REVIEW ARTICLE

Application of Chitosan Hydrogels in Traumatic Spinal Cord Injury; A Therapeutic Approach Based on the Anti-inflammatory and Antioxidant Properties of Selenium Nanoparticles

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Abstract

Purpose: The pathophysiological progression of traumatic Spinal Cord Injury (SCI) includes primary and secondary injury. Secondary injury destroys the spinal cord tissue and neurological disorders. After primary mechanical damage, inflammation is the most important factor inducing astrogliosis and scar formation. The activation of inflammatory cells in the area of damage produces free radicals, all of which damage cell membranes. A significant level of oxygen-free radical production is involved in the pathology of SCI. Therefore, limiting secondary damage is very important in the clinical treatment of acute traumatic spinal cord injury.

Materials and Methods: In this review article, the articles indexed in various databases were used. The collection of articles was evaluated without time constraints using keywords inducing traumatic SCI, inflammation, oxidative stress, chitosan, and selenium nanoparticles.

Results: Inflammation and oxygen-free radicals play a key role in secondary damage after SCI. Therefore, as a new therapeutic approach, the use of hydrogels based on chitosan has been considered in SCI. The biocompatibility and biological properties of chitosan have made it considered a suitable material for nerve regeneration.

Conclusion: The use of reactive oxygen species scavengers, including metal nanoparticles, can control inflammation and oxidative stress in spinal cord injuries. Selenium nanoparticle treatment may reduce secondary damage in SCI by using its anti-inflammatory and antioxidant properties. Therefore, the use of selenium nanoparticles in the chitosan hydrogel bed can control the degeneration and functional improvement of the nerve tissue of the spinal cord.

Keywords: Traumatic Spinal Cord Injury; Inflammation; Oxidative Stress; Chitosan; Selenium Nanoparticles.

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1. Introduction

Both traumatic and non-traumatic forms of injury can lead to spinal cord injuries. Physical injuries to the spinal cord such as sports injuries, motor vehicle accidents, falls, etc. lead to spinal cord injuries [1, 2]. Traumatic Spinal Cord Injury (SCI) leads to sensory and motor disorders, neurological bladder, and autonomic nervous system disorders throughout the patient's life [3-5]. It has been stated that spinal cord injury consists of 2 stages: the first stage which includes bleeding and direct cell death and occurs immediately after the injury [6, 7], and the second stage, which is a series of progressive complex events that occurs from a few minutes to a few weeks after the injury, and includes progressive ischemia, intravascular thrombosis, and infiltration of immune cells (macrophages, microglia, neutrophils, and lymphocytes) [8, 9]. The presence of inflammatory cells and their secretions causes the formation of inflammatory microenvironments. Hypertrophy and activation of glial cells such as microglia and astrocytes cause the formation of glial scars in the injured area [10].

Severe or prolonged inflammation is often more harmful than the initial injury. Therefore, it is necessary to reduce the severity and duration of inflammation to accelerate the tissue healing process or prevent the neurodegeneration cascade [11]. Prostaglandins, leukotrienes, thromboxane A2, and nitric oxide accelerate inflammatory processes. Prostaglandins are produced by cell membranes through the cyclooxygenase pathway and leukotrienes are produced through the lipoxygenase pathway [11]. Therefore, inhibition of these 2 pathways in damaged tissues prevents the production of these inflammatory mediators and therefore prevents the prolongation of inflammatory processes that cause damage to body tissues. Usually, a high dose of methylprednisolone sodium succinate is used as the only recommended neuroprotective drug available to reduce the adverse effects of secondary damage after acute traumatic spinal cord injury [4, 12]. However, this treatment method is very controversial because this intervention not only leads to a relatively poor therapeutic outcome, but also causes many potential complications such as pneumonia, infection, sepsis, pulmonary embolism, corticosteroid myopathy, and gastrointestinal bleeding [3, 13]. Therefore, it is very necessary to find effective treatment strategies to reduce the adverse effects of traumatic SCIs. The noticeable increase in the levels of Reactive Oxygen Species (ROS) in the injured spinal cord plays a vital role in the occurrence of secondary injuries. The excessive increase of ROS, which is mainly caused by damaged mitochondria, oxidation of Nicotinamide Adenine Dinucleotide Phosphate (NADPH), inflammatory cells, and Fenton reaction are considered unfavorable microenvironments caused by secondary damage [14, 15]. An excessive increase in ROS can cause severe lipid peroxidation, oxidative damage to proteins and Deoxyribonucleic Acids (DNAs), which leads to degeneration and demyelination of nerve fibers and apoptosis of nerve cells in the damaged areas [16-20] (Figure 1).

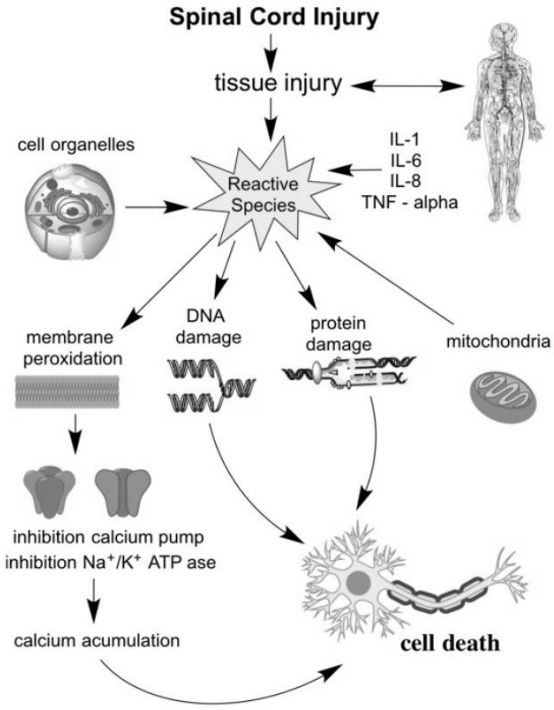

Figure 1. Oxidative stress action in spinal cord injury [21]

In addition, the easy and fast distribution of ROS in the areas adjacent to the damaged tissue often causes the expansion of the damaged area, which leads to the aggravation of secondary damage and serious movement defects [22]. Therefore, ROS scavengers can have effective positive effects in reducing the severity of complications caused by secondary spinal cord injuries, which should be the focus of attention in the treatment of traumatic spinal cord injuries [23, 24].

It should be noted that after damage to the nervous system, not only recovery is difficult, but secondary

disorders also occur in other parts of the body [25]. Therefore, the use of biological materials has been increasingly considered as a potential treatment strategy for SCI; because they can be a substitute for the extracellular matrix at the site of injury [26]. The properties of these materials are 1- supporting endogenous tissue repair; 2- axon growth guidance [27]; 3- enhancing graft survival in cell transplantation [28]; 4- local delivery of drugs [29] and 5- covering the damaged skin [30]. Chitosan, which is derived from chitin, is a type of renewable polymer (a type of cationic polysaccharide) and is the most abundant natural polymer after cellulose. Chitosan is a deacetylated polymer of acetylglucosamine obtained after the alkaline deacetylation of chitin. Chitosan consists of glucosamine and N-acetyl-glucosamine copolymers, and the specialized word chitosan includes a set of polymers that have different molecular weights (in the range of 10,000 to 2 million daltons) [31]. Currently, chitin and chitosan are commercially produced on a large scale from the outer shell of shrimp, crabs, and king shrimp. The biocompatibility and biodegradability properties of chitosan have made it considered a suitable material for nerve regeneration [32]. It has been reported that the cultured nervous cells on the chitosan membrane can grow well and chitosan can improve the peripheral nervous system [33]. The properties of adhesion to nerve cells and nerve growth of both chitin and chitosan indicate the potential of these materials for scaffolds in neural tissue engineering [34].

The combined use of other materials, including metal elements and metal nanoparticles with chitosan, has also been one of the topics of interest to researchers in the tissue engineering of spinal cord injuries. One of these materials is selenium. Selenium participates in the antioxidant defense system, the immune system, and the metabolism of thyroid hormones [35]. Selenium is an essential element and an integral part of many proteins with catalytic and structural functions. Selenium appears in different forms such as selenomethionine, selenite, selenite, and selenocysteine. Selenium deficiency is directly related to the occurrence of health disorders based on the body's immune system [36]. This element is an essential component of antioxidant enzymes that protect cells against the side effects of free radicals produced during normal oxygen metabolism. Selenium, through an activity similar to glutathione peroxidase, reduces hydroperoxides and lipoperoxidases, which is important in protecting against oxidative damage caused by the increased activity of glutathione peroxidase. Of course, the use of selenium in the form of nanoparticles has been in the spotlight for several years.

In oxidative damage induced to cells by ROS, hydrogen peroxide is produced. It has been suggested that enzymes related to glutathione peroxidase play an important role in neutralizing hydrogen peroxide [11]. Recent studies have shown the positive effects of selenium nanoparticles on acute spinal cord injury [37-39].

Drug delivery to the central nervous system is considered the main challenge due to the existence of the blood-brain barrier along with the blood-spinal barrier and the blood-ocular barrier; since the mentioned barriers cause the drug delivery to the damaged area to be very complicated and the endothelial cells of the blood-brain barrier prevent paracellular and transcellular transfer due to the lack of pores, lack of activity of endocytic vesicles, and also the activity of Metabolic rates above this barrier is limiting [40, 41]. However, it has been shown that selenium nanoparticles can cross the blood-brain barrier [42] and these nanoparticles can inhibit cyclooxygenase 1 and 2 pathways [43]. It is also stated that selenium nanoparticles reduce the expression of inflammatory mediator genes such as Tumor Necrosis Factor alpha (TNF-α) and Prostaglandin E2 (PGE2) [44]. Therefore, it is possible to use selenium nanoparticles as a promising treatment in patients with SCI.

In general, inflammation and oxidative stress are the most important mechanisms of the progression of traumatic SCI. Therefore, various compounds with antioxidant and anti-inflammatory properties can be widely used in the treatment of spinal injuries. On the other hand, by using the unique benefits of chitosan in nerve tissue engineering and the anti-inflammatory and antioxidant properties of selenium, we can expect to control progressive traumatic spinal cord injuries.

Therefore, in this article, the role of inflammation and oxidative stress in spinal cord injury is investigated first, and then the benefits of chitosan and its hydrogels in neural tissue engineering and the anti-inflammatory and antioxidant effects of selenium in the management of these injuries are reviewed. Finally, the synthesis and application of the combination of chitosan hydrogel and selenium nanoparticles as a therapeutic approach in SCI are reviewed.

2. Materials and Methods

Articles on the related topic were searched in the following databases: Science Direct, Scopus, Springer Science, Pubmed, and Google scholar to be used in writing this review article. Articles were reviewed without time limits. A total related research papers included quantitative and qualitative research in English. The review article is written according to the keywords "Surgical Stress, Systemic Inflammatory Response Syndrome, Pro-Inflammatory Cytokines, and Anti-Inflammatory Cytokines".

3. Inflammation and SCI

Inflammation is known as an important part of the immune system, which is basically a protective response against tissue ischemia, tissue damage, autoimmune responses, and infectious compounds [45]. Leukocytes, which include granulocytes, neutrophils, lymphocytes, and macrophages, migrate to the center of the spinal cord after an injury. They not only participate in promoting the inflammatory process of spinal cord injury, but are also involved in limiting the damage and healing the damage due to their anti-inflammatory effects and secretions [46]. The increase of astrocytes in the area of damage creates a physical barrier against the transfer of endogenous stem cells and effective nerve growth factors. It has been stated that the delay in the death of nerve cells prevents secondary injuries caused by the destruction of the central nervous system, which ultimately reduces the formation of the spinal cord and glial scar [47]. It has been shown that neutrophils and macrophages enter the spinal cord after SCI in a coordinated period of time. Neutrophils, which accumulate at the site of injury within 1 hour, are most abundant within 24 hours and begin to decline within 48 hours [48]. In fact, neutrophils cross the bloodstream and enter the spinal cord within the first hour after injury [49]. The presence of neutrophils is mostly limited to the acute stage of SCI and they are rarely seen in the sub-acute stage of spinal cord injury [50]. Neutrophils cause the release of inflammatory cytokines, proteases, and free radicals-which destroy the extracellular matrix - activate astrocytes and microglia, and cause neuroinflammation [51]. Although neutrophils are commonly associated with tissue injury, their depletion compromises the healing process and prevents functional improvement [52]. Stimulated neutrophils have been shown to release interleukin-1 (IL-1) receptor antagonist, which can exert neuroprotective effects following SCI [53]. Furthermore, the removal of neutrophils results in altered expression of cytokines and chemokines and downregulation of growth factors including Fibroblast Growth Factors (FGFs), Vascular Endothelial Growth Factors (VEGFs), and Bone Morphogenetic Proteins (BMPs) in the injured spinal cord, which appear to impair the healing process becomes normal [52]. Indeed, spinal cord injury induces a Central Nervous System (CNS)-specific autoimmune response in the thymus and bone marrow (T and B) cells that remain chronically activated. Autoreactive T cells can have direct toxic effects on neurons and glial cells [54, 55]. In addition, T cells can indirectly affect neuronal function and survival through in vivo production of pro-inflammatory cytokines and chemokines (including IL-1β, TNF-α, IL-12, C-C motif chemokine 2 (CCL2), CCL5, and C-X-C motif chemokine ligand 10 (CXCL10)) to be influential [54]. Genetic deletion of T cells (in athymic hairless rats) or pharmacological inhibition of T cells (using cyclosporine A and tacrolimus) leads to improved tissue preservation and function after SCI [54], suggesting the role of T cells in Pathophysiology and repair of SCI. Serological evaluation of patients with SCI shows high levels of CNS-reactive IgM and Immunoglobulin G (IgG) isotypes, confirming SCI-induced autoimmune activity of T and B cells [55].

Lymphoid organs are the source of monocytes/macrophages infiltrating the injured spinal cord area [56]. Monocytes/macrophages are seen in the injured spinal cord within the first week after injury [57], and macrophage activation is maximal within 7 to 14 days after injury [58]. M1 macrophages produce pro-inflammatory cytokines such as TNF-α and interleukin 1 (IL-1), ROS, and Nitric Oxide (NO), which are involved in inflammation and injury. M2 macrophages induced by T helper 2 (Th2) cytokines produce anti-inflammatory factors such as interleukin 10 and transforming growth factor-beta (TGF-β) and also reduce the production capacity of pro-inflammatory molecules and with these mechanisms, they play a role in wound healing and tissue regeneration. T helper 1 cells cause neuropathic pain by secreting pro-inflammatory cytokines such as interferon gamma (IFNγ) and IL-2, while helper T cells 2 by secreting anti-inflammatory cytokines such as IL-2 -10, IL-13, and IL-4 block this pain [59].

As with other inflammatory cells, the relative contribution of macrophages to injury or repair is determined by the collective effect of the function of those

cell types [60, 61]. Accumulated evidence to date suggests that type 1 inflammatory monocytes are recruited to the site of inflammation/injury via the Monocyte Chemoattractant Protein-1 (MCP-1) receptor, C-C Chemokine Receptor type 2 (CCR2) and are primarily involved in inflammation, proteolysis, and phagocytosis. Another subset of monocytes that has recently received significant attention is the so-called type 2 resident monocytes, which apparently play a role in the healing process [62]. Systemic inflammatory responses caused by SCI increase the number of inflammatory cells in the bloodstream and involved tissues, activate immune cells, and induce the secretion of pro-inflammatory cytokines. These events are involved in the pathogenesis of various organs after SCI. In fact, SCI can cause Systemic Inflammatory Response Syndrome (SIRS) that affects other organs of the body [37]. These systemic responses may affect the progression or recovery of spinal cord injury. Innate immune cells (such as monocytes and macrophages) initiate the primary inflammatory response in patients with SIRS. SIRS caused by SCI is considered a life-threatening condition [63]. After the primary spinal cord injury, the cascade of secondary injury occurs, which causes gradual degeneration in the parenchyma of the spinal cord (Figure 2).

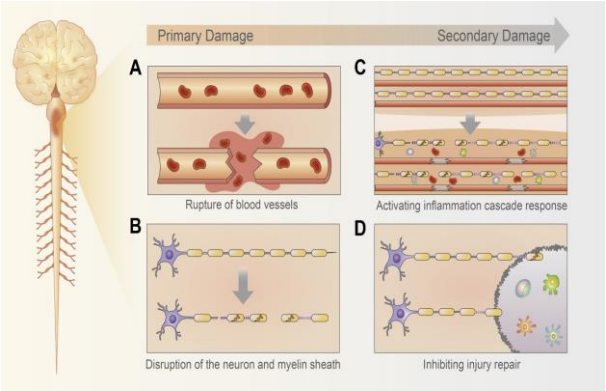

Figure 2. Primary and secondary injuries following SCI. Primary injury: (A) rupture of blood vessels in the spinal cord; (B) disruption of the myelin sheath, freeing of red blood cells from the injured area. Secondary injury: (C) various activated inflammatory cells accumulate in the injured area, producing an inflammatory cascade response; (D) forming glial scars, and inhibiting injury repair [64]

4. Oxidative Stress, ROS, and SCI

The creation of additional free radicals after any tissue damage causes an imbalance between the production of ROS and the antioxidant system. ROS includes oxygen anions, free radicals including superoxide, and hydroxyl radicals and peroxides such as hydrogen peroxide (H_2O_2) [65]. However, the overproduction of ROS in pathophysiological conditions, including SCI, may lead to oxidative stress [66]. An increase in ROS and a decrease in the level of antioxidants have been associated with the pathogenesis of neurological diseases such as depression and cognitive impairment [67]. Healthy central nervous tissues contain different concentrations of antioxidants (ascorbic acid, glutathione, ubiquinone, superoxide dismutase, catalase, and alpha-tocopherol). SCI leads to a cascade of secondary events, including the release of excitatory amino acids and disturbance in calcium homeostasis, which may cause further disruption of mitochondrial function, increased formation of ROS, and death of nerve cells [68]. In addition to mitochondria, phagocytic cells such as neutrophils and macrophages also play a role in ROS production following SCI [69]. Active parenchyma (microglia) and leukocytes (macrophages and neutrophils) are the main sources of ROS [66]. It has been shown that the formation of ROS including hydroxyl radical, superoxide and hydrogen peroxide immediately after CNS injury may contribute to the pathogenesis of SCI [70]. The influence of ROS in the pathogenesis of SCI has led to extensive studies on the neuroprotective effects of various compounds with antioxidant properties in animal models of SCI. The use of certain antioxidants may slow down the progression of spinal cord injury [65].

In fact, oxidative stress in cells is the result of an imbalance between oxidizing and antioxidant systems. When the production of free radicals exceeds the antioxidant defense capacity of the body to detoxify them, a condition called oxidative stress occurs, in such a way that it is not possible to increase the oxidants and the reduction of antioxidants is prevented and the oxidative/antioxidant balance changes to the oxidative side [71]. However, free radicals play an important role in the pathogenesis of SCI. Oxidative stress can be defined as an imbalance between high amounts of ROS and reactive nitrogen species and antioxidant defense mechanisms. Like ROS, active nitrogen species can also play an important role in the pathogenesis of many diseases, and in recent years special attention has been paid to it [71]. It has been mentioned that excessive production of reactive oxygen species has a major role in the occurrence of secondary damage caused by traumatic spinal cord injury [18, 22].

5. Chitosan and Drug Delivery Systems Based on Chitosan Hydrogel in Nervous Tissue

The use of chitosan as an important biological material is used for the development of drug delivery systems, tissue engineering scaffolds, and skin dressings due to its hemostatic properties and adhesion to mucous membranes. Chitosan is polyatomic in acidic environments and also can form gels in acidic pH environments because chitosan molecules are hydrophilic and can retain water in their structure. Acetylation of chitosan in hydrophilic environments provides the possibility of selective modification of free amino groups and is responsible for the gelatinization process. It has been shown that the density of the chain parts is an essential parameter for the formation of gels and all the factors that reduce this parameter facilitate the reduction of its large volume (swelling) and its reversibility [72]. Chitosan has been widely focused as a promising alternative therapy for nerve tissue regeneration/renewal [73-75], bone formation [76, 77], and cartilage tissue repair [78]. It should be noted that natural regeneration is a key stage for the healing of CNS injury. The unique properties of chitosan, including high compatibility, biodegradability, and non-toxicity, make it possible to use it as a scaffold in medicinal systems for the treatment of nervous tissue with nanoparticles. In particular, the three-dimensional (3D) porous scaffolds created by chitosan can create a suitable microenvironment for nerve tissue differentiation [79]. Drug delivery systems based on nanoparticles have advantages such as encapsulation of nanoparticles with CNS-targeted drugs, which increase drug effectiveness and site response. In addition, nanoparticles make it possible for the drug to be present in the blood circulation for a longer period of time, which strengthens its bioactivity as a result of a slow-release drug delivery system. It has been reported that chitosan can act as a membrane lining and most importantly as a nerve tissue protector [80]. It has been stated that chitosan may inhibit the synthesis of some inflammatory mediators such as IL-1β, reduce oxidative stress metabolites, and encourage the production of some anti-inflammatory markers such as IL-10 [81].

Hydrogels are composed of cross-linked polymeric networks that contain large amounts of hydrophilic groups and domains. These networks have a great affinity for water, but due to the chemical and physical bonds created between polymer chains, their dissolution in water is prevented. The presence of water in these networks causes swelling and hydrogel formation. Fully swollen hydrogels have some common physical properties of living tissues, including soft and elastic consistency and interfacial tension with water or biological fluids [82, 83]. Hydrogels are used to support cells during tissue regeneration and in the delivery of controlled-release drug systems [84]. A new therapeutic approach for repairing the central nervous system is using chitosan-based hydrogels [26, 85]. It has been stated that chitosan-based scaffolds are excellent support for neural stem cells [86]. The capacity of chitosan as a covering of nerve membranes has been determined, and in addition, it has been reported that chitosan hydrogel implant in the spinal cord of rats immediately after SCI causes the control of spinal cord degeneration and the reduction of glial fibrosis wound, as well as the weakening of inflammatory responses [11, 63]. In addition, it has been proven that chitosan supports the adhesion of nerve cells and nerve growth [86]. Chitosan has been reported to be a relatively inert biological material that does not cause a chronic immune response and is suitable for a long-term spinal cord repair program [26]. It should be noted that one of the most important factors determining the choice of biological materials for the treatment of the spinal cord is its biocompatibility, and it seems that chitosan achieves this goal [87].

6. Selenium as an Anti-Inflammatory in SCI

Evidence suggests that selenium regulates the inflammatory response through several complex pathways [88] and as a very important component of the antioxidant system may play a key role in modulating immunological parameters [89]. The anti-inflammatory capacity of selenium is dependent on its effect on immune cells and especially the signaling pathways of macrophages [88]. Among other anti-inflammatory activities of selenium, we can mention the regulation of the inflammatory response through the effect of selenium on the adhesion of monocytes to endothelial cells and its penetration into tissues [37]. In fact, monocytes adhere to the endothelium of blood vessels and become macrophages, which are the main agents of innate immunity in inflammation [90]. Adhesion of monocytes to vascular endothelium is mediated by L-selectin, which facilitates the migration of neutrophils during the immune response. It has been stated

that the oral administration of selenium prevents the binding of monocytes [91].

Selenium-containing supplements have reduced the endotoxin of lipopolysaccharides of gram-negative bacteria, and inhibited the gene expression of the main inflammatory cytokines such as TNF-α, and cyclooxygenase 2 by blocking the mitogen kinase active protein pathways. They restrain [37]. It has been shown that TNF-α is a strong inducer of adhesion molecules, which is important for enhancing the passage of leukocytes through the endothelium [92]. Therefore, the reduction of TNF-α after the administration of selenium causes a decrease in the adhesion of leukocytes to the endothelium [37]. It has been reported that selenium reduces the expression of TNF-α caused by adhesive molecules [93]; Therefore, increasing the levels of selenium may inhibit nuclear factor kappa light chain enhancer of activated B lymphocytes (NF-kB) by glutathione peroxidase and reduce inflammation [89].

In addition, selenium mediates the attachment of monocytes to endothelial cells and their migration to different tissues [37]. It has been reported that the binding of monocytes to vascular endothelium and their differentiation into macrophages, which are the main factors of the immune system in inflammation [90], is mediated by L-selectin. In fact, L-selectin inhibits the attachment of monocytes to endothelial cells [94]. Selenium nanoparticles can be used as micronutrients and as a substitute for antioxidant and anti-inflammatory drugs [95]. It has also been reported that selenium reduces apoptotic signaling related to the NF-kB pathway [96]. It has been mentioned that Selenium Nanoparticles (SeNPs) downregulate the mRNA expression of pro-inflammatory cytokines such as inducible Nitric Oxide Synthase (iNOS), IL-1, and TNF-α and reduce inflammation [97].

7. Selenium as an Antioxidant in SCI

Oxidative stress is one of the main factors involved in the pathogenesis of many neurological diseases. Therefore, therapy using natural antioxidant compounds has been developed to treat several neurological diseases. To protect against oxidative stress, living systems have a set of antioxidant enzymes such as Glutathione Peroxidase (GPx), Thioredoxin Reductase (TrxR), and iodothyronine deiodinases (IDD) and selenium are the main components of these antioxidant enzymes [98]. It has been mentioned that neurological disorders are often associated with oxidative stress and selenium compounds may show antioxidant and neuronal protection properties [99, 100]. Current evidence indicates the important role of selenium in redox regulation (oxidation-reduction reaction) [101]. Selenoproteins are very important for the normal function of activated T cells; Because T cells are sensitive to ROS and due to the lack of selenoproteins involved in reducing ROS, they cannot proliferate in response to the stimulation of T cell receptors [102]. Selenium nanoparticles can increase the activity of selenoenzymes with equal efficiency and less toxicity than selenite, selenium-methylselenocysteine, and selenomethionine [103].

The bioavailability, toxicity, and antioxidant activity of selenium depend on its chemical form. Selenomethionine is the most common form of selenium in food and supplements, although it is less toxic and has very good bioavailability, some have stated that its excessive use can lead to toxic effects [104]. Therefore, selenium nanoparticles can be used instead of selenomethionine due to their effects in upregulating glutathione peroxidase and thioredoxin reductase with much lower toxicity compared to it [105]. Of course, selenium is a GPx cofactor, so it can play an important antioxidant role. Selenium deficiency is associated with decreased GPx activity and thus with increased oxidative stress [106, 107]. Selenium protects against oxidative stress by enhancing a number of cellular antioxidants such as GPx, Superoxide Dismutase (SOD), catalase, and GSH [108]. Selenium is an important cofactor for the activity of GPx enzyme, which is involved in the oxidation of glutathione. Selenium is also required for the catalytic activity of mammalian thioredoxin reductase (another important antioxidant enzyme) [109]. Furthermore, when damaged and ischemic neural tissue is affected by lipid peroxidation of the cell membrane, it causes local edema and inflammation [110, 111].

As previously mentioned, the activation of inflammatory cells in the area of injury causes the production of free radicals such as nitric oxide, superoxide anion, and hydrogen peroxide, all of which are harmful to cell membranes. Therefore, inflammation and oxidative stress are both important and continuous components in the development of secondary events following primary traumatic injury. It was previously stated that selenium plays a very important role in the continuation of the physiological activity of the nervous system, such as signal transmission and its development [112]. Selenium is known to be a neuroprotective agent in a number of neurological diseases including epilepsy [112] as well as pain. The neuroprotective implications of selenium are attributed to

its ability to inhibit apoptosis [113] and modulate calcium entry through ion channels [114].

In SCI, damage or destruction of nerve cells can affect the consciousness and physical activity of the patient and may result in subsequent neurological complications. These complications and injuries cause pain, resentment, and high cost of medicine and care. On the other hand, the repair of spinal cord injuries is limited as part of the central nervous system, unlike the peripheral nervous system. Therefore, limited therapeutic approaches have been introduced during acute spinal cord injury. Therefore, the use of biocompatible scaffolds in the form of a hydrogel, especially chitosan, and its combination with efficient nanoparticles such as selenium can be a useful approach in the treatment of SCI. In the following, the synthesis of these two effective substances in the treatment of spinal injuries will be reviewed.

8. Preparation of Selenium Nanoparticles

Selenium nanoparticles are made through physical methods such as laser ablation, ultraviolet rays, and hydrothermal methods [115-117]. The chemical production of these nanoparticles is mediated by precipitation, acid decomposition, and catalytic reduction using ascorbic acid, glucose, sulfur dioxide, and sodium dodecyl sulfate [118, 119]. In the chemical synthesis method by catalytic reduction, selenium nanoparticles are made by chemical reduction of sodium selenite using glutathione (reduced form or electron absorber) and are stabilized by Bovine Serum Albumin (BSA) [63].

For this purpose, 3 milliliters (ml) of 25 millimolar (mM) ionic selenite (Na_2SeO_3) solution, 3 ml of 100 mM glutathione solution, and 0.15 g of bovine serum albumin were added to 9 ml of twice distilled water. Distillation is added in a sterile glass container. All the solutions are made in a sterile environment using a sterile glass container and double-distilled distilled water. After mixing the reactant solution, 1 mole of sodium hydroxide is added to the solution so that the pH of the solution reaches the alkaline environment. Selenium nanoparticles are formed immediately after the addition of sodium hydroxide, which is evidenced by the change in the color of the reactive solution from white to bright red. Then, the selenium nanoparticles are centrifuged with a solution centrifuge at 13000 revolutions per minute at a temperature of -4°C, and the collected selenium nanoparticles are sterilized with ultraviolet rays before being used in bacterial experiments [120]. The prepared selenium nanoparticles were collected in a 1.5 ml Eppendorf tube and kept at room temperature for further experiments.

8.1. Nanoparticle Size Analysis Using Dynamic Light Subtraction (DLS) Method

The dynamic light scattering/subtraction method is one of the most popular light scattering methods that can measure the size of particles distributed in solutions or suspensions on a scale of less than one micron to one nanometer using a Monochromatic light source to measure [121]. Dynamic Light Subtraction (DLS) analyzers are used to measure particles in solution or suspension in the range of 0.6-6000 nanometers (nm). The dynamic light subtraction method is also used to measure the hydrodynamic diameter. The main advantage of DLS is the short time required for measurement and the relatively low costs of the device; Therefore, DLS is a preferred method for measuring the size of nanoparticles [122]. Light scattering from a suspension containing nanoparticles fluctuates at a given time. In fact, light scattering has an inverse relationship with the diffusion coefficient of particles and can show information about particles with their sizes ranging from several nanometers to several micrometers. Despite this, the use of DLS to measure particle size in dilute solutions has become increasingly popular. Because the diffusion of particles in a solution is influenced by hydrodynamic and kinetic conditions. The repeatability and uncertainty of DLS measurements are often insufficient to achieve the goal of accurately measuring the true size of nanoparticles. Therefore, the mentioned effects in concentrated solutions should be considered more. Therefore, the extrapolation process of concentration and scattering angle is used in very small amounts to obtain the actual size of nanoparticles in solutions [123, 124].

It is possible to measure the particle diameter of selenium nanoparticle samples using the microtrac method and light dynamic subtraction, under the condition that the refractive index of water is 1.33 and the wavelength is 780 nm. To measure nanoparticles, the volume of 0.1 ml of selenium nanoparticles solution is placed in 2 ml of deionized water in a polystyrene cuvette and the particle size is measured at 25°C has been taken [125]. In various studies of the size of selenium nanoparticles using this method, 178 nm [126], in

the limit of 3-18 nm [127], and also in the range of 30-150 nm has been mentioned [128].

8.2. Determining the Characteristics of Nanoparticles Using a Scanning Electron Microscope (SEM)

Several microscopy techniques are commercially available today, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) being the most popular microscopes for nanoparticle analysis. A scanning electron microscope is a type of electron microscope that scans sample images using a high-energy electron beam in a horizontal line scanning pattern. The interaction of electrons with the atoms that make up the sample produces signals that have information about the topography of the sample's surface, its composition, and other properties such as electrical conductivity. This type of microscope is widely used and is regarded as the gold standard for determining the properties of nanoparticles. Therefore, the shape and size of selenium nanoparticles can be measured using SEM. In order to measure the size of nanoparticles using SEM, the sterilized nanoparticles are gently and carefully placed on the spot of the SEM, which is uniformly coated with gold, using adhesive tape. The nanoparticle sample is placed in a specific position in the SEM and scanned with different magnifications from x1500 to x3500 and at a voltage of 20-30 kilovolt (kV) [120, 125, 129, 130]. In a study using SEM, the shape of selenium nanoparticles was described as rods with a size of 74.29 nm [126]. In addition, in another study, the size of these nanoparticles using SEM was reported in the range of 50-150 nm [131].

8.3. Preparing Selenium Nanoparticles and Determining their Size Using Transmission Electron Microscopy (TEM)

In addition to the previous preparation method, to prepare selenium nanoparticles, one milliliter of 25 mM sodium selenite solution can be mixed with 4 ml of 25 mM glutathione solution containing 200, 20, and 2 milligrams (mg) of BSA. A vessel containing 200 mg of BSA was used to produce small-sized selenium nanoparticles, a vessel containing 20 mg of BSA was used to produce medium-sized selenium nanoparticles, and a vessel containing 2 mg of BSA was used to produce large-sized selenium nanoparticles (Table 1). After mixing the reactant solution, 1 mole of sodium hydroxide is added to the solution to bring the pH of the solution to the alkaline environment. The size of nanoparticles using a transmission electron microscope, the size of small nanoparticles was 15-5 nm, the size of medium nanoparticles was 20-60 nm, and the size of large nanoparticles was 80-200 nm (Table 2) [132]. In addition to another study in which selenium nanoparticles were used in spinal cord injury tissue, the size of the nanoparticles measured by transmission electron microscopy was 80-200 nm [37].

Further reduction of BSA values in glutathione and selenite oxidation reduction system makes the size of selenium nanoparticles exceed 200 nm. It has been shown that the size of selenium nanoparticles is dependent on the BSA concentration, in other words, as the BSA concentration increases, the size of the nanoparticles decreases (they have an opposite ratio). Selenium atoms have been shown to have the self-adhesive force and also these atoms can stick to proteins such as BSA [133]. The presence of BSA in the system of primary selenium atoms may disrupt the unlimited accumulation of selenium atoms, which causes them to accumulate in a certain amount. It has been said that selenium nanoparticles with a size of 20-60 nm have the same bioavailability as selenite [134].

Table 1. The size of selenium nanoparticles based on DLS, SEM, and TEM in two studies

	DLS	SEM- shape	TEM	Ref.
Size of selenium nanoparticle (nm)	178	74.29 – rod shape (rods-shape particles with the size of the particles was in the range 74.29 nm for SeNPs)	-	[126]
Size of selenium nanoparticle (nm)	-	-	80-200	[37]

Table 2. The size of selenium nanoparticles based on the amount of BSA and its measurement using TEM [132]

Amount of BSA (mg)	200	20	2
Size of selenium nanoparticle (nm)	5- 15	20- 60	80-200

9. Preparation of Chitosan Hydrogel Loaded with Selenium Nanoparticles

Chitosan is a type of linear polymer obtained through alkaline hydrolysis of chitin distillation and is considered as a stabilizing agent for the production of selenium nanoparticles [135, 136]. Due to the cationic nature and high capacity of chitosan in hydrogel construction, chitosan has been proposed as building blocks of temperature-sensitive hydrogels that can be easily produced by adding GP [137]. Temperature-sensitive chitosan/glycerol phosphate hydrogels are widely used in medicine due to their good biocompatibility, low structural degradation (degradation), and easy fabrication [138, 139]. It is possible to combine the manufacturing technologies of selenium nanoparticles and temperature-sensitive chitosan/glycerol phosphate hydrogels due to their antioxidant and anti-inflammatory properties and their use in controlled-release drug delivery systems and neural tissue engineering.

9.1. Fabrication of Chitosan/Glycerol Phosphate/Selenium Nanoparticles Nanocomposite Hydrogels

To make the mentioned hydrogel, chitosan solution (2.5% by weight) is added to 1% ascorbic acid.

About 1.5 ml of prepared selenium nanoparticle solution is added to 5 ml of chitosan solution and mixed at a temperature to obtain a homogeneous solution. Then, 1 ml of β-GP (70%) is added drop by drop to chitosan or chitosan containing selenium nanoparticles in an ice bath (at a temperature of 4°C). In a study, the concentration of selenium nanoparticles in nanocomposite hydrogels is 158 micrograms per milliliter [140].

9.2. Examining the Characteristics of the Prepared Hydrogel Using Fast Infrared Transformation Spectroscopy (FTIR) and SEM

To investigate and confirm the cross-linking of chitosan with beta-Glycerol Phosphate (β-GP), Fast Infrared Transformation spectroscopy (FTIR) is used, which is measured by the potassium bromide disk method in the frequency range of 4000-500 cm^{-1} (Figure 3). In addition, nanocomposite hydrogels are covered with gold under vacuum conditions and images of the characteristics of nanoparticles are prepared by SEM microscope at a voltage of 3 kV [140]. In a study examining the structure of chitosan hydrogel associated with β-GP using SEM microscopy, this hydrogel had a heterogeneous, porous, and interconnected structure, and the pore diameter was in the range of 100-300 nm (Figure 4).

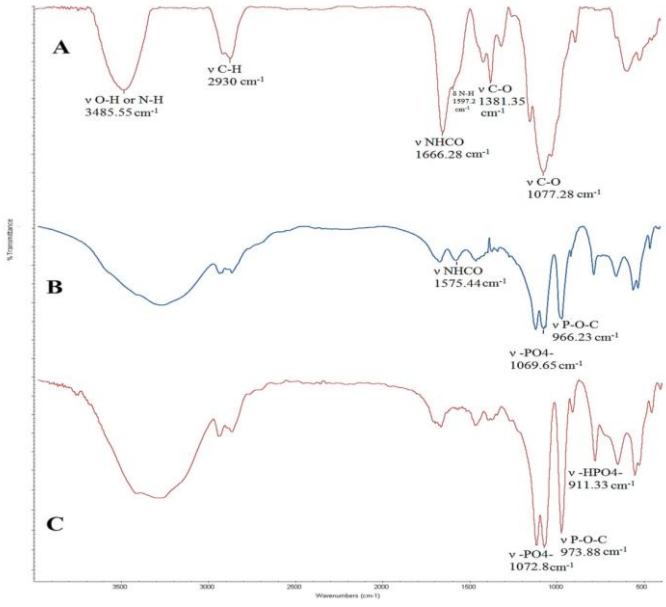

Figure 3. FTIR spectra of chitosan (a), cross-linked chitosan (B), β-GP (C) [81]

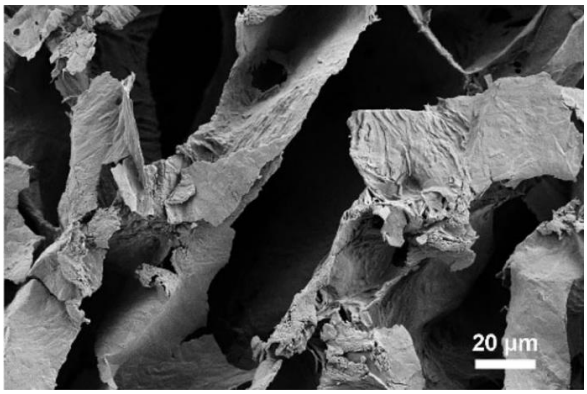

Figure 4. Micrograph prepared of chitosan hydrogel structure attached to β-GP using SEM at low magnification [140]

In another study, it is stated that the pore size of chitosan hydrogel decreases from 10-20 µm to 5-10 micrometers (µm) when cross-linked with β-GP (Figure 5). In other words, in cationic hydrogel loaded with β-GP, more complex networks are observed compared to chitosan hydrogel, which leads to the entrapment of drug molecules inside the hydrogel. These mentioned complex networks create a drug delivery system with controlled release.

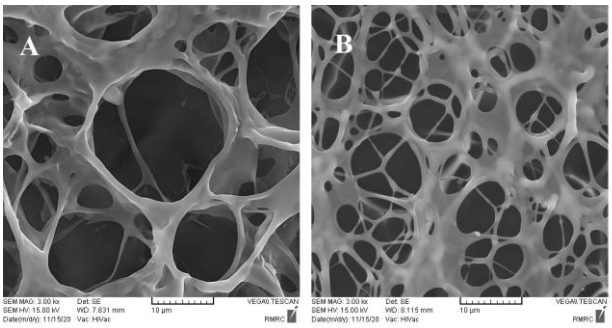

Figure 5. Micrographs prepared from chitosan hydrogel (A) and chitosan hydrogel cross-linked with β-GP by SEM [81]

It was reported that after the addition of selenium nanoparticles (158 µL/mL), it caused a highly porous structure to remain in the composite hydrogels (Figure 6). Moreover, the authors of the same paper showed that the deposited spherical selenium nanoparticles were well distributed on the surface of the composite hydrogels (Figure 7).

9.3. Measuring the duration of Gelation

The inversion method (chemical transformation) provides information about the complete gelation time of the temperature-sensitive hydrogel system and is used to determine the gelation time. Briefly, first, 2 ml of hydrogel in liquid form is poured into several clean tubes. Then the tubes containing the liquid solution are quickly transferred to a 37-degree incubator. One of the tubes is removed once every minute so that in addition to observing the color change of the solution, it is reversed and the flow of the solution is checked at the same time. When the hydrogel was completely gelled and did not flow in the tube by inverting the tube, it was recorded as the final gelation time. All experiments are repeated 6 times [141].

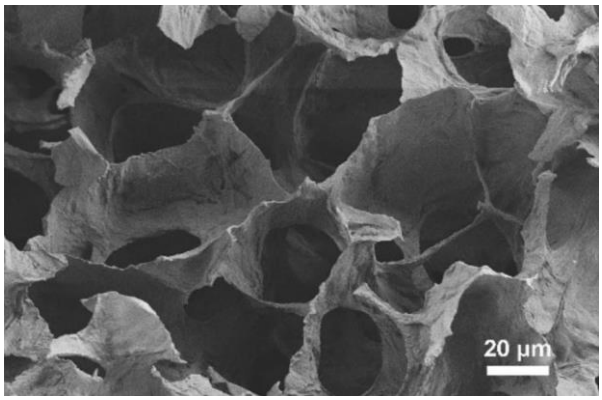

Figure 6. Micrograph prepared of chitosan hydrogel structure cross-linked with β-GP and loaded with selenium nanoparticles using SEM at low magnification [140]

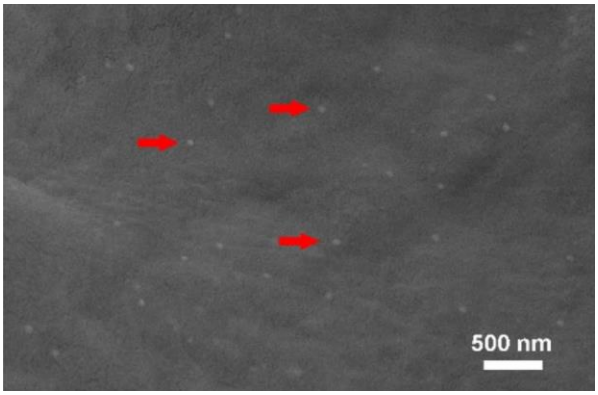

Figure 7. Micrograph prepared of β-GP bound chitosan hydrogel structure loaded with selenium nanoparticles using high magnification SEM (red arrows indicate selenium nanoparticles in the gel) [140]

9.4. Determination of Swelling Ratio of Chitosan Nanocomposite Hydrogel Loaded with Selenium Nanoparticles

Briefly, a frozen hydrogel with mass M0 is immersed in Phosphate-Buffered Saline (PBS) at

pH=7.4 at 37°C. At a certain time, the surface water of hydrogels immersed in PBS is removed using filter paper and then the sample (M1) is weighed. Three samples are prepared from each group. Finally, the hydrogel swelling ratio is calculated using the following formula (Equation 1) [142]:

$$Swelling\ Ratio\ (\%) = \frac{M1 - M0}{M0} \times 100 \quad (1)$$

$M0$: dry sample weight; $M1$: weight of sample immersed in PBS

9.5. Determining the Free Radical Scavenging property of ABTS (2,2-azinobis (3-Ethylbenzothiazoline-6-Sulfonic Acid)) Chitosan Hydrogel Loaded with Selenium Nanoparticles

Determination of 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cationic radical scavenging activity in chitosan hydrogels associated with β-GP and loaded with selenium nanoparticles (CS/GP/SeNPs), with different concentrations of selenium nanoparticles (1.25^{-25} µg/ml) can be done [143]. Briefly, by mixing 7.4 mM ABTS with 2.6 mM potassium persulfate (K2S2O8) in a dark place, the radical cation ABTS is made (it takes one night to make this radical cation). To obtain absorption of 0.4+0.02 at the wavelength of 734 nm, the obtained mixture is diluted using PBS. Then 10 microliters of CS/GP/SeNPs hydrogel were added to 100 microliters of ABTS cationic radical solution and they reacted with each other in a dark space for 6 minutes. Finally, absorbance at 734 nm wavelength is measured using a microplate reader and PBS is used as a negative control sample. ABTS cation radical scavenging activity of CS/GP/SeNPs hydrogel sample is calculated through the following formula (Equation 2) [144]:

$$ABTS\ radical\ scavenging\ activity\ (\%) = \frac{Ab - As}{Ab} \times 100 \quad (2)$$

Ab: Absorption in blank solution. As: absorption in the sample.

10. Results and Discussion

Epidemiological studies have shown a direct relationship between systemic inflammation and the pathogenesis of post-traumatic complications. In such a way, patients with SCI with positive SIRS have greater severity of damage and the incidence of complications than patients with negative SIRS [145]. Macrophage Migration Inhibitory Factor (MIF) is mainly involved in systemic inflammation and suggests that SCI-induced neuroendocrine changes promote systemic inflammation. The activation of microglia cells (neuro-immune cells of the central nervous system), which occurs chronically in the hippocampus and cerebral cortex after SCI, indicates that the disruption of the neuro-immune system is involved in systemic inflammation after SCI [146]. In the stimulation caused by the oxidative damage induced to the cells by reactive oxygen species, hydrogen peroxide is produced. Excessive production of reactive oxygen species leads to glutamate-mediated excitotoxicity, which activates astrocytes with glutamate receptors [147], and activated astrocytes secrete chondroitin sulfate causes dense glial scars [148-150]. The formation of glial scars can cut off the neural pathways in the spinal cord and suppress functional motor recovery [151, 152]. Long-term neurological deficits following traumatic spinal cord injury are partially caused by extensive activation of the apoptosis pathway in neurons and oligodendroglia in traumatic spinal cord injury areas [153].

Chitosan reduces cell membrane disruption after SCI and is a potent neuroprotective agent. Chitosan clearly targets the area of tissue injury, and the uninjured spinal cord showed a very weak affinity for chitosan [154]. In fact, chitosan layers are very compatible with neural and mesenchymal stem cells. Chitosan scaffolds have a supporting and bridging effect in damaged tissue due to the low rate of chitosan decomposition. Based on the evidence, rats with grafted chitosan scaffold for the regeneration of nerve fibers and glial cells were observed less in SCI situations [155]. It seems that chitosan is an ideal matrix for the application of nanoparticles, which is an environmentally friendly method and can be effectively used in neural tissue engineering. Also, previous studies have shown that the chitosan hydrogel implant loaded with selenium nanoparticles in the spinal cord of rats immediately after SCI caused the healing of the spinal cord and reduced glial fibrosis wounds, and also weakened the inflammatory responses [11, 63]. Therefore, by properly controlling

secondary injuries following SCI, which are mainly caused by pro-inflammatory cells and cytokines, we can try to accelerate functional recovery and reduce the destruction of neurons. Selenium nanoparticles, as a biologically active antioxidant compound, reduce the activity of enzymes that remove reactive oxygen species such as glutathione peroxidase, superoxide dismutase, and catalase in the brain of rats and mice and induce neuroprotective effects [37]. Therefore, by taking advantage of the antioxidant properties of selenium and delivering it in the form of nanoparticles to the central nervous system, we can try to control the increasing production of reactive oxygen species, the occurrence of oxidative stress, and the spread of traumatic spinal cord injury.

Finally, it can be stated that the use of β-GP is a type of physical cross-linking method that causes the production of a reversible hydrogel. This cross-linked chitosan hydrogel with β-GP is recommended for the production of hydrogels to achieve tissue repair and drug release. The cross-linking method using β-GP is a physical method that does not create any chemical bonds in the process, which claim is confirmed by examining the FTIR results of cross-linked chitosan (Figure 3). In this physical cross-linking process, adsorption interactions occur between the positive charges of chitosan molecules and the negative charges of β-GP molecules [81]. Chitosan is a type of polysaccharide that forms a polycation with its amino groups in an acidic environment. In fact, the formation of these positively charged amino groups is considered the main factor of chitosan dissolution in dilute acid solutions, which is dependent on the electrostatic repulsion forces between the positive charges of the chains of amino groups and it prevents the closeness and connection of chitosan chains and also the formation of intramolecular hydrogen bonds. Electrostatic repulsion between these groups creates chitosan hydrogel. Amino groups are reduced to normal with pH change and chitosan hydrogel assumes a gel-like structure. But the final hydrogel obtained from cross-linked chitosan is affected by pH and temperature. As the temperature increases, the polarity of the cross-linked chitosan chains decreases, and also the protons of the chitosan amino groups are converted to glycerophosphate phosphate [81].

Removing layers of water molecules around the chitosan chains strengthens the hydrophobic and hydrogen bonding forces between the adsorbent layers. By adding β-GP solution to chitosan solution, the negative charges of phosphate groups neutralize the positive charges of chitosan amino groups and the pH of the chitosan solution increases. At this time, the conditions are provided for the chains to approach each other and the hydrogen bonds between the OH...NH and O...HN groups remain in the cross chains. According to the mentioned materials, it can be concluded that the increase in salt concentration leads to a decrease in the electrostatic repulsion between the chains and an increase in the internal and external hydrogen bonds of the chains, and the time required to complete the coagulation or gelation process is reduced. As the temperature increases, the time required for the gelation process decreases. At low temperatures, strong interactions between water and chitosan molecules prevent chitosan chains from connecting to each other. Therefore, although the addition of glycerol salt neutralizes the electrostatic repulsion between chitosan chains by the phosphate group salt, there is still not enough energy to overcome the interaction between solvent/polymer and break the regular structure of water molecules around chitosan chains. Such a solution can be stable for hours at room temperature. As the temperature increases, chitosan-chitosan hydrophobic reactions are strengthened and the structure of water molecules around chitosan chains is broken. As the temperature increases, the hydrogen bonds between the chains are strengthened and finally the gelation process is completed by connecting chitosan chains to each other [81, 156].

Adding selenium nanoparticles to the β-GP cross-linked chitosan system slightly increases the pH of the said system, which can make the hydrogels more resistant to acidity. In addition, after adding selenium nanoparticles, the duration of hydrogel gelation decreases. Compared to the low strength of chitosan gel cross-linked with β-GP, the addition of selenium nanoparticles helps to enhance the strength of temperature-sensitive systems. This effect can be due to the strengthening of the interaction between nanoparticles and macromolecules, which leads to a slower and easier formation of a stable hydrogel scaffold. Studies have shown that selenium nanoparticles have excellent antioxidant activity and low cytotoxicity in vivo and in vitro environments [157-160].

Both antioxidant effects and cytotoxicity of selenium nanoparticles depend on the stabilizer, particle size, and their concentration. It has been shown that higher concentrations of selenium nanoparticles have stronger antioxidant activity and greater toxicity [140]. It has been stated that CS/GP/SeNPs hydrogel systems containing concentrations of 2.5^{-10} µg/ml of selenium nanoparticles have stronger antioxidant activity and better cellular compatibility, which are suitable for animal studies [140]. Therefore, CS/GP/SeNPs temperature-sensitive hydrogels can be used as a promising scaffold in controlled-release drug delivery systems and neural tissue engineering. As previous studies have shown that the chitosan hydrogel implant loaded with selenium nanoparticles in the spinal cord of rats immediately after SCI caused spinal cord healing and reduced glial fibrosis wound and also decreased inflammatory responses [37, 63].

Serine protease can activate the acute inflammatory response in severe traumatic spinal cord injury. Serpin is a serine protease inhibitor and an immunomodulatory biological drug that can reduce inflammation and protect neurons for the treatment of SCI. The combination of chitosan and collagen hydrogel with a stable structure resulted in the production of a highly biodegradable material that has good biocompatibility properties and low antigenicity in animal models of SCI. Therefore, scientists have shown that serpin derived from chitosan-collagen hydrogel maintains a therapeutic effect and enhances functional recovery in the rat model of traumatic spinal cord injury [161]. In addition, it is stated that chitosan-collagen hydrogel loaded with selenium nanoparticles can also strengthen functional recovery in these patients [11].

11. Conclusion

The primary spinal cord injury occurs immediately after the initial mechanical blow to the spinal cord and displacement of the vertebral column, which results in compression or severing of the spinal cord. After the primary spinal cord injury, the cascade of secondary injury occurs, which causes gradual degeneration in the parenchyma of the spinal cord, which eventually leads to chronic neurodegeneration. The activation of inflammatory cells in the area of damage causes the production of free radicals such as nitric oxide, superoxide anion, and hydrogen peroxide, all of which damage cell membranes. After primary mechanical damage, inflammation is the most important factor inducing astrogliosis and scar formation. The activation of inflammatory cells in the area of damage causes the production of free radicals, all of which damage cell membranes. It has been mentioned that excessive production of reactive oxygen species has a major role in the occurrence of secondary damage caused by traumatic spinal cord injury. It has been stated that chitosan may inhibit the synthesis of some inflammatory mediators such as IL-1β, reduce oxidative stress metabolites, and encourage the production of some anti-inflammatory markers such as IL-10 The anti-inflammatory capacity of selenium is dependent on its effect on immune cells and especially the signaling pathways of macrophages Selenium nanoparticles, as a biologically active antioxidant compound, reduce the activity of enzymes that remove reactive oxygen species such as glutathione peroxidase, superoxide dismutase, and catalase and induce neuroprotective effects. Therefore, the use of selenium nanoparticles in the chitosan hydrogel bed can control the degeneration and functional improvement of the nerve tissue of the spinal cord.

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