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Systematic Review of The Effectiveness Of Using IPSC In Spinal Cord Injury

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Abstract

Background: Damage to the spinal cord is the pathological condition known as spinal cord injury (SCI) or spinal cord injury. This ailment, which not only causes neurological impairments but also places a significant psychological and social burden on patients, has grown to be one of the most challenging worldwide health issues. A successful stem cell-based treatment has just been created and could be the answer to this medical issue. It has been demonstrated that iPSC-dNSC stem cells are efficient at both reducing post-traumatic inflammatory conditions and kicking off neuronal cell regeneration at the location of SCI lesions.
Methods: The aim of this study to investigate the the effectiveness of using iPSC in spinal cord injury. This study used the literature review method by discovering articles using the search engine Google Scholar, and PubMed. According to the search results, 413 articles were obtained in accordance with the title of the study, but 8 articles met the inclusion criteria in this study.
Result: The findings of this study showed that the iPSC methodology was applied, as well as the advantages and results of the procedure. Using iPSCs to treat SCI is still challenging and needs additional investigation.
Discussion: Several method developments in the use of iPSCs produce varying degrees of difficulty and benefits, such as increasing the ability of SCI to regenerate through the use of Engineered iPSCs using the 3D Neuronal Networks method, Electroactive Scaffolds with the biomaterial encapsulation of NSCs method, pluripotent stem cell transplantation in acute thoracic spinal cord injury with the administration of LCTOPC, and combination the methods

Keyword: Effectivity, iPSC, Spinal Cord Injury

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Introduction

The majority of spinal cord injuries (SCI) in people are contusions that, after an initial traumatic event, cause the vertebral column to dislocate. White matter around the central cavitation created by these wounds is diffuse and spared. The "primary" and "secondary" phases of SCI are divided into two parts. SCI entails a lot of interaction between different immunological cells, CNS cells that are already there, and noncellular elements in order to mount inflammatory, immune, and scar tissue responses that result in extensive tissue destruction, cyst formation, scar tissue formation, Wallerian degeneration, and Schwannosis (such as adhesion molecules, cytokines, and chemokines) [1]. The majority of data on the pathophysiology of SCI has come from experimental SCI in animal models and stereotyped responses to traumatic brain damage. Even though experimental SCI has been the subject of a plethora of research, nothing is known about how human SCI develops. There is evidence that although there may be fundamental similarities, there may also be variations that are fundamental, such as the size and significance of astrocytic responses and demyelination. [2].

Cellular transplantation strategies include replacing lost endogenous neuronal and/or glial cells, providing a better growth environment to obstruct or neutralize inhibitory molecules, and enhancing and directing any intrinsic neuronal regenerative capacity. These strategies aim to address the pathophysiology of SCI. [3,4,5,6]. (Fig. 1). In experimental SCI, mesenchymal stem cells, glial progenitors, olfactory ensheathing glial cells, and Schwann cells have historically been employed. Each of these cell types has the capacity to change how they look or behave. [7].

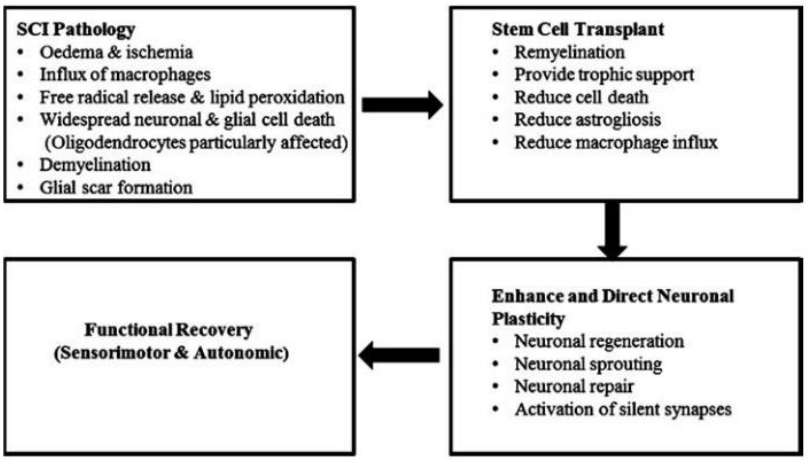

Figure 1 Stem cell transplantation for SCI. The pathophysiology of SCI is intended to be lessened by stem cell transplantation by restoring lost native cells and functions. The local milieu and microenvironment are also impacted, which improves neuronal plasticity and promotes functional recovery.

The delayed loss of myelinating oligodendrocytes is a different problem that might benefit from cellular transplantation. By replacing myelinating oligodendrocytes, damage can be contained and function can be restored. Donor oligodendrocyte precursor cells (OPCs) or a combination of neural stem/precursor cell transplants can do this. OPC transplantation has been

found to have a specific influence on the maturation of OPCs into mature oligodendrocytes, which can increase myelination by up to 50% and is connected to enhancements in locomotor performance. [8,9,10,11,12,13,14,15]

On the other hand, it is challenging to create significant quantities of pure human OPCs or brain stem/progenitor cells (which can give rise to OPCs). In order to obtain large quantities of donor OPCs that are challenging to obtain from adult stem cell sources, it is advantageous to use embryonic stem cell (ESC) lines that can differentiate into neural or glial lineages (16). Donors and recipients must, however, be immunologically compatible because embryonic cell lines are not genetically identical to the patient, and adequate immunosuppressive drugs, which are known to have adverse long-term effects, must be employed. [17]

Additionally, there is a moral controversy surrounding the usage of ESCs [18,19,20,21]. Cell fusions and somatic cell nuclear transfer (SCNT) are other sources of pluripotent stem cells [22,23,24,25,26,27]; These procedures, however, create ethical concerns because they are technically challenging, inefficient, expensive, and reliant on donor oocytes. Additionally, mitochondrial DNA is still of maternal origin even though they can produce cells with cloned nuclear DNA, which may be enough to cause immunological rejection [28].

The discovery that somatic adult cells may be dedifferentiated (or "reprogrammed") back into an ESC-like form has been a crucial turning point in clinical regenerative research. [29,30]. Simple, non-invasive approaches can be used to create human iPSCs, which can then be used as an autograft to produce OPCs or other desired cells from adult tissues. This is anticipated to reduce host immunological reactivity. iPSCs provide potential remedies for these problems. [31,32,33]. Recent research has shown that iPSCs can consistently differentiate into multiple neural lineages. iPSC technology offers a promising new alternative to cell-based therapy for several CNS illnesses, including SCI. Although there have been ethical debates about iPSCs, [34,35,36,37], The use of autologous transplants, which may reduce rejection complications, and moral issues that are inextricably linked to the use of human ESCs are among the solutions provided by iPSC technology.

Element "reprogramming" is used to make iPSCs in order to preserve pluripotency and self-renewal [38,39,40,41,42,43,44]. Using octamer-binding transcription factor 3/4, mouse embryonic fibroblasts (MEFs) were used to produce the first iPSCs. (oct3/4), region that determines sex Krüppel-like factor (klf-4), Y-box 2 (sox2), and c-myc [29]. Due to their inability to create living chimeric mice, which would have served as a real indication of pluripotency, the initial attempts created iPSCs that were only partially reprogrammed in comparison to the current norm. The field established protocols with various reprogramming variables quite fast and soon enhanced them (importantly, the oncogene c-myc was shown to be dispensable) [45,46], iPSC clone selection procedures (improved selection markers like nanog and oct3/4 [47,48,49] as opposed to F-box protein 15 (fbx15) [29] or no selection at all [50] started to make premium completely pluripotent. Rats are one of the many animals with whom this has now been accomplished [51,52], humans [53,30], pigs [54,55,56], sheep [57], horses [58], non-human

primates [59,60,61], and threatened animals [62].

Patients with the horrible neurological condition SCI, which results in the loss of both sensory and motor functions, are severely physically limited. Therapeutic interventions that partially restore function can greatly enhance patients' quality of life. Unfortunately, there are no therapeutic approaches available today that allow for a significant functional recovery after SCI. Human iPSC-based transplantation therapy may provide patients with SCI new hope. [63–65]. In fact, it has been shown that hiPSC-derived NSCs or NPCs continue to survive and grow into neurons and glial cells in the injured spinal cord following transplantation. [66–68]. This study aimed to investigate the the effectiveness of using iPSC in spinal cord injury.

Methods

The free papers on the internet were examined as part of the systematic review of the literature on iPSC in spinal cord injury. The recommended reporting items for systematic review and meta-analysis statement (PRISMA) 2020 standards were followed for reporting the findings of these research.

Inclusion and exclusion: Hafiz Ramadhan reviewed identified citations' titles and abstracts for potential inclusion in the review and searched out the full texts of any pertinent works. The search's inclusion criteria were freely available, published articles and electronic articles from January 2021 to August 2022 about the use of iPSCs in spinal cord injury research worldwide. These comprised publications from original research and reviews (systematic review or narrative review). Studies published before 2021 and works written in languages other than English were disqualified.

Study selection: Two steps of article selection were completed. Based on the inclusion criteria and search phrases, the titles and abstracts of all resources were initially evaluated. The content of the chosen titles and abstracts was then reviewed for potential answers to the review questions. The researcher eliminated irrelevant abstracts before retrieving the complete texts of the chosen abstracts. In the second stage, whole articles were evaluated to find components pertinent to the review's goals. Similar to the first stage, the whole articles were examined to ensure that they satisfied the review's goals. As indicated in Figure 1, the PRISMA flow diagram was used to pick the articles.

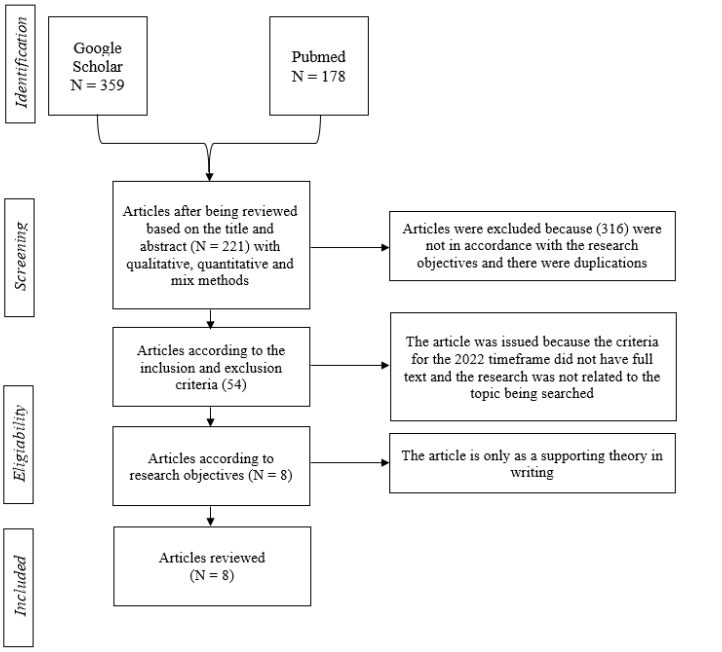

Figure 2. PRISMA screening

Search strategy and information sources: To find papers on iPSC in spinal cord injury that had been published during the previous year, a through search was first undertaken to find primary studies, reviews, and grey literature. In order to refresh the literature review before the final analysis and writing were completed, this period was extended until August 2022. In order to conduct the search, various electronic databases were used (PubMed, and Google Scholar). Based on search terms, the search strategy was created. During the literature search, the keywords (iPSC, Spinal, Cord, Injury) and associated terms were combined using boolean operators (or, and).

Data extraction: Articles were disqualified if they weren't pertinent, omitted information about iPSC in spinal cord injury and the review's goals, or had publication dates outside of January 2021–August 2022. Then, pertinent publications were evaluated in order to respond to the review questions. The following study characteristics are taken directly from publications: author name, study title, year of study, method of iPSC used, kelebihan, and results obtained from the study in question. The search results were controlled, and the complete articles' collected data was logged in Microsoft Word.

Quality appraisal: All openly available qualitative and quantitative studies that were released during the search period were evaluated for quality. Studies that were included were evaluated for relevance. The quality assessment did not result in the removal of any studies.

Data analysis: Variables related to the year of publication, the number of studies, the complications represented in the study, the scientific article, and the case report or series they were grouped into were subjected to descriptive analysis.

Results and Discussion

Eight studies in all will be examined. You can find some information in Table 1 including the author, the title, the iPSC technique utilized, the benefits of the method employed, and the outcomes of the iPSC method used in the research.

Table 1. Studies examining methods of iPSC

Year	Methods	Advantages	Results
2022	Administration of C5a Receptor Antagonist	When SCI is in its acute phase, the injection of C5aRA inhibits the inflammatory response. This positive effect increased the survival rates of transplanted hiPSC-NS/PCs and improved the restoration of motor function.	Following SCI, C5aRA treatment significantly decreased inflammatory cells and various inflammatory cytokines, including IL-1b, IL-6, and TNF.
2022	3D Neuronal Networks	Enginereed iPSCs are remarkably capable of regeneration.	The sensorimotor functional studies determined that these findings translated into a much greater level of behavioral functional recovery.
2022	Purification of iPSCs using Nestin+ Cells by Fluorescence Activated Cell Sorting (FACS)	The availability of a diversity of somatic cells without any ethical concerns regarding the use of embryos, the great potential to produce isografts without immunosuppression, and the extensive differentiation	The grooming task and the horizontal ladder test were used to evaluate the forelimb locomotor activity, and the results showed that the iPSC-NPCs were able to survive, differentiate into both neurons and astrocytes, and, importantly, increase forelimb
2022	Biomaterial Encapsulation of NSCs	When combined with ES, cells grown on electroactive scaffolds are regularly found to have significantly improved regenerative properties, which may boost the effectiveness of cell treatment in SCI.	Overall, the combination of NSC transplantation and electroactive implants represents a complicated treatment approach. However, we think this approach merits additional research given the intricacy of SCI damage and the social need for a novel therapy.

Methods	Advantages	Results
Transplanted human iPSCs-NPCs into SCI	For people with SCI, iPSC-derived sNPCs may offer a patient-specific cell source that might act as a relay system across the site of damage.	did not discover a functional improvement, while other research' findings were conflicting. There are several reasons why this may be. It's likely that the research hasn't been done for long enough for the cells to properly develop, create the right kind of synapses, and be useful.
Administration of LCTOPC1	Human pluripotent stem cell-derived oligodendrocyte progenitor cells (LCTOPC1) exhibit molecular features that allow survival and the possible repair of important cellular components and architecture at the SCI site.	gives significant first-in-person safety evidence in favor of the development of future medicines using human embryonic stem cells. Although it is impossible to rule out the possibility of future adverse events, the results of this experiment show that patients may tolerate this cell type well, with an event-free duration of up to 10 years.
Modulation by DREADD	DREADD altered the function of the locomotor system by preventing the transplanted cells' neurons from firing.	Draw attention to the importance of engrafting functionally competent neurons by hiPSC-NS/PC transplantation for adequate recovery from SCI.
Nanomaterial	The blood-spinal cord barrier, for example, might be penetrated by these nanoparticles, increasing the availability of therapeutic chemicals in the wounded area. Through the use of formulations such as injectable hydrogels and other scaffolds, nanomedicine may also be able to create the necessary conditions for the injured nerve to regenerate.	Combining stem cells with nanomaterials might result in effective therapeutic options for the treatment of SCI.

Authors	Title
Shibata, *et al* [69]	Administration of C5a Receptor Antagonist Improves the Ecacy of Human iPSCS-derived NS/PC Transplantation in the Acute Phase Of spinal Cord Injury
Wertheiem, *et al* [70]	Regenerating the Injured Spinal Cord at the Chronic Phase by Engineered iPSCs-Derived 3D Neuronal Networks
Zheng, *et al* [71]	Transplantation of Human Induced Pluripotent Stem Cell-Derived Neural Progenitor Cells Promotes Forelimb Functional Recovery after Cervical Spinal Cord Injury
Mutepfa, *et al* [72]	Electroactive Scaffolds to Improve Neural Stem Cell Therapy for Spinal Cord Injury

Authors	Title	Year
Lavoie, *et al* [73]	Human induced pluripotent stem cells integrate, create synapses and extend long axons after spinal cord injury	2022
McKenna, *et al* [74]	Ten-year safety of pluripotent stem cell transplantation in acute thoracic spinal cord injury	2022
Kitagawa, *et al* [75]	Modulation by DREADD reveals the therapeutic effect of human iPSC-derived neuronal activity on functional recovery after spinal cord injury	2022
Zarepour, *et al* [76]	Combination therapy using nanomaterials and stem cells to treat spinal cord injuries	2022

The ethical issues of using embryonic stem cells are resolved by creating artificially induced pluripotent stem cells (iPSC) from adult somatic cells. In preclinical models of SCI, a number of teams, including our own, have examined the transplant of iPSC-derived cells. Similar to ESC, many studies choose to treat SCI with NSC produced from iPSC. For instance, Fujimoto and colleagues demonstrated that in a mouse acute model of thoracic SCI, iPSC-NSC have a therapeutic potential equivalent with NSC obtained from human fetal spinal cord. The iPSC-NSC group also demonstrated improved remyelination and axon regeneration, as well as maintained endogenous neurons' survival. Through the rebuilding of the corticospinal tract, which restored broken neural circuitry in a relay fashion, the recovery of motor function was aided. Additionally, these writers used particular diphtheria toxin cell ablation techniques. When the animals with the transplants were given diphtheria toxin after the recovery of motor function was noticed, as was expected, the animals' condition deteriorated, proving that the transplanted cells were responsible for the recovery [77].

According to the literature review, several method developments in the use of iPSCs produce varying degrees of difficulty and benefits, such as increasing the ability of SCI to regenerate through the use of Engineered iPSCs using the 3D Neuronal Networks method, Electroactive Scaffolds with the biomaterial encapsulation of NSCs method, pluripotent stem cell transplantation in acute thoracic spinal cord injury with the administration of LCTOPC, and combination the methods. HiPSC-NS/PC transplantation with regulation by DREADD, hiPSC-NS/PC transplantation with injection of C5a receptor antagonists, and transplantation of hiPSCs with purification of iPSCs utilizing Nestin+Cells by fluorescence-activated cell sorting all result in increased locomotor function capacity. However, other techniques, such as the transplanting of

human iPSC-derived NPCs, still provide fewer meaningful findings as a result of the short research duration.

Conclusion

Despite being used at the pre-clinical stage, the use of iPSC in the treatment of SCI is regarded as one of the most promising treatments since it has a noticeable impact. As a result, employing iPSCs to treat SCI is still difficult and calls for more research.

References

[1]. Bruce, J. H.; Norenberg, M. D.; Kraydieh, S.; Puckett, W.; Marcillo, A.; Dietrich, D. Schwannosis: Role of gliosis and proteoglycan in human spinal cord injury. J. Neurotrauma 17:781–788; 2000.

[2]. Norenberg, M. D.; Smith, J.; Marcillo, A. The pathology of human spinal cord injury: Defining the problems. J. Neurotrauma 21:429–440; 2004

[3]. Bareyre, F.; Kerschensteiner, M.; Raineteau, O.; Mettenleiter, T.; Weinmann, O.; Schwab, M. The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. Nat. Neurosci. 7:269–277; 2004.

[4]. Madhavan, L.; Collier, T. A synergistic approach for neural repair: Cell transplantation and induction of endogenous precursor cell activity. Neuropharmacology 58:835–844; 2010

[5]. Maier, L.; Schwab, J. M. Sprouting, regeneration and circuit formation in the injured spinal cord: Factors and activity. Philos. Trans. R. Soc. Lond. Biol. Sci. 381:1611– 1684; 2006

[6]. Rossi, S. L.; Keirstead, H. S. Stem cells and spinal cord regeneration. Curr. Opin. Biotechnol. 20:552–562; 2009.

[7]. Tetzlaff, W.; Okon, E. B.; Karimi-Abdolrezaee, S.; Hill, C. E.; Sparling, J. S.; Plemel, J. R.; Plunet, W. T.; Tsai, E. C.; Baptiste, D.; Smithson, L. J.; Kawaja, M. D.; Fehlings, M. G.; Kwon, B. K. A systematic review of cellular transplantation therapies for spinal cord injury. J. Neurotrauma 28:1611–1682; 2011.

[8]. Bonner, J.; Blesch, A.; Neuhuber, B.; Fischer, I. Promoting direcftional axon growth from neural progenitors grafterd into the injured spinal cord. J. Neurosci. Res. 88:1182– 1192; 2009.

[9]. Cao, Q.; Howard, R.; Dennison, J.; Whittemore, S. Differentiation of engrafted neuronal-restricted precursor cells is inhibited in the traumatically injured spinal cord. Exp. Neurol. 177:349–359; 2002

[10]. Cloutier, F.; Siegenthaler, M.; Nistor, G.; Keirstead, H. Transplantation of human embryonic stem cell-derived oligodendrocyte progenitors into rat spinal cord injuries does not cause harm. Regen. Med. 1:469–479; 2006.

[11]. Faulkner, J.; Keirstead, H. Human embryonic stem cellderived oligodendrocyte progenitors for the treatment of spinal cord injury. Transpl. Immunol. 15:131–142; 2005.

[12]. Iwanami, A.; Kaneko, S.; Nakamura, M.; Kanemura, Y.; Mori, H.; Kobayashi, S. Transplantation of human neural stem cells for spinal cord injury in primates. J. Neurosci. Res. 80:182–190; 2005.

[13]. Keirstead, H. S.; Nistor, G.; Bernal, G.; Totoiu, M.; Cloutier, F.; Sharp, K.; Steward, O. Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. J. Neurosci. 25:4694–4705; 2005.

[14]. Mitsui, T.; Shumsky, J.; Lepore, A.; Murray, M.; Fischer, I. Transplantation of neuronal and glial restricted precursors into contused spinal cord improves bladder and motor functions,

decreases thermal hypersensitivity, and modifies intraspinal circuitry. J. Neurosci. 25:9624–9636; 2005.

[15]. Sharp, J.; Frame, J.; Siegenthaler, M.; Nistor, G.; Keirstead, H. S. Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants improve recovery after cervical spinal cord injury. Stem Cells 28:152–163; 2010.

[16]. Goh, E. L. K.; Ma, D. K.; Ming, G. L.; Song, H. J. Adult neural stem cells and repair of the adult central nervous system. J. Hematother. Stem Cell Res. 12:671–679; 2003.

[17]. Lopez, M.; Valenzuela, J.; Alvarez, F.; Lopez-Alvarez, M.; Cecilia, G.; Paricio, P. Long-term problems related to immunosuppression. Transpl. Immunol. 17:31–35; 2006.

[18]. Henon, P. R. Human embryonic or adult stem cells: An overview on ethics and perspectives for tissue engineering.Adv. Exp. Med. Biol. 534:27–45; 2003.

[19]. Jain, K. K. Ethical and regulatory aspects of embryonic stem cell research. Expert Opin. Biol. Ther. 5:153–162; 2005.

[20]. Robertson, J. A. Ethics and policy in embryonic stem cell research. Kennedy Inst. Ethics J. 9:109–136; 1999.

[21]. Romano, G. Stem cell transplantation therapy: Controversy over ethical issues and clinical relevance. Drug News Perspect. 17:637–645; 2004.

[22]. Hochedlinger, K.; Jaenisch, R. Nuclear reprogramming and pluripotency. Nature 441:1061–1067; 2006.

[23]. Jaenisch, R.; Young, R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. Cell 132:567–582; 2008.

[24]. Meissner, A.; Jaenisch, R. Mammalian nuclear transfer. Dev. Dyn. 235:2460–2469; 2006

[25]. Pralong, D.; Trounson, A. O.; Verma, P. J. Cell fusion for reprogramming pluripotency—Toward elimination of the pluripotent genome. Stem Cell Rev. 2:331–340; 2006.

[26]. Rideout, W. M.; Eggan, K.; Jaenisch, R. Nuclear cloning and epigenetic reprogram

[27]. Yamanaka, S.; Blau, H. M. Nuclear reprogramming to a pluripotent state by three approaches. Nature 465:704–712; 2010

[28]. Ishikawa, K.; Toyama-Sorimachi, N.; Nakada, K.; Morimoto, M.; Imanishi, H.; Yoshizaki, M.; Sasawatari, S.; Niikura, M.; Takenaga, K.; Yonekawa, H.; Hayashi, J. The innate immune system in host mice targets cells with allogenic mitochondrial DNA. J. Exp. Med. 207:2297–2305; 2010The innate immune system in host mice targets cells with allogenic mitochondrial DNA. J. Exp. Med. 207:2297– 2305; 2010

[29]. Takahashi, K.; Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676; 2006

[30]. Yu, J.; Vodyanik, M.; Smuga-Otto, K.; AntosiewiczBourget, J.; Frane, J.; Tian, S.; Nie, J.; Jonsdottir. G. A.; Ruotti, V.; Stewart, R.; Slukvin, I. I.; Thomson, J. A. Induced pluripotent stem cell lines derived from human somatic cells. Science 318:1917–1920; 2007.

[31]. Drukker, M.; Katchman, H.; Katz, G.; Even-Tov Friedman, S.; Shezen, E.; Hornstein, E.; Mandelboim, O.; Reisner, Y.; Benvenisty, N. Human embryonic stem cells and their

differentiated derivates are less susceptible to immune rejection than adult cells. Stem Cells 24:221–229; 2006.

[32]. Pearl, J. I.; Lee, A. S.; Leveson-Gower, D. B.; Sun, N.; Ghosh, Z.; Lan, F.; Ransohoff, J.; Negrin, R. S.; Davis, M. M.; Wu, J. C. Short-term immunosuppression promotes engraftment of embryonic and induced pluripotent stem cells. Cell Stem Cell 8:309–317; 2011.

[33]. Swijnenburg, R.-J.; Schrepfer, S.; Govaert, J. A.; Cao, F.; Ransohoff, K.; Sheikh, A. Y.; Haddad, M.; Connolly, A. J.; Davis, M. M.; Robbins, R. C.; Wu, J. C. Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. Proc. Natl. Acad. Sci. USA 105:12991–12996; 2008.

[34]. Brown, M. T. Moral complicity in induced pluripotent stem cell research. Kennedy Inst. Ethics J. 19:1–22; 2009.

[35]. Cyranoski, D. Stem cells: 5 things to know before jumping on the iPS bandwagon. Nature 452:406–408; 2008.

[36]. Magill, G.; Neaves, W. B. Ontological and ethical implications of direct nuclear reprogramming. Kennedy Inst. Ethics J. 19:23–32; 2009.

[37]. Skene, L. Recent developments in stem cell research: Social, ethical, and legal issues for the future. Ind. J. Global Legal Stud. 17:211–244; 2010.

[38]. Boyer, L. A.; Lee, T. I.; Cole, M. F.; Johnstone, S. E.; Levine, S. S.; Zucker, J. R.; Guenther, M. G.; Kumar, R. M.; Murray, H. L.; Jenner, R. G.; Gifford, D. K.; Melton, D. A.; Jaenisch, R.; Young, R. A. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 122:947–956; 2005.

[39]. Chen, X.; Xu, H.; Yuan, P.; Fang, F.; Huss, M.; Vega, V. B.; Wong, E.; Orlov, Y. L.; Zhang, W.; Jiang, J.; Loh, Y.-H.; Yeo, H. C.; Yeo, Z. X.; Narang, V.; Govindarajan, K. R.; Leong, B.; Shahab, A.; Ruan, Y.; Bourque, G.; Sung, W.-K.; Clarke, N. D.; Wei, C.-L.; Ng, H.-H. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell 133:1106–1117; 2008.

[40]. Jiang, J.; Chan, Y.-S.; Loh, Y.-H.; Cai, J.; Tong, G.-Q.; Lim, C.-A.; Robson, P.; Zhong, S.; Ng, H.-H. A core Klf circuitry regulates self-renewal of embryonic stem cells. Nat. Cell Biol. 10:353–360; 2008.

[41]. Kim, J.; Chu, J.; Shen, X.; Wang, J.; Orkin, S. H. An extended transcriptional network for pluripotency of embryonic stem cells. Cell 132:1049–1061; 2008.

[42]. Loh, Y. H.; Wu, Q.; Chew, J. L.; Vega, V. B.; Zhang, W. W.; Chen, X.; Bourque, G.; George, J.; Leong, B.; Liu, J.; Wong, K. Y.; Sung, K. W.; Lee, C. W. H.; Zhao, X. D.; Chiu, K. P.; Lipovich, L.; Kuznetsov, V. A.; Robson, P.; Stanton, L. W.; Wei, C. L.; Ruan, Y. J.; Lim, B.; Ng, H. H. The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. Nat. Genet. 38:431–440; 2006.

[43]. Takahashi, K. Direct reprogramming 101. Dev. Growth Differ. 52:319–333; 2010.

[44]. Takahashi, K.; Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676; 2006.

[45]. Nakagawa, M.; Koyanagi, M.; Tanabe, K.; Takahashi, K.; Ichisaka, T.; Aoi, T. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat. Biotechnol. 26:101–106; 2008.

[46]. Wernig, M.; Meissner, A.; Cassady, J. P.; Jaenisch, R. c-Myc is dispensable for direct reprogramming of mouse fibroblasts. Cell Stem Cell 2:10–12; 2008.

[47]. Maherali, N.; Sridharan, R.; Xie, W.; Utikal, J.; Eminli, S.; Arnold, K.; Stadtfeld, M.; Yachechko, R.; Tchieu, J.; Jaenisch, R.; Plath, K.; Hochedlinger, K. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell 1:55–70; 2007.

[48]. Okita, K.; Ichisaka, T.; Yamanaka, S. Generation of germline-competent induced pluripotent stem cells. Nature 448:313–317; 2007.

[49]. Wernig, M.; Meissner, A.; Foreman, R.; Brambrink, T.; Ku, M.; Hochedlinger, K.; Bernstein, B. E.; Jaenisch, R. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature 448:318–324; 2007.

[50]. Blelloch, R.; Venere, M.; Yen, J.; Ramalho-Santos, M. Generation of induced pluripotent stem cells in the absence of drug selection. Cell Stem Cell 1:245–247; 2007.

[51]. Li, W.; Wei, W.; Zhu, S.; Zhu, J.; Shi, Y.; Lin, T.; Hao, E.; Hayek, A.; Deng, H.; Ding, S. Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. Cell Stem Cell 4:16–19; 2009

[52]. Liao, J.; Cui, C.; Chen, S.; Ren, J.; Chen, J.; Gao, Y. Generation of induced pluripotent stem cell lines from adult rat cells. Cell Stem Cell 4:11–15; 2009.

[53]. Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861–872; 2007.

[54]. Esteban, M. A.; Wang, T.; Qin, B.; Yang, J.; Qin, D.; Cai, J.; Li, W.; Weng, Z.; Chen, J.; Ni, S.; Chen, K.; Li, Y.; Liu, X.; Xu, J.; Zhang, S.; Li, F.; He, W.; Labuda, K.; Song, Y.; Peterbauer, A.; Wolbank, S.; Redl, H.; Zhong, M.; Cai, D.; Zeng, L.; Pei, D. Vitamin C enhances the generation of mouse and human induced pluripotent stem cells. Cell Stem Cell 6:71–79; 2010.

[55]. Esteban, M. A.; Xu, J.; Yang, J.; Peng, M.; Qin, D.; Li, W.; Jiang, Z.; Chen, J.; Den, K.; Zhong, M.; Cai, J.; Lai, L.; Pei, D. Generation of induced pluripotent stem cell lines from tibetan miniature pig. J. Biol. Chem. 284:17634– 17640; 2009.

[56]. Wu, Z.; Chen, J.; Ren, J.; Bao, L.; Liao, J.; Cui, C.; Rao, L.; Li, H.; Gu, Y.; Dai, H.; Zhu, H.; Teng, X.; Cheng, L.; Xiao, L. Generation of pig induced pluripotent stem cells with a druginducible system. J. Mol. Cell Biol. 1:46–54; 2009.

[57]. Liu, J.; Balehosur, D.; Murray, B.; Kelly, J. M.; Sumer, H.; Verma, P. J. Generation and characterization of reprogrammed sheep induced pluripotent stem cells. Theriogenology 77:338–346; 2012.

[58]. Nagy, K.; Sung, H.-K.; Zhang, P.; Laflamme, S.; Vincent, P.; Agha-Mohammadi, S.; Woltjen, K.; Monetti, C.; Michael, I. P.; Smith, L. C.; Nagy, A. Induced pluripotent stem cell lines derived from equine fibroblasts. Stem Cell Rev. 7:693–702; 2011. 260. Nakagawa, M.; Koyana.

[59]. Chan, A. W. S.; Cheng, P.-H.; Neumann, A.; Yang, J.-J. Reprogramming Huntington monkey skin cells into pluripotent stem cells. Cell. Reprogram. 12:509–517; 2010.

[60]. Deleidi, M.; Hargus, G.; Hallett, P.; Osborn, T.; Isacson, O. Development of histocompatible primate-induced pluripotent stem cells for neural transplantation. Stem Cells 29:1052–1063; 2011.

[61]. Zhong, B.; Trobridge, G. D.; Zhang, X.; Watts, K. L.; Ramakrishnan, A.; Wohlfahrt, M.; Adair, J. E.; Kiem, H.-P. Efficient generation of nonhuman primate induced pluripotent stem cells. Stem Cells Dev. 20:795–807; 2011.

[62]. Ben-Nun, I. F.; Montague, S. C.; Houck, M. L.; Tran, H. T.; Garitaonandia, I.; Leonardo, T. R.; Wang, Y.-C.; Charter, S. J.; Laurent, L. C.; Ryder, O. A.; Loring, J. F. Induced pluripotent stem cells from highly endangered species. Nat. Methods 8:829–831; 2011.

[63]. Armstrong, L.; Tilgner, K.; Saretzki, G.; Atkinson, S.; Stojkovic, M.; Moreno, R.; Przyborski, S.; Lako, M. Human induced pluripotent stem cell lines show stress defense mechanisms and mitochondrial regulation similar to those of human embryonic stem cells. Stem Cells 28:661–673; 2010.

[64]. Arnhold, S.; Klein, H.; Semkova, I.; Addicks, K.; Schraermeyer, U. Neurally selected embryonic stem cells induce tumor formation after long-term survival following engraftment into the subretinal space. Invest. Opthalmol. Vis. Sci. 45:4251–4255; 2004.

[65]. Asher, R. A.; Morgenstern, D. A.; Moon, L. D. F.; Fawcett, J. W. Chondroitin sulphate proteoglycans: Inhibitory components of the glial scar. Prog. Brain Res. 132:611–619; 2001.

[66]. Baker, D. E. C.; Harrison, N. J.; Maltby, E.; Smith, K.; Moore, H. D.; Shaw, P. J.; Heath, P. R.; Holden, H.; Andrews, P. W. Adaptation to culture of human embryonic stem cells and oncogenesis in vivo. Nat. Biotechnol. 25:207–215; 2007

[67]. Ban, H.; Nishishita, N.; Fusaki, N.; Tabata, T.; Saeki, K.; Shikamura, M.; Takada, N.; Inoue, M.; Hasegawa, M.; Kawamata, S.; Nishikawa, S.-I. Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by temperature-sensitive Sendai virus vectors. Proc. Natl. Acad. Sci. USA 108:14234–14239; 2011.

[68]. Banito, A.; Rashid, S. T.; Acosta, J. C.; Li, S.; Pereira, C. F.; Geti, I.; Pinho, S.; Silva, J. C.; Azuara, V.; Walsh, M.; Vallier, L.; Gil, J. Senescence impairs successful reprogramming to pluripotent stem cells. Genes Dev. 23:2134– 2139; 2009.

[69]. Shibata, R., Nagoshi, N., Kajikawa, K., Ito, S., Shibata, S., Shindo, T., ... & Okano, H. (2022). Administration of C5a Receptor Antagonist Improves the Efficacy of Human Induced Pluripotent Stem Cell–Derived Neural Stem/Progenitor Cell Transplantation in the Acute Phase of Spinal Cord Injury. *Journal of Neurotrauma*, *39*(9-10), 667-682.

[70]. Wertheim, L., Edri, R., Goldshmit, Y., Kagan, T., Noor, N., Ruban, A., ... & Dvir, T. (2022). Regenerating the Injured Spinal Cord at the Chronic Phase by Engineered iPSCs-Derived 3D Neuronal Networks. *Advanced Science*, *9*(11), 2105694.

[71]. Zheng, Y., Gallegos, C. M., Xue, H., Li, S., Kim, D. H., Zhou, H., ... & Cao, Q. (2022). Transplantation of Human Induced Pluripotent Stem Cell-Derived Neural Progenitor Cells Promotes Forelimb Functional Recovery after Cervical Spinal Cord Injury. *Cells*, *11*(17), 2765.

[72]. Mutepfa, A. R., Hardy, J. G., & Adams, C. F. (2022). Electroactive Scaffolds to Improve Neural Stem Cell Therapy for Spinal Cord Injury. *Frontiers in Medical Technology*, *4*.

[73]. Lavoie, N. S., Truong, V., Malone, D., Pengo, T., Patil, N., Dutton, J. R., & Parr, A. M. (2022). Human induced pluripotent stem cells integrate, create synapses and extend long axons after spinal cord injury. *Journal of Cellular and Molecular Medicine*, *26*(7), 1932-1942.

[74]. McKenna, S. L., Ehsanian, R., Liu, C. Y., Steinberg, G. K., Jones, L., Lebkowski, J. S., ... & Fessler, R. G. (2022). Ten-year safety of pluripotent stem cell transplantation in acute thoracic spinal cord injury. *Journal of Neurosurgery: Spine*, *1*(aop), 1-10.

[75]. Kitagawa, T., Nagoshi, N., Kamata, Y., Kawai, M., Ago, K., Kajikawa, K., ... & Okano, H. (2022). Modulation by DREADD reveals the therapeutic effect of human iPSC-derived neuronal activity on functional recovery after spinal cord injury. *Stem cell reports*, *17*(1), 127-142.

[76]. Zarepour, A., Öztürk, A. B., Irmak, D. K., Yaşayan, G., Gökmen, A., Karaöz, E., ... & Mostafavi, E. (2022). Combination therapy using nanomaterials and stem cells to treat spinal cord injuries. *European Journal of Pharmaceutics and Biopharmaceutics*.