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Systematic Review

Regenerative Medicine Therapies for Chronic Spinal Cord Injury, a Systematic Review of Reviews for Evidence-Based Medicine

Managol Kayyal [1], Navid Jamali [2], Reyhaneh Abolghasemi [3*], Esmat Davoudi- Monfared [4], Fakhri Allahyari [5], Gholamreza Farzanegan [6], Mahdi Sharif-Alhoseini [7]

[1] Student research committee, Faculty of medicine, Qazvin University of Medical School, Qazvin, Iran.
[2] Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran.
[3] MD, Ph.D., New Hearing Technologies Research Center, Clinical Sciences Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
[4] MD, Assistant Professor, Health Management Research Center & Department of Community Medicine, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran.
[5] MD, Assistant Professor, Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
[6] MD, Associate Professor, Trauma Research Center & Department of Neurosurgery, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran.
[7] MD, Assistant Professor, Sina Trauma and Surgery Research Center, Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran.
*** Corresponding Author**: [3] MD, Ph.D., New Hearing Technologies Research Center, Clinical Sciences Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
Email: Aborey110@gmail.com

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Abstract

Introduction: Chronic spinal cord injury has complicated pathophysiology, and current therapies cannot restitute deficits in neurological functions. This review summarizes assembled knowledge of evidence-based medicine and highlights possible challenges.

Methods: Review in systematic reviews refer to the review compiling evidence from several reviews into accessible and usable documents. The PubMed, Google Scholar, and Cochrane Library databases were searched by keywords in title and abstracts for articles satisfying the inclusion criteria through September 30, 2022. Two trained investigators independently performed the critical appraisal of systematic review papers with the PRISMA 2020 checklist.

Results: The results were presented in the PRISMA flow diagram. After the exclusion, significant findings resulted in the nine final reviewed studies. The Neuropathology of the chronic phase is determined by the stabilization of the lesion, including white matter demyelination, fibrotic scar, and cystic cavitation. Multiple stem cell types and glial cells are studied in regenerative medicine therapies for chronic spinal cord injury. Also, cell and tissue products, gene therapy products, and xenogeneic cell products are other candidates for clinical applications. Synthetic or natural tissue engineering materials can be implanted in solid scaffolds or injectable in situ forming hydrogels, acting as vehicles for the cells or as vectors of therapeutic agents, or filling the cysts in the case of chronic lesions.

Conclusion: Combinatorial therapeutic process that employs cells, biomaterials, and soluble molecules to address different appearances of injury will probably provide the definitive solution by regulating the balance between the inhibitory and excitatory factors implicated in chronic spinal cord injury.

Keywords: Cell therapy; Biomaterials; Biomolecules.

Introduction

Spinal cord injury (SCI) represents one of the most devastating neurological disorders with high economic and social impact. The most common mechanisms of injury are road traffic crash, fall, and penetrating injuries[1]. The majority of patients are young males[2]. Chronic spinal injury takes more than six months after the initial traumatic lesion to the spine[3,4]. Different cellular and molecular phenomena arise in temporal stages, leading to a chronic phase after the first lesion[5,6] (Figure 1). At this stage, patients have many symptoms caused by neurological weakness, such as paraplegia, which is called paralysis in Farsi[7].

Human efforts to treat SCI patients have been numerous to the extent that the use of herbal treatments for paralysis has been suggested[8]. Recently, regenerative medicine clinical trials have been proposed as a new treatment for chronic spinal cord injuries. Different types of somatic and glial cells and cell products have been used. Also, natural or synthetic tissue engineering products in injectable solids or hydrogels are used to carry therapeutic agents or fill cysts at the injury site. Multiple systematic reviews have been written for some of this research. However, according to the articles in the typical medical databases, these review articles have not been summarized. The result of this article can determine the future treatment path. Also, open the gaps in the research to the researchers.

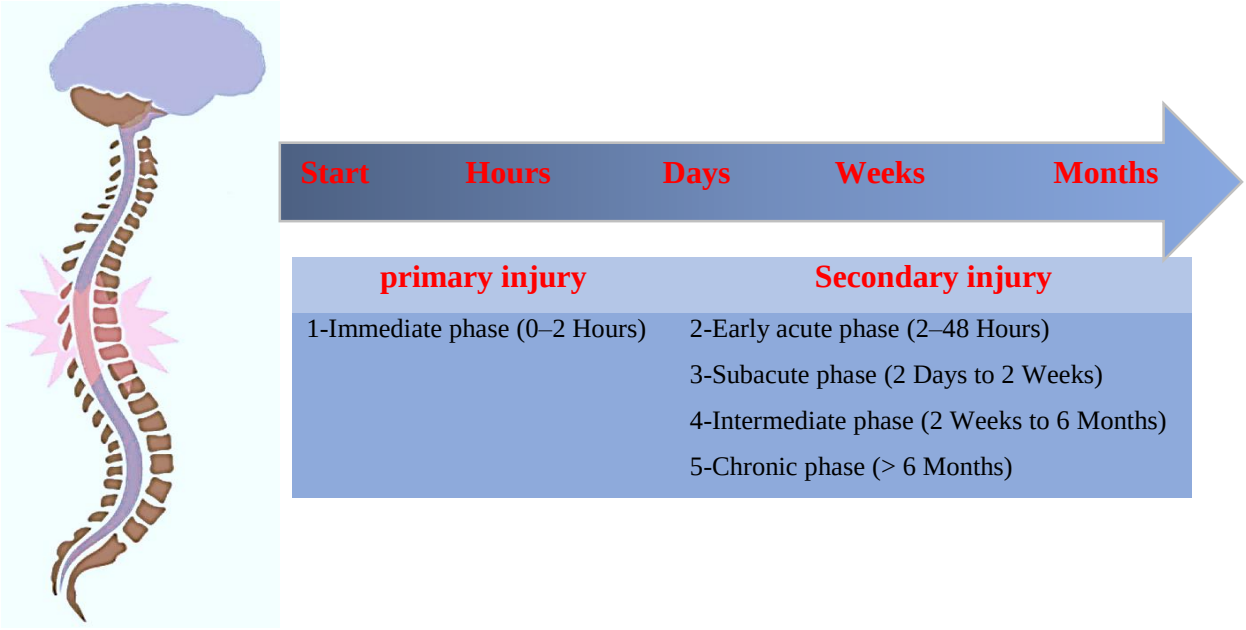

Figure 1: Temporal phases of spinal cord injury.

Methods

The review of systematic reviews has an influential role in evidence-based medicine and decision-making in health care services. This systematic review follows the guidelines of Valerie Smith et al.[9] and Edris Hasanpoor et al.[10].

Two reviewers accomplished an independent electronic literature search in the PubMed, Google Scholar, and Cochrane Library databases from initiation until September 30, 2022. Also, they independently performed the critical appraisal of systematic review papers with the PRISMA 2020 checklist[11]. Any disagreements were resolved through discussion, agreement, or consultation with a third researcher.

The specific keywords used for the search were as follows: "chronic spinal cord injury," AND "regeneration," OR "regenerative therapy," AND "systematic review." We searched titles and abstracts. The reference lists of the selected articles were also searched to discover the studies not found in the primary search. The eligible studies were included for review.

Inclusion criteria: a systematic review of regeneration or regenerative therapy, chronic SCI, English language, full text, without time limitation (from initiation to September 30, 2022).

Exclusion criteria: original article, case report, duplication, other treatments than regenerative therapy, acute and sub-acute SCI, languages other than English, only abstract, grey literature (books, news, dissertations, preprints…).

Results

The PRISMA flow diagram of the literature screening and results is shown in Figure 2. The total number of data from the searches was 926 records. After excluding irrelevant findings (original article (n =132), case report (n =36), duplication (n =126), other treatments than regenerative therapy (n =167), acute and sub-acute SCI (n =287), other languages than English (n =15), only abstract (n =8), grey literature (n =146)) in the nine final reviewed studies (Table1), essential findings are recorded in following.

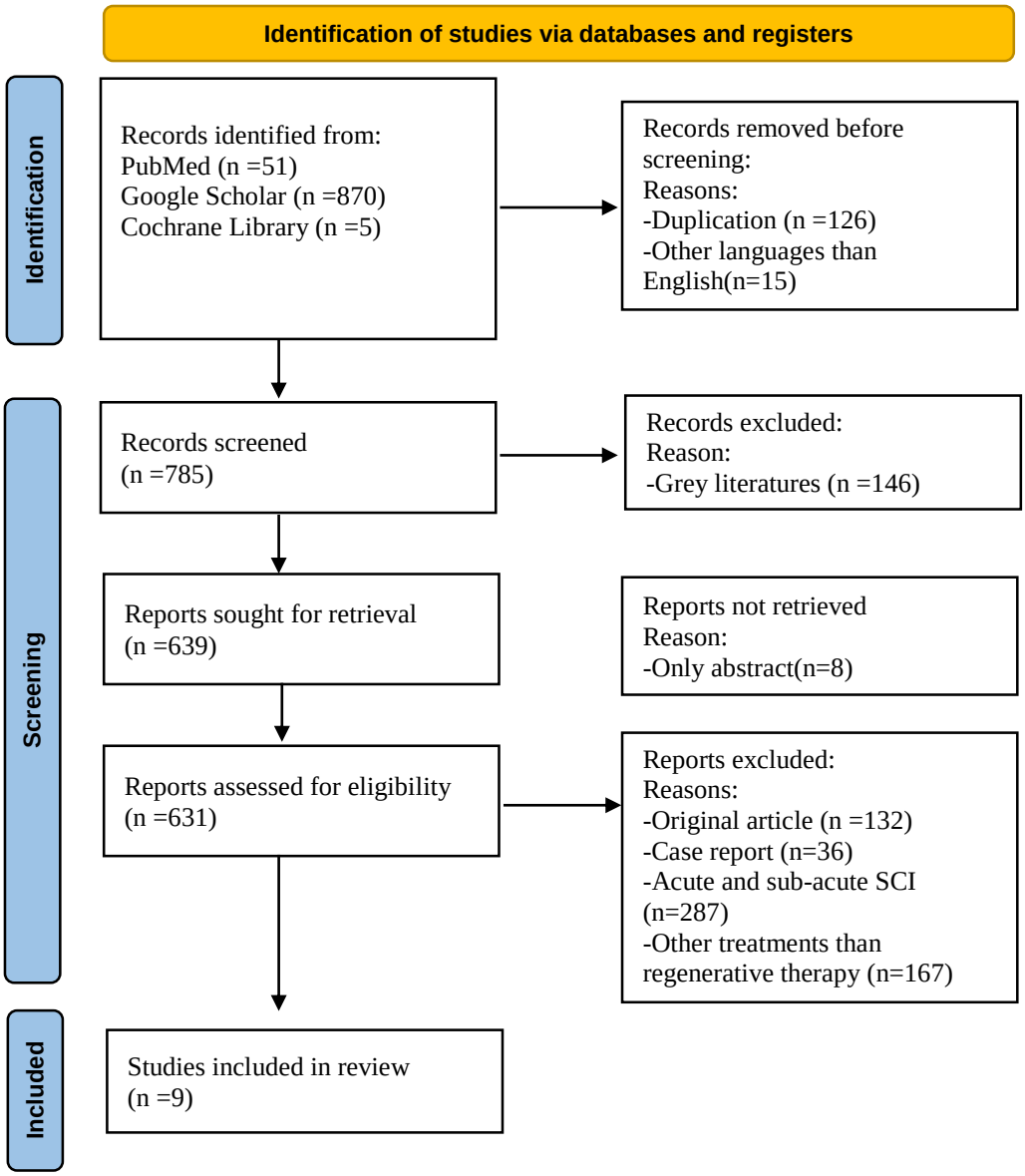

Figure 2: The PRISMA flow diagram records the number of articles found and then makes the selection process transparent by reporting on decisions made at various stages of the systematic review. n: numbers of articles are recorded at the different stages.

Table1: Summary of review articles reported

ID	First author	Year	Journal name (2021-2022 ISI Impact Factor)	Regenerative medicine approaches	Number of References in paper	Reference number
1	Hettiaratchi MH	1998	Current Opinion in Neurobiology(7.07)	The intrinsic capacity of mature CNS neurons, Alter the terrain at the injury site itself, The role of inhibitors in restricting axonal growth, The axonal growth to recovery of motor function.	75	23
2	Houle JD	2003	Experimental Neurology (5.62)	Neurotrophic factor, Cell therapies, Molecular therapies	50	17
3	Peˆgo AP	2012	Experimental Neurology(5.62)	Transplantation of fetal spinal cord (FSC), Neurotrophic or growth factors, Genetically modified fibroblasts, Combination therapies	56	22
4	Tsintou M	2015	Neural Regeneration Research(6.058)	Cell-based treatment strategies, Biomolecules delivery treatment strategies, Scaffold-based or combination strategies	149	21
5	Assunção-Silva RC	2017	Current Opinion in Biomedical Engineering(4.164)	Cell transplantation, Drug delivery	253	24
6	Dalamagkas K	2019	Tissue Engineering Part B Reviews (7.376)	Cell-based therapies, Soluble bioactive molecule-based therapies, Biomaterial-based therapies, Combinatorial therapeutic approaches	81	18
7	Papa S	2020	Expert Opinion on Biological Therapy (3.224)	Cell-based therapies, Biomaterials, Combinatorial approaches	143	27
8	Ashammakhi N	2020	Tissue Engineering Part B Reviews (7.376)	Cell-based approach, Biomaterials-based approach	225	14
9	Chen X	2021	Biomedicine & Pharmacotherapy (7.419)	Stem cells, Nanoscaffolds, Scaffold and stem cell combinations	73	20

Neuropathology of Chronic Spinal Cord Injury

Sequential secondary events begin within the injured cord following the primary physical (mechanical) lesion[12,13]. The lesion's maturation/stabilization determines the chronic phase, including white matter demyelination, gray matter dissolution, astroglial and fibrotic scar, and the development of cystic cavitation[14] (Table2). Failure of axonal regeneration and sprouting responses no farther than 1 mm are due to the intrinsic and extrinsic inhibitory mediators expressed by myelin, oligodendrocytes, astrocytes, pericytes, fibroblast-like cells, ependymal cells, and some neurons[15,16]. The atrophic cord extends more than two injury levels, rostrally and caudally. Myelomalacia and syringomyelia represent the final stage of necrotic death[17-19]. The list of upregulated and downregulated soluble bioactive molecules is presented in Table3. The most mentioned molecules in the reviewed articles were the increase of Myelin-associated glyproteins (MAG) and BDNF and the decrease of Nogo-A and Neurotrophic factors molecules.

Regenerative therapies

Based on the Food & Drug Administration's (FDA) interpretation in section 506(g), Regenerative medicine therapies (RMTs) include cell therapies, therapeutic tissue engineering products, human cells, tissue products, and combination products using any such approaches. Also, human gene therapy products, including genetically modified cells that conduct a sustained effect on cells or tissues, and xenogeneic cell products may affiliate with the definition of an RMT[20]. In the following, we will explain different regenerative therapy approaches.

Table2: Histopathology and pathophysiology of human chronic spinal cord injury.

Histopathology
• Neuronal loss (apoptosis and necrosis) • Prolonged wallerian degeneration • Retro-grade axonal dieback • Dystrophic axonal growth cones • Spared, demyelinated axons • Thinner myelin sheaths • Few myelinated axons crossing the injury site • Subpial rim axons, around the entire perimeter of the cord or along one side only • Dense fibrotic scar • Extended cord atrophy • Thinner spine, at beneath the pia and close to the contusion site • Decreased anteroposterior diameter of cord • Spinal cord tethering • Myelomalacia • Syrinx and syringomyelia
Pathophysiology
• Circumscribed areas of infarcts • Local disruption of CNS connections • Neural conduction damage • Permanent hyper excitability • Inhibited the axonal regeneration

Table3: : Upregulated and downregulated soluble bioactive molecules in chronic SCI.

Upregulated reference number	**Downregulated** reference number
Extracellular amino acids[14,20,27], Myelin-associated glyproteins (MAG) [14,17,20,21,22,23,27], Rho-associated protein kinase (ROCK)[14,21,23] , Semaphorin 3A[17,23], ephrins[23], BDNF[14,17,21,22,23,24,27], TrKB[27], GFAP[14,24,27], Chondroitin sulfate proteoglycans(CSPGs)[17,20,21,23,24], Tenascin[17,20], Metallothioneins [27], Vimentin[27], GAP-43[17,22,27].	SNAP-25A and B[27], Nogo-A[14,17,18,21,22,23,27], Neurotrophic factors[14,17,18, 21, 22, 23,24,27], MAP-2 [27], MOG[27], Glutamate receptor[27], Oligodendrocyte myelin glycoprotein(OMgp) [21,23]

1-Allogeneic and autologous Cell therapy

Transplanted cells can stimulate synergistic regenerative plans[21]. Cell-based therapies in the spine may enable numerous direct benefits to repair and replace the damaged cells, including neuroprotection, neuroregeneration, angiogenesis, immune modulation, and generation of new neural cells. Besides, paracrine mediators secreted by delivered cells may indirectly help activate occupant progenitors[15], secreting trophic factors, and modifying the surroundings, making it more incisive for regeneration[22].

The widest cells studied included stem cells (embryonic stem cells (ESCs), neural stem and progenitor cells (NS/PCs), mesenchymal stem cells (MSCs), subventricular zone of the Hippocampus, spinal cord stem cells, umbilical cord-derived cells, bone marrow-derived mononuclear cells (BMMCs), induced pluripotent stem cells (iPSCs)) and glial cells (oligodendrocyte precursor cells (OPCs), Schwann cells (SCs), and olfactory ensheathing cells (OECs))[15,16,21].

1-1-Embryonic stem cells (ESCs)

The ESCs can generate glial or neuronal lineage, multipotent neural precursors, motor neurons, and oligodendroglial progenitors[23] or generate agents that can restrict damage and maintain tissue regeneration[16]. The ESC-derived oligodendroglial progenitor cells have been safe and improved the remyelination of host axons[23].

1-2- Neural stem and progenitor cells (NS/PCs)

The sources of multipotent NS/PCs are human fetal brains, human fetal spinal cord[18], other ESCs[15], and iPSCs[21]. Human NSCs can retain their ability to regenerate and differentiate into the three major neurogenic and glycogenic cell types of the central

nervous system (CNS) (cholinergic and GABA-ergic neurons, motor neurons[23], astrocytes, and oligodendrocytes)[19], which generally make up the spinal cord. Also, NSCs can produce pro-regenerative factors, preserve damaged cells and axons, and help reform neural orbits generated into synaptic formation [16]. NSCs increase the regenerated fibers at the lesion site and the caudal position and improve remyelination [23].

1-3-Subventricular zone of the Hippocampus and Spinal cord stem cells

Adult self-renewing endogenous progenitor NSCs present in the subventricular zone of the Hippocampus and ependymal region of the central canal of the spinal cord[16]. The anatomical place of NSCs impresses their ability to cultivate axonal protrusion. The cells from the spinal cord enhance substantial axonal regeneration more than cells harvested from the brain[24].

In addition, ependymal progenitor cells in the glial scar can restrict secondary injury by sustaining the tissue totality and diffusing bioactive factors that increase neuronal survival. Thus, mobilization of resident progenitor cells or handling the local ependymal stem cell niche could stimulate spinal self-repair[23].

1-4-Mesenchymal stem cells (MSCs)

MSCs can be isolated from various perinatal or adult tissues, including adipose tissue, bone marrow, dermis, dental pulp, peripheral blood, umbilical cord blood, Wharton's jelly, amniotic liquid, and placenta. These cells separate and expand for autologous application quickly. MSCs promote remyelination and neuronal regeneration through neurotrophic protection and immunomodulation[15]. The therapeutic efficacy of MSC in the neural tissue is due to their ability to incorporate into the host tissue and differentiation into neural lineage[19,25]. MSC provides a favorable microenvironment by secreting a variety of anti-apoptotic and neurotrophic growth factors, cytokines, microvesicles, and exosomes that target residing cells, modulate the immune response, reduce lesion volume, and prevent cell death[21,23] rather than morphological regeneration, and replacement changes[16,19]. The secretome intercedes both paracrine and autocrine MSCs acting. The secretome suppresses the local immune response, enhances angiogenesis, and inhibits scarring and cell apoptosis[25].

1-5-Umbilical cord-derived cells

The stem cells of umbilical cord blood cells (UCB) consist of hematopoietic stem cells, endothelial cell precursors, mesenchymal progenitors, and multipotent/pluripotent lineage stem cells[26].

Umbilical cord (UC) derived- MSCs (UC-MSCs) have demonstrated neurotrophic, anti-apoptotic, and angiogenic actions[16]. They release growth factors, upregulate matrix metalloproteinase, downregulate tissue plasminogen activator, mediate in the myelination process, and decrease gliosis[19].

1-6-Bone marrow-derived mononuclear cells (BMMCs)

Bone marrow mononuclear cells could be mobilized by granulocyte colony-stimulating factors resulting in smaller cavities and increased white matter[23]. Also, they have neuroplastic proprieties that contribute to neuroplasticity and/or paracrine results[19].

1-7-Induced pluripotent stem cells (iPSCs)

The iPSCs, overcome issues with the immunogenic rejection of allogeneic transplants[15]. The human iPSC-derived neurospheres (hiPSC-NSs) can survive, migrate, and differentiate into three major neural lineages. hiPSC-NSs express the neurotrophic factors, enhance axonal sparing/regrowth and angiogenesis, prevent the lesion epicenter demyelination, promote the configuration of synapses between grafted cells and host neurons, and advance the myelination in the injured area[25].

1-8-Oligodendrocyte precursor cells (OPCs)

The OPCs differentiate into myelinating oligodendrocytes[19]. A remarkable portion of SCI pathology is the absence of oligodendrocytes. The autologous OPCs are limited. Thus, demyelination leads to the delayed and progressive degeneration of remaining axonal tracts. The promising approach to salvaging the remaining axons is the stimulation of endogenous oligodendroglial progenitors. Also, the oligodendroglial progenitors (derived from ESCs, neural progenitors, or iPSCs) can transplant[25].

1-9-Schwann cells (SCs)

The SCs promote axonal regeneration via axonal regrowth and myelination. The regenerated axons reduce cyst formation[19]. They can produce different trophic growth factors such as neural growth factor

(NGF), fibroblast growth factor-2 (FGF-2), brain-derived neurotrophic factor (Brain-derived neurotrophic factor (BDNF), or neurotrophin-3(NT-3), cell adhesion molecules, and extracellular matrix proteins that can increase recovery after SCI[16,23].

1-10-Olfactory ensheathing cells (OECs)

The OECs are glial cell types that ensheath olfactory axons within both the CNS and PNS parts of the olfactory pathways. They coexpress the phenotypic features and functions with astrocytes and SCs[21]. The OECs cross the injured site, promote axonal growth and long-distance axonal remyelination, increase serotonergic axons, and provide several neurotrophic growth factors[16,19,23,27]. The dorsal root ganglion neurites myelination near and far from the cell injection site by these glial cells was observed, denoting large-scale migration of OECs within the lesion. OECs secrete neuregulins, NGF, BDNF, and glial cell line-derived neurotrophic factor (GDNF)[25].

Significant challenges and expert opinions about cell therapy

- The best cell source is still an argument. An essential item in stem cell choice of transplantation is their adaptability with the host tissue. Thus, the autologous transplantation of stem cells is preferred.
- The optimal number of cells for transplantation that equilibrates efficacy and safety, the characterization, expansion, increasing the survival of transplanted cells, addressing cell differentiation, and functional adhesion with the host circuitry are fundamental issues.
- The optimal dose of stem cells, single dose or continuous administration within several days, the suitable timing for delivery, delivery vehicle, a reservoir of pro-survival factors, and the minimally invasive but highly effective delivery methods (systemic or intrathecal transplantation) should be in attendance.
- The fate of transplanted cells in the host tissue must be traced to ensure proper cell release, holding them in the target site and preventing the scattering in cerebrospinal fluid.
- Decrease in the risks of stem cell treatment, such as the formation of aberrant stem cell colonies, teratomas, and differentiation into unwanted, non-regenerative cell types, are important issues.
- Ethical issues. The iPSCs, MSCs, OECs, and SCs are relatively free from ethical issues.
- Donor genetic patterns, the location from which cells were captured, and the developmental phase at which the cells were isolated differ.

Cell transplantation protocols could be applied to improve cell survival, such as biomaterials or growth factor modulations, to certify that the ideal cell types are refilled in the injury site. For example, applying polymeric scaffolds can decrease the number of cells needed, guarantee proper cell release, prohibit cerebrospinal fluid dispersion, and maintain them in the target site. The potency of cell products is difficult to define. Thus current good manufacturing practice (cGMP) requires examining the indicators of potency in desired cells and contaminants.

Multiple cell types may be used simultaneously to increase the efficacy of cell therapy[20].

2-Tissue engineering products

Cell transplantation cannot promote tissue regeneration in the chronic stage of spinal cord injury with central cystic cavities. Therefore, tissue repair requires "bridging" the lesion with an allowable material, which fills the gap and provides constructive support for axonal regrowth. Biomaterials in SCI are applied based on two strategies. At first, the biomaterials are provided as a supporting vehicle or carrier scaffold for encapsulating specific cells, drugs, growth factors, or genes for delivery to promote endogenous tissue integration and axonal growth[21]. The second tactic is using biomaterials that imitate the electromechanical properties of soft tissue, bridge the lesion and fill the cavity, fix the injury site, and furnish mechanical support for interactions between the environment with the host or transplanted cells[15,16,19].

Synthetic or natural scaffolds (degradable or non-degradable) can be implanted in solid with guiding tubular channels/fibers or injectable in situ forming hydrogels for small or irregular cavities with their advantages and disadvantages (Table4). Acellular biomaterials are avoided intricate problems associated with the use of cells. Decellularized spinal cord tissue preserves the extracellular matrix (ECM) composition and structure and enhances the axonal regeneration of the spine[15].

Table4: Summary of advantages and disadvantages of tissue engineering products.

Tissue engineering products reference number	Advantages	Disadvantages
Synthetic materials[14,20,21,22,24]	electrostatic forces, steric hindrance,polymeric entanglement, high flexibility, no toxicity, gas permeability, good mechanical properties, reversible, remain localized wherever they are injected, deliver the loaded molecules to the spinal cord,easy to sterilize, easily controlled and modified according to our needs	inadequacies to control the delivery, the adverse loading of hydrophobic molecules, lack recognition signals, poor biocompatibility
Natural products[14,20,21,22,24]	easily obtained from natural sources, predictable physical, highly controlled synthesis, resulting in regular structures, biodegradable, contain signals for cell adhesion	hard to be sterilized, low reproducibility of the research results, low mechanical strength

2-1-Hydrogels

Hydrogels are 3D biocompatible polymeric soft matter, either chemically with high water content or physically cross-linked materials, that can closely mimic and integrate the aqueous environment of the ECM molecules of the CNS architecture[23,25]. Hydrogels are injected in a noninvasive intrathecal manner and fill lesion cavities. These materials can act as reservoirs for a sustained release of molecules and cells. Therefore, hydrogels support cell survival and integration, modulate the innate immune response, reduce scarring, and guide axonal regeneration[15,19,22,25]. Loaded hydrogels release drugs and biomolecule factors secreted by stem cells or maintain them to fill the gap at the lesion site[15,16].

2-2- Scaffolds

At the chronic stage, using fibers and conduits could better guide neural regeneration[15]. Scaffolds are based on particular extracellular matrix molecules (fibrin, collagen, fibronectin), other natural polymers (alginate, agarose, chitosan) or synthetic polymers (as poly (α-hydroxy acids), poly (2-hydroxyethyl methacrylate), polyethylene glycol). They supply structural and active growth support for damaged axons. Some biomaterials have biologically active peptide sequences. Scaffolds act as the mechanical and trophic support, enhance cell survival and integration of stem cells at the seeding site, deliver the therapeutic factors locally, avoid any systemic side effects, and discourage the scar through the lesion site[22].

Significant challenges and expert opinions about tissue engineering products

- The keynote of the success of scaffolds is the use and proper biomaterial selection to support nerve growth through the cavity lesion, tailored drug releases, the delivery system, and timing of intervention.

· For injectable hydrogels, some indexes such as mesh size, set time, and softness should be improved during the systematic assessment.

- Non-biodegradable synthetic materials have problematic safety issues regarding foreign body reactions in clinical implantation. Safety in polymeric devices involves biomaterials and their degradation products.
- Advanced studies on how biomaterials adjust cellular activity, biosafety, and efficacy must be inscribed in their clinical applications.
- An essential problem with naturally harvested biocompatible materials is higher batch-to-batch variability. Thus immune reaction is introduced.
- Because of irregular or multiple-shaped cavities in complex SCIs, the configuration of the scaffolds may not fit into the cavity and thus include integration difficulties. Also, the surgery of scaffold implantation may be complicated.

The evolution of the 3D architecture of scaffolds to currently two-dimensional structures is a novel era. The

biomaterials utilized in a scaffold should have mechanical and chemical properties similar to the spinal cord. Gel pattern is a relatively new technique in this area, and hydrogels have preferable properties for SCI repair. Expanding the newest biomaterials, which imitate the natural stem cell niche's microenvironment for supporting cell growth effectively while supplying structural reliance simultaneously, could maintain the key to success in neuroregeneration for SCI. The next generation of scaffolds likely would include innovative, multifunctional materials that can sense, react, stimulate, and announce with remote control.

3-Human cell and tissue derivatives

Stem cells release helpful factors in the treatment of SCI. Stem cells secrete extracellular vesicles (EVs): microvesicles and exosomes. They are mediators between cells and can simulate the action of stem cells by transporting the bioactive molecules from stem cells to damaged cells. The stem cell-conditioned medium contains different regenerative factors such as vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), transforming growth factor β1 (TGF-β1), and hepatic growth factor (HGF). Furthermore, stem cell-derived exosomes have immunomodulatory action, such as the recruitment and modulation of the function of macrophages[23].

Significant challenges and expert opinions about human cell and tissue derivatives

- The big challenge is the low quantity of factors secreted and the minimum amount needed to obtain a functional outcome.
- The cell-derived exosomes are minimally-invasive procedures for SCI. Therefore, it is essential to have a profound realization of their effect to provide progress in novel therapeutic methods.

The transfer of specific growth factors may cause more desirable results regarding regeneration rather than stem cells.

4-Human gene therapy products

Stem cells are the primary goal for gene therapy to generate several trophic factors necessary for the regeneration of injured nerve tissue and increase the efficacy of cell therapy[27]. The exogenous utilization of neurotrophic factors has severe functional limitations as fast degradation. Thus, in gene therapy, nontoxic, non-immunogenic viral vectors with long-term transgene expression are developed[23].

The NSCs are often genetically modified to create a variety of growth-stimulating factors, including BDNF, NGF, GDNF, neurotrophin 3, or their receptors[25,28]. The genetically modified MSCs represent a multi-neurotrophin that attaches to the growth factor receptors, reduces cystic cavity size, enhances axonal growth, promotes angiogenesis, and modifies glial scar formation[16]. The genetically modified fibroblasts secrete BDNF and NT-3, intensifying the regeneration of spinal axons[27].

Besides the cells, scaffolds can be genetically modified. Collagen-based neural conduits, which are utilized with the NT-3 gene, increase NT-3 levels in surrounding tissues and cause axonal regeneration[15].

New technologies for gene editing, such as the clustered regularly interspaced short palindromic repeats (CRISPR), can aid in expanding methods to raise the ability of stem-cell-based therapies.

Significant challenges and expert opinions about gene therapy

- It is necessary to determine which Adeno-Associated Viral Vector (AAV) serotypes might be selected to achieve a safe, specific, low variable immunogenicity, widespread, and controlled transduction of the spine.
- Delivery of the cell is a trouble for the clinical application of small interfering RNA (siRNA). Because the naked siRNA is susceptible to degradation in the bloodstream, renal clearance and incomplete influx into cells are problematic.

Lentivirus delivery could be incorporated into biomaterial scaffolds to improve lentivirus retention and efficacy through the injury site to mitigate gene delivery risks. Besides, gene editing methods such as CRISPR/Cas9 can efficiently handle the overexpression of neurotrophic factors in spinal cord tissue.

5-Xenogeneic cell products

Table5 summarizes the xenogeneic cell biomolecules for regenerative medicine therapies in chronic SCI. In chronic SCI, the overall balance is changed towards an inhibitory environment because of the lack of neurotrophic support. Therefore, one essential strategy is stimulating axonal regeneration with neurotrophic

factors. The exogenous use of neurotrophins (BDNF, NT-3, and neurotrophin 4/S [NT-4/S]) augments the amount of supraspinal axonal growth within the transplant[24,27].

One of the critical inhibitory components of the glial scar is chondroitin sulfate proteoglycans (CSPGs). The bacterial enzyme chondroitinase ABC (ChABC) can degrade the CSPGs, leads to axonal regeneration, and increases synaptic plasticity[24].

Significant challenges and expert opinions about xenogeneic cell products

- It is unclear what is the optimal timeframe and duration of molecules to counteract the inhibitory environment of the spine.
- The therapeutic application of neurotrophins is restricted by their short half-life and difficulty passing the blood-spinal cord barrier (BSCB). Since the BSCB confers a significant obstacle to the systemic delivery of drugs, a better figuring out of the BSCB will aid in setting up a precise therapeutic strategy for increased regenerative treatment efficacy.
- New products will require comprehensive preclinical characterization, targeting more specific SCI pathologies, and the use of new imaging technologies and biomarkers to improve the evaluation of outcomes.

Improvements in our understanding of the pathophysiology of SCI offer advances in regenerative therapies.

Table5: Xenogeneic cell biomolecules for regenerative medicine therapies in chronic SCI.

Mechanism of action [reference number]	**Examples**
Anti-inhibitory molecules[14,17, 18, 21,22, 23, 24,27]	Bacterial enzyme chondroitinase ABC (ChABC), Pregabalin, Rho-ROCK inhibitor, Granulocyte-colony stimulating factor (G-CSF), Cethrin/VX-210, Anti-Nogo-A monoclonal antibody
Blocking receptors of inhibitory molecules[14,17, 18, 21, 22,23, 27]	NgR receptor of Nogo-A antagonists, EphrinB3 and sema4D antibodies, PTPs receptor of CSPGs blocking, Inhibition of phosphatase and tensin homolog (PTEN)
Enhancing endogenous repair mechanisms[14,17,18, 21, 22, 23,24,27]	Neurotrophin-3 (NT-3), Brain-derived neurotrophic factor (BDNF), Neurotrophin 4/S (NT-4/S)
Promoting axonal regeneration[14,17,18, 20,21, 22, 23, 24, 27]	Epothilone B, Taxol, Monoamine receptors or L-amino acid decarboxylase (AADC) inhibitors, CNTF, Transforming growth factor B, Insulin-like growth factor, Basic fibroblast growth factor (bFGF)
Stem cell recruitment and reprogramming[23]	Stromal cell-derived factor-1a (SDF-1a), Transcription factor Sox2, Valproic acid
Neuroprotective therapies[14,17, 21,27]	Riluzole, Magnesium, Minocycline, Hepatocyte growth factor (HGF), G-CSF
Immunomodulation biomolecules[14]	IL-4, IL-10, blocking the IL-7, Fractalkine
Neural regenerating growth factors[14,18, 20, 22, 27]	Connective tissue growth factor (CTGF), Nerve growth factor, Brain-derived neurotrophic factor (BDNF), Beurotrophin-3, Ciliary neurotrophic factor, Glial cell-derived neurotrophic factor, Leukemia inhibitory factor, Epidermal growth factor, Vascular endothelial growth factor, Noggin, Sonic hedgehog, Bone morphogenetic protein, , NT-3, CNTF, FGF2, Transforming growth factor-β
Scar formation disruption[21]	Protein tyrosine phosphatase σ (PTPσ), Chondroitinase ABC (ChABC), Hyaluronidase
Extracellular matrix proteins[14, 21,24]	Laminin, Fibronectin, Collagen I/III and IV
Promote healing by promoting the production of growth factors[17]	Epigallocatechin-3-gallat [EGCG], Plumbagin, Olive oil, Luteolin, Resveratrol, Polydatin, Curcumin, Withaferin A
Oligodendrocyte proliferation and myelination of regenerating axons[14,17,21,22, 23,24,27]	NT-3, BDNF
Target the neuron's intracellular metabolic response (axonal regeneration through a cAMP-independent pathway)[17,22]	Inosine, Daidzein (a soy isoflavone)

6-Combination procedures

The neuropathology changes of SCI have complex nature. Therefore, combinatorial methods, including biomaterials, cells, and molecular therapy, that address different appearances of the injury are more efficient [15,16,21,25].

6-1-Biomaterials and cells

Natural or synthetic biomaterials can serve as a carrier for improving the survival and neuronal differentiation of transplanted stem cells[21]. Biomaterials can express neurotrophic factors and direct axonal regeneration[15]. The cells encapsulated in biocompatible scaffolds gradually release, and the nerve growth and repair process is guided and tuned[19]. Thus, the number of cells needed to receive a therapeutic result decreases.

Different combinations of biomaterials with cells, including hyaluronic acid hydrogels with NSPCs, self-assembling peptides with NSPCs, PEG hydrogels with human iPSC-derived NSPCs, MSC-seeded alginate hydrogel, MSCs combined with a collagen sponge, polyurethane-based gel with MSCs, Matrigel with SCs, and fibronectin-mimetic, peptide-grafted gellan gum hydrogel with ADSCs and OECs led to increased axonal regeneration[15].

6-2- Biomaterials and soluble biomolecules

By mixing soluble diffusible bioactive molecules (e.g., growth factors, drugs, or other therapeutic agents) and even gene therapies in polymeric structure, delivery of the therapeutic agents can control, therapeutic effects are maximized, and the restrictions of the systemic providing, short half-life, and cytotoxicity are overcome[23]. Biomaterials can also retain proper local therapeutic concentrations at or close to the injury site for extended periods than bolus administration or even trigger endogenous regeneration within stem cell niches [15,19].

6-3- Cells and soluble biomolecules

In some studies, cells are applied as delivery vehicles for specific factors. For example, the astrocytes transfected to overexpress NGF and encapsulated in a collagen scaffold, significantly enhancing axonal growth. ESC-derived OPCs, in combination with ciliary neurotrophic factor (CNTF), lead to significant improvement in hind limb locomotor function[15].

6-4- Biomaterials, cells, and soluble biomolecules

Combining stem cells, biomaterials, and biomolecules demonstrates a promising strategy that may better recover after SCI. A simple fibrin matrix, including a growth factor cocktail and human NSPCs, improves extensive engraftment with host neuronal circuits. In the other study, a scaffold containing SCs modified to overexpress NT-3 and adult stem cells modified to overexpress the NT-3 receptor TrkC. Results show that NSPCs can be incorporated into the host tissue and act as neuronal boosters through SCI. A hydrogel delivery platform in combination with chondroitinase ABC and human iPSCs results in better survival of both host and transplanted cells. In another study, alginate is seeded with SCs, and adenovirus encoding BDNF is delivered caudally to the lesion to encourage axons to regenerate along the rostral-caudal axis[15]. A fibrin delivery vehicle combined with nine growth factors and a neural cell death inhibitor increases the differentiation of large portions of transplanted NSCs into neurons, forming synapses with spinal projections and integrating into the neuronal network [24].

Discussion

The pathophysiology of SCI is multidimensional and complex. Understanding how damage is detected at the cellular level and which internal signaling events trigger and coordinate inhibitory responses in the axon and soma is critical to understanding the mechanisms that control innate regenerative capacity. Following an injury in the adult CNS, regenerating axons need to grow long distances to reach their targets. This long regrowth period requires a steady supply of membranes, cytoskeleton, organelles, and other building blocks for the growth tip (growth cone)[29].

Even though this is only a tiny review of the literature supporting spine repair, it is evident that cell therapy and biomaterial are excellent candidates for clinical applications as regenerative therapeutic strategies in chronic SCI. Cell-based therapies propose the most promising results because these cells can comply with multifactorial functions. Even multicellular transplantation may be more beneficial for the patient's functional improvement[30]. Further studies are needed to evaluate the effects and clarify the mechanisms of these factors on stem cell therapy in SCI[31]. Also, the role of biomaterials is gaining importance, both acting as vehicles for the cells or as vectors of therapeutic agents and filling the cysts in the case of chronic lesions. Although, combination therapies will probably be required to attain satisfactory outcomes.

Conclusion

This review summarizes accumulating knowledge about regenerative medicine therapies for chronic spinal cord injury. In addition, we highlight potential challenges. The ultimate goals for managing chronic SCI are to reduce cell death, minimize the extent of the injury, and facilitate the process of neuro-regeneration.

We did not differentiate in-vitro research, animal studies, and clinical trials. While there are differences between animals and humans in biological properties, the extrapolation of results can be difficult. There is not a detailed study for comparing different methods of identical situations. Research grades to stem cell therapies differ from clinical grade cells; thus, translating results is problematic.

Axonal regeneration does not always equal functional recovery. Specific research should be made to identify unique populations of axons responsible for functional recovery.

In the future, successful strategies for the treatment of chronic SCI will likely include the integration of several advances in various fields of regenerative therapies in minimally invasive approaches, such as regulating the growth of particular pathways in particular ways, with blocking the axonal sprouting of some neural pathways, while increasing the regenerative growth of other pathways. The regeneration of axons below the spinal cord lesion remains a significant problem.

Moreover, a multidisciplinary approach involving researchers, clinicians, pharmacologists, materials scientists, and bioengineers will be essential to treat chronic SCI successfully.

This review aimed not to comprehensively analyze the various studies conducted in regenerative medicine regarding chronic SCI. There is a large amount of information, in vivo, in vitro, and clinical trials on nerve regeneration for SCI, making it hard to follow the advances in the field and the advantages and disadvantages of each method. This article aims to gather all the puzzle pieces to provide insight into the recent advances in regenerative therapies for chronic SCI. Many questions should be inscribed to maximize future research studies' output.

It is understood that a combinatorial therapeutic approach to the scaffolds with stem cells and/or growth factors and biomolecules such as the enzyme ChABC will likely provide the definitive solution to regulate the balance between the inhibitory and excitatory factors implicated in neuro-regeneration.

Glossary

Neuropathology: Pathology of the nervous system.

Histopathology: A branch of pathology concerned with the tissue changes characteristic of disease. The tissue changes that affect a part or accompany a disease.

Pathophysiology: The physiology of abnormal states.

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Authors' Contributions

Dr. Reyhaneh Abolghasemi is the principal manager and contributor in writing the manuscript. She created the primary conception and design of the paper. Dr. Mahdi Sharif-Alhoseini revised and approved the manuscript. Mrs. Managol Kayyal and Mr. Navid Jamali approved the final manuscript for publication. Dr. Esmat Davoudi-Monfared, Dr.Fakhri Allahyari, and Dr.Gholamreza Farzanegan helped revise the article and fix its problems.

Ethical Statement

This review was written under the supervision of the Ethics Committee of the Ministry of Health, Medical Education and Treatment of Iran (Ethical code: IR.BMSU.REC.1401.101). The authors have respected the ownership rights of the articles used for the review. The authors avoided plagiarism. The authors inform us that the present manuscript has neither been published nor is currently under consideration for publication by any other journal.

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