA, Wang X, Jin L, Zhang Y, Zheng L, et al. Effects of transplanted heparin-poloxamer hydrogel combining dental pulp stem cells and bFGF on spinal cord injury repair. *Stem Cells International*. 2018;**2018**.
85. Grant RL. The uptake of Bayesian methods in biomedical meta-analyses: A scoping review (2005-2016). *J Evid Based Med*. 2019;**12**(1):69-75.
86. Ward RE, Huang W, Kostusiak M, Pallier PN, Michael-Titus AT, Priestley JV. A characterization of white matter pathology following spinal cord compression injury in the rat. *Neuroscience*. 2014;**260**:227-39.
87. Song W, Song G, Zhao C, Li X, Pei X, Zhao W, et al. Testing Pathological Variation of White Matter Tract in Adult Rats after Severe Spinal Cord Injury with MRI. *Biomed Res Int*. 2018;**2018**:4068156.
88. Dumont CM, Carlson MA, Munsell MK, Ciciriello AJ, Strnadova K, Park J, et al. Aligned hydrogel tubes guide regeneration following spinal cord injury. *Acta Biomater*. 2019;**86**:312-22.
89. Chedly J, Soares S, Montembault A, von Boxberg Y, Veron-Ravaille M, Mouffle C, et al. Physical chitosan microhydrogels as scaffolds for spinal cord injury restoration and axon regeneration. *Biomaterials*. 2017;**138**:91-107.
90. Zhu J, Lu Y, Yu F, Zhou L, Shi J, Chen Q, et al. Effect of decellularized spinal scaffolds on spinal axon regeneration in rats. *J Biomed Mater Res A*. 2018;**106**(3):698-705.
91. Kushchayev SV, Giers MB, Hom Eng D, Martirosyan NL, Eschbacher JM, Mortazavi MM, et al. Hyaluronic acid scaffold has a neuroprotective effect in hemisection spinal cord injury. *Journal of neurosurgery Spine*. 2016;**25**(1):114-24.
92. Imani S, Zagari Z, Rezaei Zarchi S, Jorjani M, Nasri S. Functional Recovery of Carbon Nanotube/Nafion Nanocomposite in Rat Model of Spinal Cord Injury. *Artificial cells, nanomedicine, and biotechnology*. 2016;**44**(1):144-9.
93. Kaneko A, Matsushita A, Sankai Y. A 3D nanofibrous hydrogel and collagen sponge scaffold promotes locomotor functional recovery, spinal repair, and neuronal regeneration after complete transection of the spinal cord in adult rats. *Biomedical Materials (Bristol)*. 2015;**10**(1).
94. Tamosaityte S, Galli R, Uckermann O, Sitoci-Ficici KH, Later R, Beiermeister R, et al. Biochemical Monitoring of Spinal Cord Injury by FT-IR Spectroscopy--Effects of Therapeutic Alginate Implant in Rat Models. *PloS one*. 2015;**10**(11):e0142660-e.
95. Cho Y, Shi R, Borgens RB. Chitosan produces potent neuroprotection and physiological recovery following traumatic spinal cord injury. *J Exp Biol*. 2010;**213**(Pt 9):1513-20.
96. Yoshii S, Ito S, Shima M, Taniguchi A, Akagi M. Functional restoration of rabbit spinal cord using collagen-filament scaffold. *J Tissue Eng Regen Med*. 2009;**3**(1):19-25.
97. Fan L, Liu C, Chen X, Zou Y, Zhou Z, Lin C, et al. Directing Induced Pluripotent Stem Cell

Derived Neural Stem Cell Fate with a Three-Dimensional Biomimetic Hydrogel for Spinal Cord Injury Repair. *ACS applied materials & interfaces*. 2018;**10**(21):17742-55.
98. Zaviskova K, Tukmachev D, Dubisova J, Vackova I, Hejcl A, Bystronova J, et al. Injectable hydroxyphenyl derivative of hyaluronic acid hydrogel modified with RGD as scaffold for spinal cord injury repair. *J Biomed Mater Res A*. 2018;**106**(4):1129-40.
99. Raynald, Li Y, Yu H, Huang H, Guo M, Hua R, et al. The hetero-transplantation of human bone marrow stromal cells carried by hydrogel unexpectedly demonstrates a significant role in the functional recovery in the injured spinal cord of rats. *Brain Research*. 2016;**1634**:21-33.
100. Gao M, Lu P, Bednark B, Lynam D, Conner JM, Sakamoto J, et al. Templated agarose scaffolds for the support of motor axon regeneration into sites of complete spinal cord transection. *Biomaterials*. 2013;**34**(5):1529-36.
101. Kang KN, Kim DY, Yoon SM, Lee JY, Lee BN, Kwon JS, et al. Tissue engineered regeneration of completely transected spinal cord using human mesenchymal stem cells. *Biomaterials*. 2012;**33**(19):4828-35.
102. Yang JT, Kuo YC, Chiu KH. Peptide-modified inverted colloidal crystal scaffolds with bone marrow stromal cells in the treatment for spinal cord injury. *Colloids Surf B Biointerfaces*. 2011;**84**(1):198-205.
103. Du B-L, Xiong Y, Zeng C-G, He L-M, Zhang W, Quan D-P, et al. Transplantation of artificial neural construct partly improved spinal tissue repair and functional recovery in rats with spinal cord transection. *Brain research*. 2011;**1400**:87-98.
104. Kim BG, Kang YM, Phi JH, Kim Y-H, Hwang DH, Choi JY, et al. Implantation of polymer scaffolds seeded with neural stem cells in a canine spinal cord injury model. *Cytotherapy*. 2010;**12**(6):841-5.
105. Pritchard CD, Slotkin JR, Yu D, Dai H, Lawrence MS, Bronson RT, et al. Establishing a model spinal cord injury in the African green monkey for the preclinical evaluation of biodegradable polymer scaffolds seeded with human neural stem cells. *Journal of neuroscience methods*. 2010;**188**(2):258-69.
106. Maeda K, Kanno H, Yamazaki Y, Kubo A, Sato F, Yamaguchi Y, et al. Transplantation of Von Hippel-Lindau peptide delivered neural stem cells promotes recovery in the injured rat spinal cord. *Neuroreport*. 2009;**20**(17):1559-63.
107. Bighinati A, Focarete ML, Gualandi C, Pannella M, Giuliani A, Beggiato S, et al. Improved Functional Recovery in Rat Spinal Cord Injury Induced by a Drug Combination Administered with an Implantable Polymeric Delivery System. *J Neurotrauma*. 2020;**37**(15):1708-19.
108. Liu XY, Liang J, Wang Y, Zhong L, Zhao CY, Wei MG, et al. Diffusion tensor imaging predicting neurological repair of spinal cord injury with transplanting collagen/chitosan scaffold binding bFGF. *J Mater Sci Mater Med*. 2019;**30**(11):123.
109. Oudega M, Hao P, Shang J, Haggerty AE, Wang Z, Sun J, et al. Validation study of neurotrophin-3-releasing chitosan facilitation of neural tissue generation in the severely injured adult rat spinal cord. *Exp Neurol*. 2019;**312**:51-62.
110. Pan Q, Guo Y, Kong F. Poly(glycerol sebacate) combined with chondroitinase ABC promotes spinal cord repair in rats. *Journal of biomedical materials research Part B, Applied biomaterials*. 2018;**106**(5):1770-7.
111. Rao JS, Zhao C, Zhang A, Duan H, Hao P, Wei RH, et al. NT3-chitosan enables de novo regeneration and functional recovery in monkeys after spinal cord injury. *Proc Natl Acad Sci U S A*. 2018;**115**(24):E5595-E604.
112. Yin W, Li X, Zhao Y, Tan J, Wu S, Cao Y, et al. Taxol-modified collagen scaffold implantation

promotes functional recovery after long-distance spinal cord complete transection in canines. *Biomater Sci*. 2018;**6**(5):1099-108.
113. Li X, Zhao Y, Cheng S, Han S, Shu M, Chen B, et al. Cetuximab modified collagen scaffold directs neurogenesis of injury-activated endogenous neural stem cells for acute spinal cord injury repair. *Biomaterials*. 2017;**137**:73-86.
114. Chen B, He J, Yang H, Zhang Q, Zhang L, Zhang X, et al. Repair of spinal cord injury by implantation of bFGF-incorporated HEMA-MOETACL hydrogel in rats. *Sci Rep*. 2015;**5**:9017.
115. Grulova I, Slovinska L, Blaško J, Devaux S, Wisztorski M, Salzet M, et al. Delivery of Alginate Scaffold Releasing Two Trophic Factors for Spinal Cord Injury Repair. *Scientific reports*. 2015;**5**:13702.
116. Ni S, Xia T, Li X, Zhu X, Qi H, Huang S, et al. Sustained delivery of chondroitinase ABC by poly(propylene carbonate)-chitosan micron fibers promotes axon regeneration and functional recovery after spinal cord hemisection. *Brain Res*. 2015;**1624**:469-78.
117. Fouad K, Pearse DD, Tetzlaff W, Vavrek R. Transplantation and repair: combined cell implantation and chondroitinase delivery prevents deterioration of bladder function in rats with complete spinal cord injury. *Spinal Cord*. 2009;**47**(10):727-32.
118. Johnson PJ, Parker SR, Sakiyama-Elbert SE. Controlled release of neurotrophin-3 from fibrin-based tissue engineering scaffolds enhances neural fiber sprouting following subacute spinal cord injury. *Biotechnol Bioeng*. 2009;**104**(6):1207-14.
119. Xu HL, Tian FR, Xiao J, Chen PP, Xu J, Fan ZL, et al. Sustained-release of FGF-2 from a hybrid hydrogel of heparin-poloxamer and decellular matrix promotes the neuroprotective effects of proteins after spinal injury. *Int J Nanomedicine*. 2018;**13**:681-94.
120. Li G, Che MT, Zhang K, Qin LN, Zhang YT, Chen RQ, et al. Graft of the NT-3 persistent delivery gelatin sponge scaffold promotes axon regeneration, attenuates inflammation, and induces cell migration in rat and canine with spinal cord injury. *Biomaterials*. 2016;**83**:233-48.
121. Xu H-L, Tian F-R, Lu C-T, Xu J, Fan Z-L, Yang J-J, et al. Thermo-sensitive hydrogels combined with decellularised matrix deliver bFGF for the functional recovery of rats after a spinal cord injury. *Scientific reports*. 2016;**6**:38332-.
122. Li X, Han J, Zhao Y, Ding W, Wei J, Han S, et al. Functionalized Collagen Scaffold Neutralizing the Myelin-Inhibitory Molecules Promoted Neurites Outgrowth in Vitro and Facilitated Spinal Cord Regeneration in Vivo. *ACS applied materials & interfaces*. 2015;**7**(25):13960-71.
123. Lowry N, Goderie SK, Lederman P, Charniga C, Gooch MR, Gracey KD, et al. The effect of long-term release of Shh from implanted biodegradable microspheres on recovery from spinal cord injury in mice. *Biomaterials*. 2012;**33**(10):2892-901.
124. Conova L, Vernengo J, Jin Y, Himes BT, Neuhuber B, Fischer I, et al. A pilot study of poly(N-isopropylacrylamide)-g-polyethylene glycol and poly(N-isopropylacrylamide)-g-methylcellulose branched copolymers as injectable scaffolds for local delivery of neurotrophins and cellular transplants into the injured spinal cord. *J Neurosurg Spine*. 2011;**15**(6):594-604.
125. Johnson PJ, Tatara A, Shiu A, Sakiyama-Elbert SE. Controlled release of neurotrophin-3 and platelet-derived growth factor from fibrin scaffolds containing neural progenitor cells enhances survival and differentiation into neurons in a subacute model of SCI. *Cell Transplant*. 2010;**19**(1):89-101.
126. Deng WS, Ma K, Liang B, Liu XY, Xu HY, Zhang J, et al. Collagen scaffold combined with human umbilical cord-mesenchymal stem cells transplantation for acute complete spinal cord injury. *Neural Regen Res*. 2020;**15**(9):1686-700.

Table 1. Summary of included studies on testing tissue engineering strategies in animal models of SCI.

aFGF: acidic fibroblast growth factor; BDNF: brain-derived neurotrophic factor; bFGF: basic fibroblast growth factor; BMHP1: bone marrow homing peptide; BMSC: bone marrow stromal cells; ChABC: chondroitinase ABC; CNTF: ciliary neurotrophic factor; DTX: docetaxel; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor; EnSC: endometrial-derived stromal cells; ESNPC: embryonic stem cell-derived neural progenitor cell; FHPS: fragmented physical hydrogel suspension; HA: hyaluronic acid; HEMA-MOETACL: hydroxyl ethyl methacrylate [2-(methacryloyloxy)ethyl] trimethylammonium chloride; HP: heparin-poloxamer; IKVAV-PA: IKVAV-functionalised peptide amphiphile; iPSC: induced pluripotent stem cell; MSC: mesenchymal stem cell; NPC: neural progenitor cells; NSC: neural stem cell; NT-3: neurotrophin-3; OEC: olfactory ensheathing cell; PDGF: platelet-derived growth factor; PEG: polyethylene glycol; PLGA: poly (lactic-co-glycolic acid); PLL: poly-L-Lysine; PNIPAAm: poly(N-isopropylacrylamide); PGS: poly(glycerol sebacate)

Biomaterials alone

Study	Analogous clinical study design	Animal species	Injury pattern	Timing of intervention / outcome measurement	Type of intervention	Main findings	Likely effects of the biomaterial
Dumont 2019	Prospective	Mouse	Hemisection	Immediately	Porous PEG	Reduced glial scar and	Axon guidance

[88]	cohort study		(T9)	after injury / 8 weeks	hydrogel tubes with intermediate microsphere phase	robust axon growth along tube surface; axon density was 3-fold higher compared to control and 30% of axons within the tube were myelinated; enhanced functional recovery	
Gao 2019 [53]	Prospective cohort study	Rat and pig	Contusion (T10)	Rat: 6 hours post-injury / 24 hours or 1 week after intervention Pig: 3 hours post-injury / 2 hours after	PLGA nanoparticles	Dose-dependent increase and significantly greater localisation of nanoparticles at lesion site than uninjured regions, which was not seen in sham animals; nanoparticles were retained at the lesion site	Carrier for drug delivery

				intervention (n=1) and 30 min post injury / 5 hours after intervention (n=1)			
Hejčl 2018 [46]	Prospective cohort study	Rat	Hemisection (T8)	Immediately after injury / 12 weeks	Modified methacrylate hydrogels	Hydrogels significantly increased connective tissue infiltration, blood vessel ingrowth, axonal ingrowth and formation of some neurofilaments	Axon guidance; Neuronal regeneration

Sitoci-Ficici 2018 [76]	Prospective cohort study	Rat	Hemisection (T9-10)	Immediately after injury / 20 weeks	Non-functionalised soft alginate hydrogel	Significantly improved locomotor recovery in animals with 2 mm lesions but not 4 mm lesions; reduced fibrous scarring in spinal cord	Axon guidance
Chedly 2017 [89]	Prospective cohort study	Rat	Hemisection (T8-9)	Immediately after injury / 12 weeks	Chitosan microhydrogels with FPHS (3μL)	Promoted reconstitution of spinal tissue and vasculature, and reduced fibrous glial scarring	Axon guidance; Alleviate the secondary response
Zhu 2017 [90]	Randomised controlled study	Rat	Complete transection (T9-10)	Immediately after injury / 12 weeks	Decellularised spinal cord scaffold	Scaffold provided contact guidance for axonal regrowth, and allowed better recovery of motor function	Axon guidance

Kushchayev 2016 [91]	Randomised controlled study	Rat	Complete transection (T9-10)	Immediately after injury / 16 weeks	Hyaluronic acid hydrogel	Smaller lesion size, decreased fibrous scarring and presence of inflammatory cells; no differences in behavioural assessments; no axonal or neuronal regeneration	Alleviate the secondary response
Imani 2015 [92]	Randomised controlled study	Rat	Complete transection (T9-10)	1 week post-injury / 4 weeks after intervention	Carbon nanotubes functionalised with a sulfonated tetrafluoroethylene copolymer (Nafion)	Decreased lesion volume, increased neurofilament-positive fibres and corticospinal tract fibres in the lesion, and no increase in gliosis; modest improvement in hind limb locomotor recovery	Alleviate the secondary response

Kaneko 2015 [93]	Prospective cohort study	Rat	Complete transection (T9-11)	Immediately after injury / 20 weeks	3D nanofibrous hydrogel and collagen sponge scaffold	Greater neuronal regeneration, spinal repair and locomotor recovery; promoted differentiation and maturation of neurons and astrocytes	Neuronal regeneration
Suzuki 2015 [83]	Prospective cohort study	Rat	Complete transection (T9)	Immediately after injury / 8 weeks	Collagen scaffold containing 4 bundles (5 mm length), total 4000 filaments (20 μm diameter)	Myelinated nerve fibres found in the scaffold and 10-25% of rubrospinal tracts were repaired; the graft could function as a nerve tract and might provide a permissive microenvironment for axon elongation	Axon guidance

Tamosaityte 2015 [94]	Randomised controlled study	Rat	Hemisection (T9)	Immediately after injury / 24 weeks	Non-functionalised soft calcium alginate hydrogel	Significantly reduced injury-induced demyelination and fibrotic scarring; hydrogel had long-term persistence *in vivo*	Alleviate the secondary response
Roman 2011 [29]	Randomised controlled study	Rat	Complete transection (T9)	1week post-injury / 4 weeks after intervention	Single-walled carbon nanotubes functionalised with PEG	Decreased lesion volume, increased neurofilament-positive fibres and corticospinal tract fibres in the lesion, and did not increase reactive gliosis; modest improvement in hind limb locomotor recovery	Axon guidance

Cho 2010 [95]	Prospective cohort study	Guinea pig	Contusion (midthoracic)	Immediately after injury / 2 weeks	Chitosan solution	Restored the conduction of nerve impulses through the length of the spinal cord	Alleviate the secondary response
Johnson 2010 [58]	Prospective cohort study	Rat	Hemisection (T9)	2 weeks post-injury / 4 weeks after intervention	Fibrin scaffold	Fibrin was conducive to regeneration and cellular migration; higher levels of neural fibre staining and delayed accumulation of reactive astrocytes in the lesion	Alleviate the secondary response
Tysseling 2010 [54]	Prospective cohort study	Rat and mouse	Contusion (T13 for rat, T10 for mouse)	1 day post-injury / 9 weeks after intervention	Self-assembling IKVAV-PA scaffold	Improved functional recovery required the bioactive sequence; may be due to increased serotonergic innervation	Axon guidance

						caudal to the lesion, and regeneration of motor and sensory axons	
Yoshii 2009 [96]	Prospective cohort study	Rabbit	Complete transection (T10)	Immediately after injury / 24 weeks	Collagen scaffold (5 mm length) with 6000 filaments	Scaffold grafted parallel to the axis of the spinal cord supported axonal regeneration and improvement in locomotion; functional restoration appeared to be permanent	Axon guidance
Biomaterials with cells							
Study	**Analogous clinical study**	**Animal species**	**Injury pattern**	**Timing of intervention / outcome**	**Type of intervention**	**Main findings**	**Likely effects of the biomaterial**

	design			**measurement**			
Koffler 2019 [28]	Prospective cohort study	Rat	Complete transection (T3)	Immediately after injury / 20 weeks	Polyethylene glycol gelatin methacrylate loaded with rat NPCs	Channelled scaffolds produced using 3D printing supported axon regeneration and formation of new 'neural relays' between host and transplanted cells; restored synaptic transmission and significantly improved functional outcomes	Axon guidance
Marchini 2019 [42]	Randomised controlled study	Rat	Hemisection (T9-10)	1 week post-injury / 8 weeks after	Self-assembling peptide hydrogel with human NSCs	Decreased astrogliosis and immune response; scaffolds with pre-differentiated cells	Alleviate the secondary response; Axon guidance;

				intervention		showed higher percentages of neuronal markers, better engraftment, and improved behavioural recovery	Cell homing
Fan 2018 [97]	Randomised controlled study	Mouse	Complete transection (T9-10)	Immediately after injury / 6 weeks	Gelatin methacrylate (GelMA) hydrogel with mouse iPSC-derived NSCs	More robust neurite outgrowth and neuronal differentiation; greater functional recovery; reduced cavity area, inflammation and glial scar formation	Induce neurodifferentiation; Cell homing; Axon guidance; Alleviate the secondary response
Han 2018 [41]	Randomised controlled study	Dog	Complete transection (T8)	Immediately after injury / 36 weeks	Linear-ordered collagen scaffold with human placenta-derived	Better hind limb locomotor recovery; regenerated tissue well integrated with host tissue; more neurons,	Axon guidance; Cell homing

					MSCs	axonal regeneration, remyelination and synapse formation in lesion site; enhanced sprouting of motor and sensory fibres in lesion site	
Zaviskova 2018 [98]	Prospective cohort study	Rat	Hemisection (T8)	1 week post-injury / 8 weeks after intervention	Modified hyaluronic acid hydrogel with human Wharton's jelly-derived MSCs	Promoted axonal ingrowth into the lesion; no effect on locomotor recovery, blood vessel ingrowth or density of glial scar around the lesion	Axon guidance; Cell homing
Wang 2017 [63]	Randomised controlled study	Rat	Complete transection (T9-10)	Immediately after injury / 8 weeks	PLGA scaffold with rat OECs	Enhanced locomotor recovery, axon myelination and better protected	Extracellular matrix substitution; Cell homing

						neurons compared with scaffold alone	
Raynald 2016 [99]	Randomised controlled study	Rat	Hemisection (T9)	Immediately after injury / 8 weeks	HA-PLL hydrogel with human BMSCs	Improved survival of transplanted cells, axonal growth and functional recovery	Axon guidance; Cell homing
Gao 2013 [100]	Randomised controlled study	Rat	Complete transection (T3)	Immediately after injury / 4 weeks	Agarose scaffold with bone marrow stromal cells secreting BDNF	Templated scaffold supported motor axon regeneration and organised axons into highly linear fascicles; BDNF significantly enhanced axonal growth	Axon guidance; Extracellular matrix substitution; Cell homing
Kang 2012 [101]	Randomised controlled	Rat	Complete transection	Immediately after injury / 8	PLGA scaffold with human MSCs	Improved recovery of hind limb locomotion, with	Axon guidance; Cell homing

	study		(T8-9)	weeks		higher amplitude of motor evoked potentials; cells survived in implant site at 8 weeks and differentiated into nerve cells	
Yang 2011 [102]	Prospective cohort study	Rat	Contusion (T10)	Immediately after injury / 4 weeks	Inverted colloidal crystal scaffold grafted with two defined peptides, with rat bone marrow stromal cells	Neuronal survival and axonal growth were highest in scaffold with peptide, and higher in scaffold compared to cells alone; construct inhibited formation of glial scar tissue and inflammatory cytokines	Alleviate the secondary response; Axon guidance; Cell homing

Du 2011 [103]	Randomised controlled study	Rat	Complete transection (T9)	Immediately after injury / 8 weeks	Macroporous PLGA scaffold with rat NSCs	Significantly improved locomotion recovery; grafted cells had higher survival rate and could differentiate into neuronal phenotype; higher nerve fibre regrowth but limited corticospinal tract axon regeneration	Induce neurodifferentiation; Axon guidance; Cell homing
Kim 2010 [104]	Prospective cohort study	Dog	Hemisection (T11)	Immediately after injury / 12 weeks	PLGA scaffold with human NSCs	Scaffold bridged tissue defects and integrated with host tissue; grafted cells survived implantation and showed migratory behaviour	Cell homing

Pritchard 2010 [105]	Prospective cohort study	Non-human primate	Hemisection (T9-10)	Immediately after injury / up to 16 weeks	PLGA scaffold with human NSCs	Scaffold persisted for >40 days and degraded within 82 days; differences in structural and functional improvements were not significant between animals, but only one animal per treatment was used	Cell homing; Axon guidance
Maeda 2009 [106]	Randomised controlled study	Rat	Contusion (T10)	1 week post-injury / 6 weeks after intervention	Von Hippel–Lindau peptide with rat NSCs	Improved behavioural recovery and increased differentiation of engrafted NSCs into neuronal marker positive cells	Induce neurodifferentiation; Cell homing
Biomaterials with drugs or biomolecules							

Study	Analogous clinical study design	Animal species	Injury pattern	Timing of intervention / outcome measurement	Type of intervention	Main findings	Likely effects of the biomaterial
Bighinati 2020 [107]	Randomised controlled study	Rat	Contusion (T9)	Immediately after injury / 8 weeks	Poly (l-lactic acid) scaffold loaded with ibuprofen and triiodothyronine	Reduced lesion volume and percentage of astrocytes; increased locomotion recovery, myelin and neurofilament formation	Alleviate the secondary response; Drug delivery
Hassannejad 2019 [72]	Randomised controlled study	Rat	Contusion (T7-8)	1 day post-injury / 6 weeks	IKVAV-PA hydrogel loaded with BDNF (20 µL of 0.05 mg/mL)	Axon preservation and reduction of astrogliosis; no difference in locomotor functional recovery compared to control	Alleviate the secondary response; Axon guidance

Liu 2019 [108]	Randomised controlled study	Rat	Complete transection (T10)	Immediately after injury / 8 weeks	Collagen/chitosan mixture adsorbed with bFGF (50 ng)	Significantly improved locomotor function, axonal repair and regeneration of nerve fibre tracts	Axon guidance
Oudega 2019 [109]	Randomised controlled study	Rat	Complete transection (T7-8)	Immediately after injury / 12 weeks	Chitosan tubes containing chitosan carriers loaded with NT-3 (100 ng)	Neural tissue bridged the transection gap; hind limb movement was significantly improved	Axon guidance; Alleviate the secondary response
Pan 2018 [110]	Randomised controlled study	Rat	Complete transection (T10)	Immediately after injury / 12 weeks	PGS scaffold with ChABC (6 μL of 10 U/mL) injected separately	Improved nerve regeneration and recovery of movement function, compared to scaffold alone or biomolecule alone	Alleviate the secondary response; Axon guidance; Drug delivery
Rao 2018 [111]	Randomized controlled	Non-human	Hemisection (T8)	Immediately after injury / 1	Chitosan tube scaffold with NT-3	Enabled robust neural regeneration accompanied	Axon guidance

	study	primate		month to >3 years	(100 ng)	by motor and sensory functional recovery; motor axons in the corticospinal tract entered the injury site within the biomaterial and also grew across the lesion area into the distal spinal cord	
Tom 2018 [27]	Prospective cohort study	Rat	Contusion (T9-10)	1 week post-injury / 10 weeks after intervention	PNIPAAm-g-PEG scaffold loaded with BDNF and NT-3	Significant restoration in the rate depression property of H-reflex for animals with treadmill training, with or without the implant; implant alone was ineffective	Drug delivery; Extracellular matrix substitution

Yin 2018 [112]	Prospective cohort study	Dog	Complete transection (T8)	Immediately after injury / 24 weeks	Linear-ordered collagen scaffold with Taxol (0.24 mg)	Significantly promoted motor evoked potentials and locomotion recovery; significantly increased neurogenesis and axon regeneration to reconnect the spinal cord stumps; reduced glial scar formation	Alleviate the secondary response; Axon guidance; Drug delivery
Li 2017 [113]	Randomised controlled study	Rat and dog	Complete transection (T8)	Immediately after injury / 36 weeks	Linear ordered collagen scaffold with PBS containing cetuximab	Neuronal regeneration in both rodent and canine models, including neuronal differentiation, maturation, myelination, and synapse formation leading to significant locomotion	Neuronal regeneration; Alleviate the secondary response; Drug delivery

						recovery	
Chen 2015 [114]	Prospective cohort study	Rat	Complete transection (T9)	5 days post-injury / 8 weeks after intervention	HEMA-MOETACL hydrogel loaded with bFGF (2 μg)	Allowed ingrowth of regenerating tissue; promoted nerve tissue regeneration and functional recovery	Alleviate the secondary response; Axon guidance
Grulova 2015 [115]	Randomised controlled study	Rat	Contusion (T8)	1 week post-injury / 7 weeks post-injury	Alginate scaffold loaded with EGF and bFGF	Enhanced sparing of spinal cord tissue and outgrowth of corticospinal tract axons, and increased number of surviving neurons and sensory fibres; improved functional recovery	Extracellular matrix substitution; Alleviate the secondary response

Ni 2015 [116]	Prospective cohort study	Rat	Hemisection (T7-9)	Immediately after injury / 4 weeks	Polypropylene carbonate electrospun fibres with chitosan microspheres loaded with ChABC	Promoted axon sprouting and functional recovery, and reduced glial scarring; fibres without ChABC did not have the same effects	Drug delivery; Alleviate the secondary response; Axon guidance
Wang 2014 [14]	Randomised controlled study	Rat	Complete transection (T8)	Immediately after injury / 15 weeks	Sodium hyaluronate gelatinous particles containing CNTF	Powerful functional recovery (open-field locomotion, cortical motor/somatosensory evoked potentials), possibly due to increased axonal regrowth and neuron-like cells	Axon guidance; Extracellular matrix substitution

Fouad 2009 [117]	Prospective cohort study	Rat	Complete transection (T8)	Immediately after injury / 12 weeks	Matrigel-filled guidance channels with rat Schwann cells, olfactory ensheathing glia and ChABC (2 µL of 10 µg/mL)	Prevented collagen deposition in bladder walls and maintained the animal's ability to void efficiently; controls with Matrigel only had thicker bladder walls	Axon guidance; Drug delivery
Johnson 2009 [118]	Prospective cohort study	Rat	Hemisection (T9)	2 weeks post-injury / 2 weeks after intervention	Fibrin scaffold with NT-3 (500 or 1000 ng/mL)	500 ng/mL NT-3 increased neural fibre density compared to scaffold alone; scaffolds with or without NT-3 had lower astrocyte density compared to control	Axon guidance; Extracellular matrix substitution
Biomaterials with a combination of additional factors							

Study	Analogous clinical study design	Animal species	Injury pattern	Timing of intervention / outcome measurement	Type of intervention	Main findings	Likely effects of the biomaterial
Luo 2018 [84]	Randomised controlled study	Rat	Contusion (T10)	Immediately after injury / 4 weeks	HP hydrogel combining human dental pulp stem cells and bFGF	Improved neuronal repair, functional recovery and tissue regeneration	Induce neurodifferentiation; Neuronal regeneration; Cell homing
Wang 2018 [48]	Randomised controlled study	Rat	Contusion (T9-10)	Immediately after injury / 8 weeks	HP hydrogel containing liposomes with aFGF, BDNF and DTX	The multiple drugs were effectively delivered to the injury site, where their combined application improved neuronal survival and plasticity, and	Drug delivery; Axon guidance; Alleviate the secondary response

						promoted axonal regeneration	
Xu 2018 [119]	Randomised controlled study	Rat	Hemisection (T9-10)	Immediately after injury / 4 weeks	HP hydrogel with decellularised matrix and FGF2 (20 μL of 3 μg/μL)	Scaffold promoted better recovery of neuron functions and tissue morphology compared to free FGF2; increased expression of neurofilament protein and axon density in scaffolds	Axon guidance; Extracellular matrix substitution; Alleviate the secondary response
Fan 2017 [68]	Randomised controlled study	Rat	Complete transection (T8)	Immediately after injury / 12 weeks	Linear-ordered collagen scaffold modified with a collagen-binding EGFR antibody	Promoted neurogenesis of endogenous injury-activated NSCs, which matured into functional neurons to reconnect the	Axon guidance; Induce neurodifferentiation

					fragment	injured gap	
Li 2016 [71]	Randomised controlled study	Rat	Complete transection (T9-10)	Immediately after injury / 12 weeks	Collagen scaffold functionalised with a cocktail of neutralising proteins and collagen-binding neurotrophic factors	Designed to antagonise myelin inhibitory molecules while providing neurotrophic protection; reduced the volume of cavitation, facilitated axonal regeneration, and promoted neuronal regeneration; new neurons in the lesion enhanced locomotion recovery	Axon guidance; Neuronal regeneration; Alleviate the secondary response
Li 2016 [120]	Prospective cohort study	Rat and dog	Hemisection (T10)	Immediately after injury / 4	Gelatin sponge scaffold coated with	Significantly reduced cavity areas in the injury site, due	Axon guidance; Extracellular matrix

				weeks	NT-3/fibroin, with rat bone marrow-derived MSCs	to tissue regeneration and axonal extensions with myelin sheath through the glial scar into the implant; decreased inflammation	substitution; Alleviate the secondary response
Tavakol 2016 [73]	Randomised controlled study	Rat	Contusion (T9-10)	10 days post-injury / 6 weeks after intervention	Injectable self-assembling peptide nanofibre scaffold containing BMHP1, with human EnSCs	Improved axon regeneration and myelination, and motor neuron function with less inflammatory response	Axon guidance; Extracellular matrix substitution; Cell homing
Xu 2016 [121]	Randomised controlled study	Rat	Hemisection (T9-10)	Immediately after injury / 4 weeks	Acellular spinal cord scaffold loaded with bFGF and encapsulated into a HP hydrogel	Enhanced inhibition of glial scars and improved functional recovery through regeneration of nerve axons and differentiation of neural	Axon guidance; Induce neurodifferentiation; Alleviate the secondary response

						stem cells	
Li 2015 [122]	Randomised controlled study	Rat	Complete transection (T9-10)	Immediately after injury / 12 weeks	Collagen scaffolds loaded with two collagen-binding proteins	The proteins were used to neutralise axon guidance molecules that inhibit nerve fibre regeneration; constructs improved axonal regeneration and locomotion recovery	Axon guidance
Shi 2014 [62]	Randomized controlled study	Rat	Hemisection (T9)	Immediately after injury / 8 weeks	Collagen scaffold with collagen binding bFGF	Improved survival rates; higher improvement in motor function compared to scaffold alone; guided fibres to growth through the implant	Axon guidance

Lowry 2012 [123]	Prospective cohort study	Mouse	Partial transection or contusion (T9-10)	Immediately after injury / 7 weeks	PLGA microspheres loaded with Shh protein	Increased proliferation of endogenous oligodendrocyte lineage cells, decreased astrocytic scar formation, and increased sprouting and growth of corticospinal and raphespinal tract fibres	Axon guidance; Extracellular matrix substitution; Alleviate the secondary response
Conova 2011 [124]	Prospective cohort study	Rat	Hemisection (C4-5)	3 days post-injury / 2 weeks after intervention	Injectable PNIPAAm scaffold lightly crosslinked with PEG or methylcellulose, loaded with BDNF	Scaffolds did not contribute to injury-related inflammatory response; both were permissive to axonal growth and allowed local BDNF delivery	Axon guidance; Extracellular matrix substitution

Johnson 2010 [125]	Prospective cohort study	Rat	Hemisection (T9)	2 weeks post-injury / 2 weeks after intervention	Fibrin scaffold with ESNPCs transplanted as embryoid bodies, containing a heparin-binding delivery system, NT-3 and PDGF	Fibrin scaffold with NT-3 and PDGF increased total number of ESNPCs in the lesion; inclusion of heparin-binding delivery system with growth factor increased number of ESNPC-derived neurons	Axon guidance; Induce neurodifferentiation; Extracellular matrix substitution

Table 2. Biomaterials that have been tested in clinical trials for spinal cord injury (SCI) repair.

Study	Type of SCI	Biomaterial composition	Trial registration information
Deng 2020 [126]	Acute complete SCI	Collagen scaffold combined with human umbilical cord mesenchymal stem cells (MSCs)	Ethics Committee of the Characteristic Medical Center of Chinese People's Armed Police Force on 3 February 2016 (Approval No. PJHEC-2016-A8)
Chen 2020 [77]	Acute complete SCI	NeuroRegen scaffold (bovine aponeurosis) combined with autologous bone marrow mononuclear cells (BMMCs)	National Institutes of Health database (ClinicalTrials.gov: NCT02510365)
Xiao 2018 [75]	Acute complete SCI	NeuroRegen scaffold combined with allogeneic umbilical cord MSCs	National Institute of Health database (ClinicalTrials.gov: NCT02510365)
Xiao 2016 [21]	Chronic complete SCI	NeuroRegen scaffold combined with autologous BMMCs	National Institutes of Health database (ClinicalTrials.gov: NCT02352077)
Theodore 2016 [22]	Acute traumatic SCI	Neuro-Spinal Scaffold (poly(lactic-co-glycolic acid) covalently conjugated to	National Institute of Health database (ClinicalTrials.gov: NCT02138110)

		poly(L-lysine))	
UC Davis Medical Center Sacramento, California, United States	Complete thoracic SCI	Neuro-Spinal Scaffold	National Institutes of Health database (ClinicalTrials.gov: NCT03762655)
Affiliated Hospital of Logistics, University of CAPF, Tianjin, China	Acute or chronic complete SCI	Functional neural regeneration scaffold	National Institutes of Health database (ClinicalTrials.gov: NCT03966794)
Affiliated Hospital of Logistics, University of CAPF, Tianjin, China	Chronic complete SCI	NeuroRegen scaffold combined with mesenchymal stem cells or neural stem cells	National Institutes of Health database (ClinicalTrials.gov: NCT02688049)
First Affiliated Hospital of PLA General Hospital, Beijing, China	Complete thoracic SCI	NeuroRegen scaffold combined with BMMCs	National Institutes of Health database (ClinicalTrials.gov: NCT02688062)
Toronto Western Hospital,	Traumatic acute	Neuro-Spinal Scaffold	National Institutes of Health database

Toronto, Ontario, Canada (withdrawn)	cervical SCI	(ClinicalTrials.gov: NCT03105882)