IJAER/ May-June 2023/Volume-12/Issue-3 ISSN: 2278-9677
International Journal of Arts & Education Research

MESENCHYMALSTEM CELLS FOR SPINAL CORD REGENERATION

Dr. Dhankesh Meena

Assistant professor, scrs government college

Sawai Madhopur (Rajasthan)

ABSTRACT

The process of spinal cord regeneration (SCR) has a significant influence on the physical, mental, and financial well-being of these patients. There is a correlation between secondary SCR and inflammation, as well as the destruction of blood vessels and the ensuing permanent dysfunction of the neurological system. Mesenchymal stem cells, also known as MSCs, are a hopeful approach to the therapy of systemic chorionic reticulum (SCR) since they possess anti-inflammatory qualities, promote vascular regeneration, and have the ability to produce neuronutrients. It has been demonstrated through preclinical research that MSCs facilitate the recovery of sensory and motor function in rats. During clinical trials, it has been claimed that mesenchymal stem cells (MSCs) have improved sensory and motor scores as measured by the American Spinal Injury Association (ASIA). In spite of this, there is still debate regarding whether or not MSCs are effective in treating patients who have SCR. The survival of mesenchymal stem cells (MSCs) in the harsh environment of spinal cord injuries (SCI) is a challenge. MSCs foster the development of tumors. Within the scope of this paper, we investigate the evidence concerning the pathophysiological alterations that take place following SCR. Following that, we briefly discuss the possible applications of mesenchymal stem cells (MSCs) in therapeutic settings and examine the mechanisms that underlie the use of MSCs in the treatment of SCR. In conclusion, we provide an overview of the difficulties that are associated with the utilization of MSCs in the therapy of SCR and address potential future applications.

Keywords : *Spinal cord, Mesenchymalstem cells.*

INTRODUCTION

Spinal cord regeneration (SCR) is one of the most severe sorts of impairments. It has a significant impact on the patient's life and leads the individual to face a wide range of limitations because of the detrimental effects it has on the patient's ability to control their motor functions. When compared to the rates of non-developed countries, which ranged from 13 to 220 per million people, the incidence rates of developed countries ranged from 13 to 163 per million people, according to the figures. In addition, the prevalence of SCR ranges from 490 to 526 per million population in industrialized countries, while the stated prevalence for nondeveloped countries is approximately 440.3) Having said that, the expense of providing care for an SCR is also rather substantial. As an illustration, it is projected that spinal cord injuries cost more than $9 billion annually in the United States. Additionally, the cost of treating a patient with tetraplegia is anticipated to be over $900,000 in the first year and $170,000 over the subsequent years.4. As a result of the Iraq-Iran conflict, there are over 2,000 veterans in Iran who have suffered from spinal cord injuries, and more than 15,000 cases of spinal cord injuries have been diagnosed up until the present

day. The research indicates that the most prevalent causes of spinal cord regeneration include injuries sustained while driving or participating in sports, injuries sustained as a result of violent acts or war, injuries sustained from falling from a height, certain disorders, and medical occurrences. The loss of whole or partial movement ability (such as walking) and sensation in the afflicted area are examples of complications that can arise as a result of spinal cord injuries. The intensity of the complications will increase in proportion to the amount of upper body activity that is involved. Unfortunately, spinal cord axons that have been damaged have the capacity to repair themselves; however, the maximum amount of axon growth that can occur is approximately one millimeter, and this restoration process is not occurring at a level that is sufficient. On the other hand, the glial scars formation and the lack of growth boosting symptoms such as semaphorin and ephrin and on the contrary the existence of inhibition signals such myelin sheath components (myelinassociated glycoprotein, Nogo-A) are viewed as inhibitory elements of axon growth. It is for this reason that several efforts have been done in order to improve the effects of the damages stated, taking into consideration the magnitude and severity of the disabilities that are brought about by spinal cord injuries as well as the rising incidence of these injuries. It is dependent on the nature and extent of the damage to determine whether or not the injury may be effectively treated; nevertheless, the high expenses of therapy can have an impact on the therapeutic process. One of the answers that has been widely discussed over the course of the past two decades is the utilization of cell therapy. This is mostly owing to the rapid expansion of regenerative medicine, which is founded on tissue engineering and stem cell transplantation.

OBJECTIVES

1. To study Mesenchymalstem cells.
2. To study spinal cord regeneration.

Mesenchymal cells

It is possible for these cells to regenerate and develop into mesoderm-derived cell types such as myocytes, chondrocytes, osteoblasts, and adipocytes. They are multipotent cells having the ability to do so. Multipotent stem cells (MSCs) that have been isolated from various tissues exhibit a variety of cell surface markers that can be utilized for a variety of therapeutic applications. Their differentiation and neural regeneration capacity are primarily responsible for the fact that they are currently the primary focus of study and have demonstrated the most promising outcomes in cell therapy for the treatment of traumatic spinal cord injuries (TSCI). (1) antiapoptotic properties: stem cells have the ability to inhibit programmed cell death because they are able to secrete a variety of factors and molecules that promote cell survival and prevent cell death. (2) anti-inflammatory properties: stem cells have the ability to secrete anti-inflammatory cytokines and other molecules that inhibit the activation and proliferation of immune cells, which allows them to regulate the immune response and prevent immune cells from becoming activated and multiplying. (3) angiogenic properties: because they have the ability to release proangiogenic substances, which stimulate the proliferation of endotelial cells, they encourage the development of new blood vessels. The use of mesenchymal stem cells (MSCs) in stem cells as a treatment for traumatic spinal cord injuries (TSCI) is justified for a number of reasons. Their capacity to sustain viability after cryopreservation, their great potential for multilineage differentiation, and their low or nonexistent immunogenicity following allogeneic transplantation are all characteristics that make them simple to

separate. Additionally, they are able to migrate to the location of the injury, which gives them the ability to have homing capabilities. Because of their seclusion, patients are required to undergo bone marrow aspiration while under the influence of local anesthetic. This is a drawback. Bone marrow, adipose tissue, cord blood, and placenta are all potential sources of these compounds; nevertheless, there are significant therapeutic distinctions across these four sources. Accordingly, the ones that have been derived from bone marrow, the umbilical cord, and adipose tissue have been the ones that have been used the most frequently and have demonstrated the most value in the treatment of TSCI. Included in these cells are

Spinal cord

One of the components that make up the central nervous system is the spinal cord. It is a lengthy structure that resembles a pipe and originates from the medulla oblongata, which is a region of the brain that is composed of a collection of nerve fibers. It travels through the vertebral column of the backbone. This structure is split by a pair of roots, known as the dorsal and ventral roots, which are composed of nerve fibers that connect together to form the spinal nerves.

Research Methodology

Both the Institutional Review Board (Approval No. 7022 dated 16.12.2009) and the Institutional Animal Ethics Committee (IAEC) gave their approval to the experiments that were carried out. Furthermore, these experiments were carried out in accordance with the guidelines established by the Committee for the purpose of Control and Supervision of Experimentation of Animals (CPCSEA), which is established by the Government of India.

Bone marrow mesenchymal stem cells (MSC)

Collection of Bone marrow

Following the administration of an overdose of ketamine and xylazine through intraperitoneal injection with a 31-gauge needle, a male Albino Wistar rat was weighed and then put under anesthesia. After the muscles were removed, the femur was exposed by disarticulating the hind limb at the hip joint and then clearing the muscles. A dissection was performed on the tibia and femur of a rat. After that, incisions were made at the knee joint in order to separate the femur from the other bones in the leg. Using bone rongeurs, the ends of the femur and tibia bones were opened up in order to expose the chamber that contains the marrow. Following the insertion of a syringe that contained two milliliters of phosphate buffered saline (PBS) into the narrow cavity, the bone marrow was collected in a test tube by flushing the marrow cavity with phosphate buffer saline. After that, the suspension of bone marrow was sent to the culture lab in order to undergo processing. the procedures that were carried out as outlined below

Isolation of MSC

A Rosette Sep antibody cocktail, manufactured by Stemcell Technologies Inc., was utilized in order to differentiate the mesenchymal stromal cells from the hematopoietic cells. After incubating the bone marrow cell suspension with the Rosette Sep mixture for twenty minutes at room temperature, the results were recorded. This concoction creates immunological rosettes by generating cross-links between cells in the bone marrow that are undesirable. This results in a rise in the density of the undesired cells, which are

rosetted, to the point that they pellet together with the liberated RBCs when the cells are centrifuged at 1200g for twenty minutes over a buoyant density medium such as Ficoll-Paque (GE Healthcare). At the interface (buffy coat) between the buoyant density medium and the plasma, the desired cells that are not attached to the antibody were easily collected as a highly enriched population. This was accomplished with relative ease.

Culture of MSC

The cell culture media consisted of Dulbecco's Modified Eagle media (DMEM) (Gibco), supplemented with 20% fetal bovine serum (Gibco), 2mM L-glutamine (GibcoInvitrogen), 100U/mL penicillin, 100µg/ml streptomycin and 25ng/ml of AmphotericinB. Trypsinization and passage to the second passage are performed on the cells once they have reached a confluency level of 80–90%. Immunostaining, flow cytometry, and patch-clamp characterization were all performed on cells that were obtained from the second passage.

Characterization of MSC by IHC

At a cell density of 8000 cells per square centimeter, second passage cells were grown on a round coverslip of 12 millimeters in diameter. Paraformaldehyde at a concentration of 4% was used to fix the cells for fifteen minutes at room temperature. The cells were then washed three times with PBS (Invitrogen, Gibco) to remove any remaining debris. Both blocking and permeabilization were performed in a solution containing 0.1% Triton X-100 in 2% goat serum and 2% bovine serum albumin (BSA). The cells were subjected to an overnight incubation with primary antibody at a temperature of 4 degrees Celsius. Following the washing of the cells with PBS, secondary antibodies were allowed to incubate for a period of two hours at room temperature. They were then mounted with DAPI (Vectashield mounting media with DAPI) after being cleaned and mounted first. It was immediately followed by the transfer of coverslips to glass slides, which were then studied using a fluorescence microscope. The following markers were utilized: CD54, CD90, CD73, CD29, and CD105 representing mesenchymal stem cells; CD45, CD34, and CD14 representing haematopoietic cells; and NeuN, Neurofilament, and MAP2 representing neuronal cells.

Characterization of MSC by flow cytometry

After being trypsinized, the cells from the second passage were washed with PBS. Each antibody was produced using between two and five lakh cells, which were placed in a separate test tube. Incubation of 5 to 10 microliters of primary antibody on ice for a duration of 20 minutes. Those antibodies that were not bound were washed with PBS and then removed. The secondary antibody, which is fluorescently tagged and consists of 5 to 10 microliters, was incubated for a duration of twenty minutes. After that, the secondary antibody that was unbound was rinsed with PBS. In order to determine the presence of mesenchymal stem cell markers (CD54, CD90, CD73, CD29, and CD105) and haematopoietic markers (CD45, CD34, and CD14), the cell solution was aspirated and then utilized for flow cytometry analysis.

Neuronal induction of MSC

Subsequent quotation Neuronal induction media, which included DMEM/F12, 2% FBS, B27 supplement, 20mM Retinoic acid, and 12.5ng/ml bFGF, was utilized in order to stimulate mesenchymal stem cells

(MSC) to differentiate into neuronal cells.(189) For a period of twelve days, the cells were kept suspended in neuronal induction media. These cells were stained for neural marker after a period of twelve days.

Characterization of neuronal induced cells

These cells were stained for neural marker after a period of twelve days. by using the immunohistochemical approach as stated above (Beta III tubulin, MAP2, NeuN, Neurofilament, O4, and Nav1.1).

List of primary and secondary antibodies used:

CD54-FITC: Mouse anti-rat CD54-FITC(1:50); BD Pharmingen.

CD29: Monoclonal mouse anti-beta 1 integrin(1:50); Millipore.

Sec: Goat anti-mouse IgG2b-RPE (1:50); Southern Biotech.

CD90: Monoclonal mouse anti-rat CD90-FITC conjugated(1:100); Millipore

CD73: Monoclonal mouse anti-rat CD73 (1:50); BD Pharmingen.

Sec: Goat anti-mouse IgG1-PE conjugated (1:50); Southern Biotech.

CD105: Goat polyclonal IgG (1:25); Santa cruz Biotechnology.

Sec:Donkey anti-goat IgG-perCp conjugated (1:100).

Mouse monoclonal IgG1 anti-CD34-FITC conjugated (1:100); santa cruz Biotechnology

Mouse monoclonal anti-rat CD45-PE conjugated (1:100); BD Pharmingen.

CD14(1:50); Goat anti-rabbit IgG-RPE(1:50); Jackson Immunoresearch)

Mouse monoclonal anti-BetaIII tubulin(1:50); Millipore.

Sec: Goat anti-mouse Rhodamine(1:50); Millipore.

Mouse monoclonal anti-MAP2 IgG1(1:100);Millipore.

Sec:Goat anti-mouse IgG1-FITC (1:50).

Mouse monoclonal anti-NeuN IgG1(1:50); Millipore.

Sec: Goat anti-mouse IgG1-FITC(1:50)

Mouse monoclonal anti-Neurofilament IgG1(1:100); Millipore.

Sec: Goat anti-mouse IgG1-FITC(1:50).

Mouse monoclonal IgG1 anti-Glial fibrillary acid protein (GFAP) Alexa Fluor 488 conjugated(1:50); eBioscience.

Mouse monoclonal IgM anti-Oligodendrocyte(O4) (1:50); Sigma.

Sec:Donkey anti-mouse IgM-CY3 conjugate (1:50): Jackson Immunoresearch.

Rabbit polyclonal anti-Nav1.1 (1:100); alomone labs.

Sec: Goat anti-rabbit IgG-PE (1:50).

Mouse IgG2A Neuron specific β-III tubulin PerCp conjugated (1:100); R&D systems.

Result and Discussion

Figure 1 depicts the nasal septum of a rat, which reveals the sites of the olfactory mucosa (OM) and the respiratory mucosa (RM). The bony septum is shown by the semicircular line, which is the location where the olfactory mucosa was segregated from the posterior section of the septum. In front of the olfactory mucosa is where you'll find the respiratory mucosa.

According to the illustration in (b), the olfactory mucosa can be found on either side of the nasal septum (NS). The nerve fascicle (NF) is visible in the lamina propria, and the olfactory epithelium (E) can be found on its periphery nearby. The lamina propria is rich in olfactory ensheathing cells and olfactory nerve fibroblast, whereas the olfactory epithelium is composed of HBC, GBC, and immature neurons, which are responsible for the process of neurogenesis.

In the lamina propria, there is a single nerve fascicle that contains the nuclei of olfactory ensheathing cells, olfactory receptor neurons, and olfactory nerve fibroblast. The olfactory ensheathing cells (OEC) that wrap the bundle of olfactory sensory axons are visible under electron microscopy of the lamina propria. Additionally, the olfactory nerve fibroblast (ONF) is located in the periphery of the nerve fascicle. An OEC is a cell that has been flattened and bent, with an outside surface that is connected by basal lamina and an inner surface that encloses a large number of axons surrounding it.

Both olfactory ensheathing cells (OEC) and olfactory nerve fibroblast (ONF) were characterized by immunohistochemical analysis using p75NTR-FITC and Fibronectin-PE, respectively. The culture of olfactory ensheathing cells exhibited expression of S100β, which is a marker for Schwann cells, and GFAP, which is a marker for astrocytes. However, the culture of olfactory ensheathing cells showed a negative result for Galc, which is a marker for oligodendrocytes (Figure 2). In the flow cytometry investigation, the P75NTR-FITC demonstrates a positive result of 40% for OEC and a positive result of 35% for anti-fibronectin.

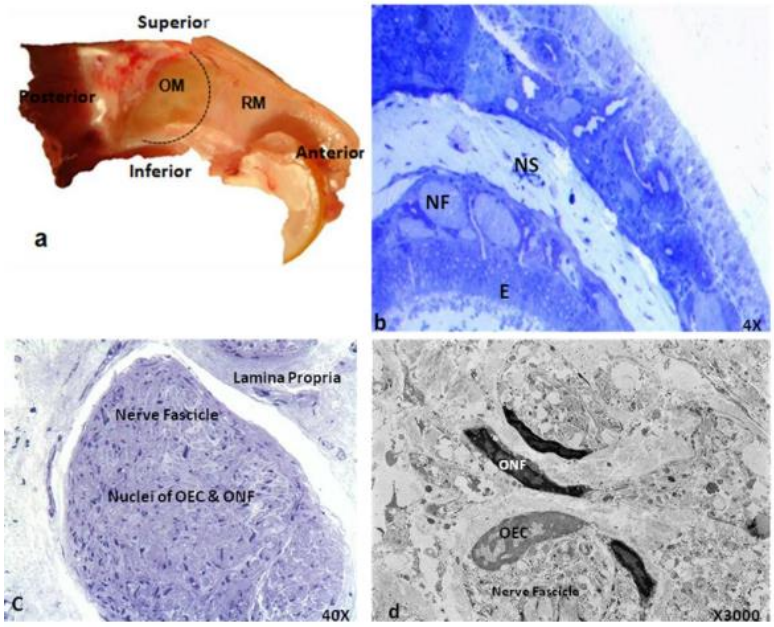

Figure 1 Olfactory mucosa – light and electron microscopic features

a. The olfactory mucosa is seen in the rat septum, which is shown in the first image. It is important to take note of the mucosa's yellow hue as well as the line that separates the respiratory mucosa (RM) from the olfactory mucosa (OM).

b. semi-thin segment of the rat olfactory mucosa stained with toluidine blue is shown here and below. olfactory nerves are visible in the nasal septum (NS) and the olfactory mucosa shown here.

c. single nerve fascicle demonstrates the presence of nuclei of olfactory ensheathing cells (OEC) and olfactory nerve fibroblasts (ONF).

d. EM demonstrating the presence of nerve fascicles (OEC and ONF)

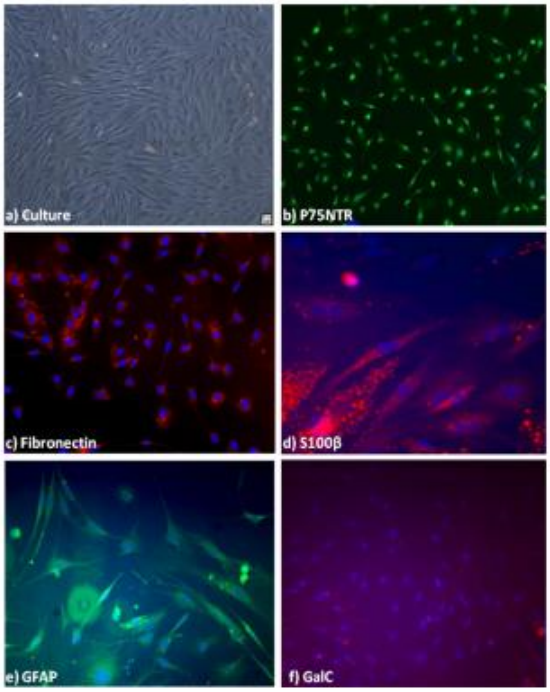

Figure 2 Immunocytochemical characterization of olfactory ensheathing cells and olfactory nerve fibroblasts.

a. A phase contrast picture of OEC and ONF in culture.

b. A photo of OEC showing it stained with P75NTR-FITC. Cells have a favorable response to the marker.

c. anti-fibronectin-PE staining of the oncotic nuclei (ONF). Cells have a favorable response to the marker.

d. S100β-PE was used to stain the OEC image. OEC labeled with GFAP-Alexa Fluor 488 reveals that the cells display a positive response to the marker

e. OEC stained with Galc-PE reveals that the cells have a positive response to the marker

f. When the marker is present, cells are negative.

Conclusions

Patients who have had catastrophic spinal cord injuries can experience neurological improvement through the use of mesenchymal stem cell treatment. There were reported to be positive changes in the AIS grade as well as the ASIA sensory and motor scores in different patients. In addition, it was established that this therapy appears to have the potential to be safe in both the short and medium term.Due to the lack of scientific evidence and the absence of a broad recommendation for their application, it is still unclear what the clinical and functional relevance of these advancements will be, as well as whether or not they will be safe over the long term.Based on this research, it is possible to draw the conclusion that stem cell therapy is the treatment that is believed to be the most promising for traumatic spinal cord injury, and it is also the

treatment that is the primary focus of all efforts. For this reason, there is a need for more multicenter, randomized, and controlled trials with larger numbers of patients. Additionally, there is a need for the standardization of complementary assessment tests and their parameters, such as new injury biomarkers, in order to evaluate the potential benefits of this treatment in a manner that is more objective and consistent, which will allow us to draw conclusive conclusions. Nevertheless, there are a number of variables that continue to be substantial obstacles for multicenter, randomized, and controlled trials with greater numbers of patients. These limitations include, among other things, limited patient recruitment as a result of the low incidence of TSCI, stem cell production, and financing.

References

1. Kooijmans, H.; Post, M.W.M.; Stam, H.J.; van der Woude, L.H.V.; Spijkerman, D.C.M.; Snoek, G.J.; Bongers-Janssen, H.M.H.; van Koppenhagen, C.F.; Twisk, J.W.; ALLRISC Group; et al. Effectiveness of a Self-Management Intervention to Promote an Active Lifestyle in Persons with Long-Term Spinal Cord Injury: The HABITS Randomized Clinical Trial. Neurorehabilit. Neural Repair 2017, 31, 991–1004.

2. Montoto-Marqués, A.; Ferreiro-Velasco, M.E.; Salvador-De La Barrera, S.; Balboa-Barreiro, V.; Rodriguez-Sotillo, A.; Meijide-Failde, R. Epidemiology of traumatic spinal cord injury in Galicia, Spain: Trends over a 20-year period. Spinal Cord 2017, 55, 588–594.

3. Galeiras Vázquez, R.; Ferreiro Velasco, M.E.; Mourelo Fariña, M.; Montoto Marqués, A.; Salvador de la Barrera, S. Actualización en lesión medular aguda postraumática: Parte 1. Med. Intensiva 2017, 41, 237–247.

4. Gaitán Pérez, N.; Montoto Marqués, A. Disfunción Vesical e Intestinal en La Lesión Medular; Méderic Ediciones, S.L.: Barcelona, Spain, 2019; pp. 1–25.

5. Ahuja, C.S.; Wilson, J.R.; Nori, S.; Kotter, M.R.N.; Druschel, C.; Curt, A.; Fehlings, M.G. Traumatic spinal cord injury. Nat. Rev. Dis. Primers 2017, 3, 17018.

6. Venkatesh, K.; Ghosh, S.K.; Mullick, M.; Manivasagam, G.; Sen, D. Spinal cord injury: Pathophysiology, treatment strategies, associated challenges, and future implications. Cell Tissue Res. 2019, 377, 125–151.

7. Padilla-Zambrano, H.; Ramos-Villegas, Y.; Raphael Alvis-Miranda, H.; Joaquin, A.F.; Rafael Moscote-Salazar, L.; Rafael Moscote, L. Fisiopatología del trauma raquimedular. Pathophysiology of spinal trauma. Rev. Mex. Neurocienc. 2017, 18, 46–53.

8. Muthu, S.; Jeyaraman, M.; Gulati, A.; Arora, A. Current evidence on mesenchymal stem cell therapy for traumatic spinal cord injury: Systematic review and meta-analysis. Cytotherapy 2020, 23, 186–197.

9. Ahuja, C.S.; Mothe, A.; Khazaei, M.; Badhiwala, J.H.; Gilbert, E.A.; van der Kooy, D.; Morshead, C.M.; Tator, C.; Fehlings, M.G. The leading edge: Emerging neuroprotective and

neuroregenerative cell-based therapies for spinal cord injury. Stem Cells Transl. Med. 2020, 9, 1509–1530.

10. Eckert, M.J.; Martin, M.J. Trauma: Spinal Cord Injury. Surg. Clin. N. Am. 2017, 97, 1031–1045.
11. Galvagno, S.M.; Nahmias, J.T.; Young, D.A. Advanced Trauma Life Support® Update 2019: Management and Applications for Adults and Special Populations. Anesthesiol. Clin. 2019, 37, 13–32.
12. Kreinest, M.; Gliwitzky, B.; Schüler, S.; Grützner, P.A.; Münzberg, M. Development of a new Emergency Medicine Spinal Immobilization Protocol for trauma patients and a test of applicability by German emergency care providers. Scand. J. Trauma Resusc. Emerg. Med. 2016, 24, 71.
13. American Spinal Injury Association. International Standards for Neurological Classification of Spinal Cord Injury. Richmond (VA): ASIA. 2019.
14. Kirshblum, S.; Snider, B.; Rupp, R.; Read, M.S. International Standards Committee of ASIA and ISCoS. Updates of the International Standards for Neurologic Classification of Spinal Cord Injury: 2015 and 2019. Phys. Med. Rehabil. Clin. N. Am. 2020, 31, 319–330.
15. El Tecle, N.E.; Dahdaleh, N.S.; Bydon, M.; Ray, W.Z.; Torner, J.C.; Hitchon, P.W. The natural history of complete spinal cord injury: A pooled analysis of 1162 patients and a meta-analysis of modern data. J. Neurosurg. Spine 2018, 28, 436–443.
16. Montoto Marqués, A. Lesión Medular Traumática en Galicia (1995–2014): Estudio de la Evolución Epidemiológica y Resultados Clínico-Funcionales. Ph.D. Thesis, Universidade da Coruña, A Coruña, Spain, 2018.
17. Griffin, J.M.; Bradke, F. Therapeutic repair for spinal cord injury: Combinatory approaches to address a multifaceted problem. EMBO Mol. Med. 2020, 12, e11505.
18. Roquilly, A.; Vigué, B.; Boutonnet, M.; Bouzat, P.; Buffenoir, K.; Cesareo, E.; Chauvin, A.; Court, C.; Cook, F.; de Crouy, A.C.; et al. French recommendations for the management of patients with spinal cord injury or at risk of spinal cord injury. Anaesth. Crit. Care Pain Med. 2020, 39, 279–289.