Chapter

Use of Mesenchymal Stem Cells in Pre-Clinical Models of Spinal Cord Injury

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Abstract

Spinal Cord Injury (SCI) is a devastating disease that causes disruption of sensorimotor function below the site of injury. Current management is based on surgical decompression of the neural tissue and pharmacotherapy; however, there is no gold standard treatment readily available for patients in the clinic. This indicates that novel therapeutic strategies for the treatment are still needed in the clinical setting. There are several alternatives that are currently under investigation for the treatment of this disease, with increasing focus in regenerative medicine treatments. Mesenchymal stem cells (MSCs) are one of the most promising candidates for stem cell therapy in SCI, as they are easily obtained, have high safety profiles, and help with neural regeneration in SCI mainly via release of trophic factors, neovascularization, and immunomodulation. In this work, authors provide an insight of the available MSC for neural regeneration, their therapeutic role, and the potential MSC-based therapies for SCI.

Keywords: mesenchymal stem cells, adipose-derived stem cells, spinal cord injury, animal model, stem cell therapy

1. Introduction

Traumatic Spinal cord injury (SCI) is a devastating disease that results in severe neural disruption and severe disabilities below the site of injury. Patients are unable to regenerate neural tissue after injury, leading to a lifelong disability. The pathophysiology of SCI is complex, consisting of a primary insult to the cord followed by a secondary cascade of events characterized mainly by inflammation, ischemia, ionic imbalance, excitotoxicity, and apoptosis [1]. This disease comprises a significant portion of health care expenditure in the United States, with an estimated annual cost of 7.7 billion dollars [2]. According to the National Spinal Cord Injury Statistical Center (NSCISC) in 2019, the incidence of SCI was about 54 cases per million people in the United States. SCI is caused by motor vehicle collisions in about 50% of cases, but other common etiologies include falls (30%), violent crime (11%), and sports-related injuries (9%) [2, 3]. SCI induced paraplegia and quadriplegia causes a significant

physical and emotional toll on those inflicted, thus, there is a need for optimized strategies to better treat these patients. Although there has been significant investment into development of novel therapeutic strategies to improve outcomes for these patients, there remains little with proven benefit besides aggressive supportive care.

2. Spinal cord injury: standard of care

Spinal cord injuries can be subdivided into multiple groups depending on the mechanism of injury, anatomic location of the lesion, type and severity of the injury. The basis of treatment is surgical decompression of the spinal cord to prevent secondary damage associated with hypoxia and ischemia [4]. Besides surgery, there have been numerous neuroprotective drugs that have been assessed in clinical trials, including methylprednisolone, thyrotropin-releasing hormone, nimodipine, and naloxone [5–7]. However most of these drugs were ineffective, and some were associated with wound healing complications and infections that represents a limitation in the management of this population of patients.

The current standard of care for these patients consists of aggressive medical management. This includes prevention of secondary injury with strict maintenance of mean arterial pressure (MAP) [8, 9]. SCI patients are prone to cardiovascular instability, neurogenic shock, respiratory insufficiency, particularly when cervical levels are involved, which then leads to further secondary injury [10]. Multiple studies have shown improved outcome when these patients are managed in the intensive care unit (ICU), with strict monitoring of blood pressure parameters [11, 12]. Studies have shown that augmentation of MAPs to greater than 85 for 7 days is associated with improved outcomes as assessed by American Spinal Cord injury Association (ASIA) impairment scale.

Stems cells have become a hot topic of great interest in various fields such as cancer biology, regenerative medicine, and SCI. There are multiple types of stem cells, with varying capabilities, including embryonic stem cells, (ESC), tissue-specific stem cells, mesenchymal stem cells (MSCs), and induced pluripotent stem cells. (iPSC). MSCs were first discovered in the bone marrow, but since then have been grown from other sources such as adipose tissue, amniotic fluid and umbilical cord blood, making them more easily accessible. MSCs are typically defined as plastic adhering cell populations that can be directed to differentiate *in vitro* into cells of osteogenic, chondrogenic, adipogenic, myogenic, and various other lineages. They are known to have a beneficial effect in SCI, via release of trophic factors for neuroprotection, neovascularization, and immunomodulation [13–15]. These cells naturally secrete various trophic factors, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and vascular endothelial growth factors (VEGF). BDNF is of particular interest since it has been shown to induce sprouting of corticospinal tracts in animal models of SCI [16]. MSCs derived from adipose tissue present unique advantages over other mesenchymal stem cell types as bone marrow, umbilical cord, dental pulp and others. In this chapter we'll focus in how MSCs can help in promoting spinal cord recovery after traumatic injury.

3. Mesenchymal stem cell use in the central nervous system

MSCs have been suggested for the treatment of various diseases. MSCs have also been proposed as a potential treatment for diabetes, inflammatory bowel disease, Parkinson disease, Alzheimer's disease, osteoporosis, bone regeneration, wound healing, skin aging, different inflammatory skin conditions, and others [17–27].

Among neurologic diseases, the use of MSCs in hypoxic-ischemic encephalopathy, multiple sclerosis, and glioma has been considered. The majority of studies investigating MSCs' impact on the treatment of stroke reported a decrease in the size of stroke volume and improvement in behavioral outcomes [28]. In animal studies, functional improvement, along with decreased seizures and increased long term potentiation, was seen in the hypoxic-ischemic encephalopathy model. In animal models of multiple sclerosis, demyelination and infiltrates were reduced after the treatment with MSCs. In murine studies, targeting of glioma cells with MSCs while loading them with viruses, were effective in impeding the growth of the tumor [29].

Different stem cell types have been used to treat SCI (**Figure 1**). Among them, MSCs are preferred due to several reasons:

- The simplicity of the isolation process
- The simplicity of cryoprecipitation
- Preservation of regenerative capacity and viability after cryoprecipitation at very low temperatures (−80)
- Minimal chances of cellular reaction induction
- High replication speed with high-quality progenitor cells and high potential of multilineage differentiation [30]
- Hypoimmunogenicity [31]

3.1 Bone marrow mesenchymal stem cells (BM-MSCs)

The collection of bone marrow tissue for extraction of MSCs is done by aspiration, which is not only invasive and painful for the patient, but also distressing but also carries a risk of infection [32]. Nevertheless, these risks are partially negated by the intriguing properties of BM-MSCs for neuronal regeneration. One of this

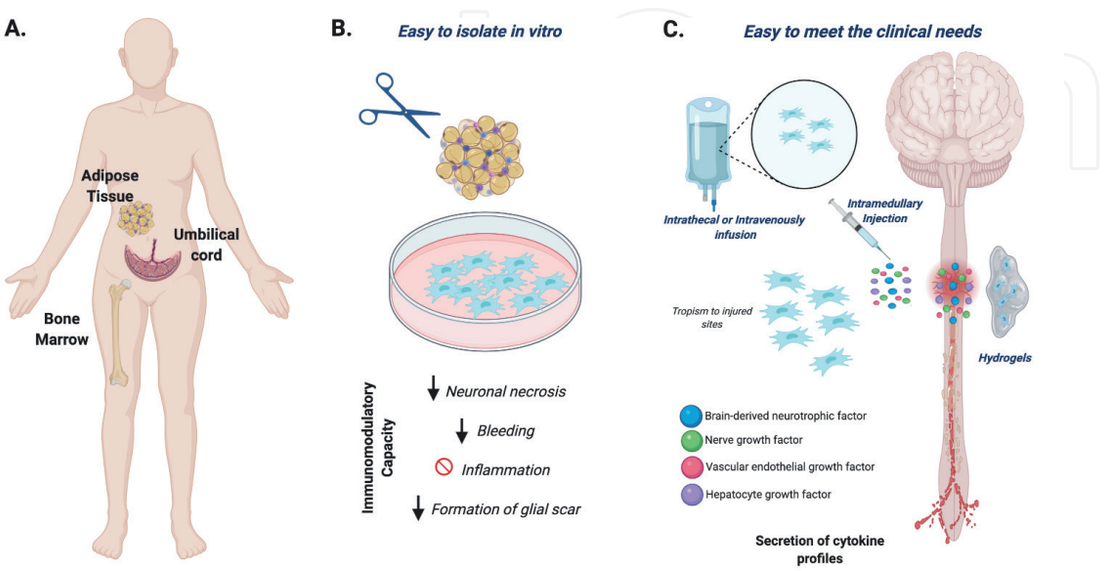

Figure 1.
Mesenchymal stem cells can be isolated from different sources (a), and can be expanded in vitro; when cultured they have a high proliferative rate (b). When applied to the SCI, they show homing properties, they are attracted by chemotactic signals and migrate towards the injured sire (c). Created with BioRender.com

properties is their plasticity potential as it allows them to differentiate into a broad spectrum other than mesodermal lineage cells as described by Wislet-Gendebien et al. [33] in which bone marrow stem cells were cultured with cerebellar granule neurons, inducing the expression the genes sox2, sox10, pax6, fzd, erbB2, and erbB4 in nestin-positive MSCs. Furthermore, with the help of electrophysiological analyses, they could establish that BM-MSCs neuron-like cells were able to fire single-action potentials and respond to the stimulation of distinct neurotransmitters such as GABA, glycine, and glutamate, concluding that nestin-positive bone marrow-derived MSCs can differentiate *in vitro* into excitable neuron-like cells.

Numerous authors have described another characteristic of this type of stem cell's source and are the capacity of MSCs to migrate to the injured tissue – a mechanism described as 'homing' - especially in BM-MSCs [34]. This characteristic makes BM-MSCs source very attractive due to the range of alternatives for applying treatment with MSCs to patients other than invasive procedures. *Andersen et al.* [35] put in practice the migration ability by injecting with BMSCs subcutaneously to an immune-deficient mouse with a bone fracture. Besides observing the homing capacity of MSCs, and is mediated by a wide range of growth factors such as PDGF and IGF-1.

BMSCs in a chimeric mice contusion SCI model was more effective in reducing the neuropathic pain and motor and thermal sensitivity if BMSCs were injected 3 days after the injury compared to injections at day 1, 7, or 14 days. This effect was mediated through the suppression of p38 MAPK and ERK1/2 activation in microglia and macrophages, CREB and PKC-c in dorsal horn neurons in the site of the injury and around it, and decreased macrophage infiltration to the epicenter. The latter reduces inflammation and restores Blood Spinal Cord Barrier [36]. Quertainmont et al. observed improved locomotor skills using open field test in the rats treated with BMSCs [37].

3.2 Adipose-derived mesenchymal stem cells (ADSC)

The abundant availability of adipose tissue, its easy accessibility under local anesthesia, a less painful procedure for the patients, and no adverse effects on animal models treated make this source desirable for the extraction, process, and administration to patients [38, 39]. A study conducted by *Ohta Y. et al.* [40] revealed that the secretion of specific growth factors, cytokines, proteases, immunomodulatory factors, and cellular matrix molecules promotes ADSCs' ability to regenerate neural tissue. However, the lack of full functional recovery results and the gap of knowledge on the description of the pro-regenerative effects are some of the limitations described on the literature on the use of MSCs that needs to be addressed in order to improve outcomes on complete functional recovery in *in vivo* models of SCI [41–43].

3.3 Umbilical cord mesenchymal stem cells

Ryu et al. [44] conducted a comparison between four sources of MSCs (Bone marrow, adipose-derived, umbilical cord blood, and Wharton's Jelly) to treat a canine model with spinal cord injury. Even though data revealed no significant differences in functional recovery among the MSCs groups, they identified essential properties such as the promotion of neuronal regeneration and anti-inflammatory activity. Umbilical cord stem cells group showed more nerve regeneration, neuroprotection, and less inflammation with reduced IL-6 and COX-2 levels than other MSCs. Moreover, researchers establish improvement in locomotion measured using the Olby and modified Tarlov scores eight weeks after the application of MSCs

compared with the control group, suggesting that the use of MSCs promotes functional recovery after SCI. Additionally, preclinical studies such as the one carried by *Chua S. J. et al.* [45] have detected cytokines and growth factors known by its neuroprotective, angiogenic, and anti-inflammatory effects.

In a compression SCI rat model, both BMSCs and umbilical cord-derived stem cells caused similar results and improvement in allodynia, hyperalgesia, and functional recovery. However, UCSCs were more effective in decreasing wind-up levels [46]. 13 of 22 patients treated with UC-MSCs were better in daily living activities. They had a better motor function, better motor and tactile sensation [47].

3.4 Amniotic fetal mesenchymal stem cells

There are few preclinical studies in animal models that have to use amniotic fetal derived stem cells identified in the literature [48]. Nevertheless, the specific characteristics of this source of MSCs were observed. For instance, the multipotency, the low risk of immunogenic reaction, the ease of sample processing, and the high proliferative capacity, makes amniotic fetal derived stem cells an attractive alternative for regenerative medicine [49]. These properties are supported by data observed which showed promotion of angiogenesis and support of the surrounding tissue surplus the decreased inflammatory response and apoptosis [50–52].

In a rat model of SCI, the impact of two types of MSCs: Human umbilical cord blood-derived and Human amniotic epithelial cells were assessed for the treatment of SCI-induced thermal hyperalgesia and mechanical allodynia. None of them were effective in treating the thermal hyperalgesia. Though both improved the mechanical allodynia, human amniotic epithelial stem cells were more efficacious [53].

4. Animal models in spinal cord injury

Multiple studies described the administration of MSCs to treat spinal cord injury in a variety of animal models such as rodents, primates, sheep, dogs, cats, bovine, and even humans. Rodents are the most common animal model used [54], and the most appropriate model for spinal cord injury studies [55] since large animals and non-human primates are very expensive to care, demand additional managing requirements, and have ethical implications to consider when choosing. However, the experiments of the latter approximate more to SCIs [56].

The efficacy of MSCs were also observed in cats with SCI. Improvement in the cutaneous trunci (panniculus) reflex, pain sensation, bowel, and bladder function were noted. However, no significant change in proprioception and hyperreflexia of ataxic hind limbs were observed [57]. In dogs with SCI, treatment with BMSCs also caused the same clinical improvement with no significant recovery of low proprioceptive and hyperreflexic ataxic hind limbs [58]

4.1 Stem cell delivery methods in SCI animal models

There are currently 3 different methods to deliver mesenchymal stem cells (MSCs) in animal models of spinal cord injury (SCI). These are direct implantation, intravenous (IV) infusion, and intrathecal infusion. Direct implantation refers to the injection of MSCs directly in the injured area of the spinal cord. IV infusion refers to the injection of MSCs in a major vein of the animal model (e.g. the tail vein of a mouse or rat). Lastly, intrathecal infusion refers to the injection of MSCs directly into the subarachnoid space, in the cerebrospinal fluid. The delivery methods

described in the following paragraphs concern mainly Sprague-Dawley rats. For a summary of advantages and disadvantages of delivery methods, see **Table 1**.

Direct implantation of the MSCs is done using a small syringe capable if precisely injecting cells in the damaged area [59], guaranteeing delivery to the desired site [60]. This method has been largely favored due to its high cell viability and improved survival [61], observed in higher engraftment rates in both acute and chronic SCI [59, 61, 62]. However, some authors have expressed concerns regarding the translation of this technique to clinical practice. Agglomeration of cells in the injection site, needle damage to the adjacent non-injured spinal cord, and failure of cell migration to the central parenchyma are some of the most noteworthy disadvantages of this delivery method [59–64]. Additionally, if done in humans, direct implantation of MSCs would require the patient to undergo general anesthesia and an invasive surgical procedure [61].

Since direct implantation of MSCs in humans might pose substantial risks, IV and intrathecal infusion were deemed appropriate less invasive surrogates that could potentially be clinically used. Damage to the blood-brain barrier (BBB) in SCI (particularly in traumatic SCI) allows infiltration of cells and toxic mediators that promote further neurologic damage [65]. It was initially thought that this process could improve diffusion of MSCs into the spinal cord. However, cell infusion in the first 48 hours of injury has shown conflicting evidence, with some authors reporting either the presence or absence of MSCs at the lesion site [65, 66]. Nevertheless, when present, the cell's engraftment rate with IV delivery in the spinal cord was very low in comparison to other methods [59].

Delivery method	**Advantages**	**Disadvantages**
Intralesional delivery	• Precise positioning of cells at lesion site.	• Agglomeration of cells at injection site.
	• Higher engraftment, translating into higher cell viability and increased survival.	• Needle damage to adjacent spinal cord.
	• High opportunity of differentiation	• Failure of cell migration to the central parenchyma.
		• Complicated surgical approach in animal models.
		• Would require an invasive surgical approach in humans.
Intravenous delivery	• Promotion of an anti-inflammatory environment that prevents BBB leakage and progressive damage.	• Low engraftment rates.
	• Easy administration.	• Lowest limb function recovery scores.
	• Administration of multiple doses	• Limited beneficial effects
Intrathecal delivery	• Migration of cells to different regions of the spinal cord.	• Cell attachment to other regions of the central nervous system.
	• Highest limb function recovery scores.	
		• Complicated surgical approaches in animal models.

Table 1.
Advantages and disadvantages of the three main MSC delivery methods.

The low engraftment rates in the spinal cord with IV delivery methods are considered a consequence of the cells' first pass through the systemic circulation. MSCs have been observed in the lungs and liver in the first 24-48 hours of IV infusion [66, 67], with progressive increase of cell numbers in the spleen in the following days [61, 67]. Cell engraftment in the spleen is associated with increased levels of anti-inflammatory cytokines (e.g. IL-10, TIMP-1) [67] in plasma, which is believed to decrease BBB permeability by inhibiting monocyte adhesion to the vasculature, preventing metalloproteinase release and vascular basement membrane degradation [67]. Therefore, the anti-inflammatory environment promoted by cells engrafting outside the spinal cord prevents vascular leakage at the lesion site, decreasing hemorrhage, inflammation and further damage [66, 67].

IV infusion of MSCs can be done through the femoral vein or the tail vein. In rodents, peripheral circulation is mostly accessed through the tail vein [61, 67, 68] due to its simplicity when compared to the approach required to access the femoral vein [59, 65, 66]. Additionally, inflammation around the site of injury is higher than with intrathecal administration but lower than direct injection [59]. Even though cells administered IV have low engraftment rates, animals still score better outcomes in limb function recovery scores and grip strength [67], develop less scarring [59, 68], and have higher vascularization, myelination, and axonal density than controls [68].

Intrathecal delivery of MSCs can be done via the intracisternal approach (i.e. injection into the fourth ventricle) [61, 69, 70] or by laminectomy with injection of cells through the dura [63]. When initially injected, cells occupy the whole subarachnoid space, but progressively decrease their number in this anatomical region [63]. In contrast to intralesional delivery of MSCs, intrathecally administered cells show a more extensive migration in the neural tissue, extending from the dorsal spinal cord to its center [61]. Although the number of viable cells is only second to the intralesional delivery method [61], a decrease in engrafted cells to 5% of the original cell number has been observed after 6 weeks, with some cells attaching to the pia mater [63]. Animals with intrathecally delivered MSCs obtain higher scores in limb function recovery scores when compared with intralesional and intravenous deliveries [61].

The impact of MSCs on SCI resolution can be explained by the following characteristics: immunomodulatory, anti-inflammatory, neurotrophic/neuroprotective, and angiogenetic effects. The direct impact on the regeneration of the neurons is mainly exerted by neurotrophic and neuroprotective functions. These functions are usually mediated by the secretion of neurotrophic factors.

The spinal cord injury occurs in 2 phases. The first phase occurs immediately after the trauma and is mediated by damage to the microvascular elements, cellular membrane, and the blood-spinal cord barrier. Damage to these three structures evokes series of events that give rise to axonal fragmentation, demyelination, cyst formation, and expansion and accumulation of the microglia and macrophages in and around the injury site, which leads to the secondary injury. The secondary injury is characterized by inflammation, ischemia, disruption of ion channels, free radical production, glutamatergic excitotoxicity, necrosis, axonal demyelination, and glial scar formation [30, 71].

As previously described, MSCs from different sources have been used to treat SCI. They can directly be injected to the injury site or intravenously as they have the ability to migrate to the epicenter of the injury, demonstrating their homing abilities.

Chen *et al*. reviewed 12 randomized controlled trials on rats and mice and showed that stem cell treatment improved the mechanical reflex threshold. For the mice, improvement in thermal withdrawal latency was observed. However, no

improvement was seen in rat studies [72]. In a rat spinal cord hemisection model, BMSCs were noted to promote astrocyte migration to the injury epicenter. In the group treated with a combination of the BDNF with platelet-rich plasma, more astrocyte migration, and higher rates of remyelination has been documented. This group also showed remyelination and oligodendrocytes with higher activity, while only the BMSCs group showed axonal demyelination, vacuole and whirled body formation [73].

The human ADSC have been shown to be able to convert to the oligodendrocytes and to attract oligodendrocyte precursor cells, which, in turn, mediates remyelination. Unsurprisingly, the treatment caused an improvement in the motor function of the animals with focal demyelination [74]. Differentiation of neurotrophic factors secreting cells from human ADSC to oligodendrocytes was noted in a damaged spinal cord rat model. Neurotrophic factors secreting cells promoted remyelination and increased thickness of the myelin and the diameter of the axons [75].

In a rat model of spinal cord contusion, rats treated with bone marrow-derived Schwann cells experienced better functional recovery. At the same time, the size of the cystic cavity decreased, and axonal regeneration was observed [76]. Treatment with bone-derived MSCs stimulated axonal growth in the subtotal cervical hemisection rat model. An increase in the length of the axons was observed [77]. Bone marrow mesenchymal stem cells decreased the cavity volume and increased the spared white matter, the length of the neurites, the number of axons, and the neurites in a SCI rat model [78]. Rats with a moderate contusion model of spinal cord injury showed better recovery in 2 behavioral tests (Bresnahan Locomotor Rating Scale [79] and exploratory rearing [80]). All rats experienced a decrease in the size of the cyst cavity and more axons in the injury site either through an increase in spared axons or through axon regeneration [80]. In a complete spinal cord transection rat model, human umbilical mesenchymal stem cells promoted axonal regeneration, and more neurofilament-positive fibers around the epicenter of the corticospinal tract injury was observed. Additionally, proximal and dorsal to the injury site, fewer microglia and astrocytes with reactive features were found [81].

Some studies suggest that the MSCs do not have the ability to convert to neural cells. As an example, Quertainmont et al. could not detect the stem cells after grafting. The authors described tissue sparing, vascularization in the injury epicenter, but no evidence of axonal regrowth [37].

5. Use of mesenchymal stem cells in spinal cord injury in the clinical setting

The use of MSCs in the clinic has been studied thoroughly; however, results among studies are not consistent. For example, 5 patients with complete spinal cord injury were treated with autologous bone marrow cells and granulocyte macrophage-colony stimulating factor, and 4 of them showed neurologic (sensory or motor) improvement [82]. There are controversial evidences for both MSC's ability to cause and treat neuropathic pain. In one study, 9 people with SCI got treated with MSCs, and 8 of them experienced a reduction or resolution of the neuropathic pain. Improvement in peripheral nerve conduction, motor power, and sensitivity was noted. Enhancement of the voluntary muscle contraction was explained with active muscle reinnervation [83]. All 14 patients with chronic traumatic complete SCI treated with BMSCs felt an improvement in sensitivity to light touch and pinprick. The majority also experienced sacral sparing, and improvement in urologic and motor function [84].

Treatment with MSCs of 6 traumatic syringomyelia patients with paraplegia reduced the size of the syrinx. Additionally, the neuropathic pain either resolved or decreased [85]. In one case report, the patient was treated with MSCs and G-CSF, and his motor function, tactile and pain sensation were improved after the treatment. However, the patient still reported neuropathic pain, etiology of which was unknown [86].

6. Limitations in mesenchymal stem cell delivery

MSC type, dosage, delivery method, and timing of therapy are current obstacles in spinal cord injury cell therapy that limit its translation to clinical practice. Bone marrow stromal cells, bone marrow mononuclear cells, and neural stem/progenitor cells are just some examples of the cell types currently under study for this purpose, with no clear best candidate yet. Cell dosage needs further research since the appropriate therapeutic dose is still unclear. Dosage standardization is required to build a homogenized body of literature that allows for an appropriate comparison of delivery methods. The most appropriate delivery method is still debated since all poses unique benefits. Innovative methods such as subpial delivery and stem cell-derived nanovesicle are promising new alternatives that will be added to the battery of delivery techniques [87, 88].

7. Conclusions

Mesenchymal stem cell therapy has shown promising results in spinal cord injury repair. Animal studies and clinical trials performed in human patients using mesenchymal stem cells suggested that this therapy can be a promising candidate for patients suffering from spinal cord injury. MSCs derived from different sources offer potential healing in different spinal cord injury conditions.

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Conflict of interest

The authors declare no conflict of interest.

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