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# Design and Local Application of Controlled Released Drug Delivery System of Chitosan Hydrogel Loaded with Selenium Nanoparticle in Spinal Cord Injury-Induced Rat; Evaluation of Antioxidant Changes in Neural Tissue

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**ABSTRACT**

Traumatic spinal cord injury (SCI) is one of the serious injuries of the central nervous system. Oxidative stress is considered one of the signs of the secondary phase of SCI. Therefore, the design and local application of controlled released drug delivery system of chitosan hydrogel loaded with selenium nanoparticles in rats with spinal cord injury and also the evaluation of antioxidant changes in nerve tissue were considered as the aim of the present study. For this purpose, experimental spinal cord injury was created in 60 female adult rats and they were randomly divided into three equal groups; 1- Control group; 2- Chitosan hydrogel group and 3- Chitosan hydrogel loaded with selenium nanoparticles group. The activity of some antioxidant enzymes in spinal cord tissue was measured on the 3rd, 7th, 21st, and 28th days after injury. The results clearly showed that the changes in the amount of superoxide dismutase, malondialdehyde, and glutathione peroxidase on the 3rd and 7th days after trauma in the treatment groups were significantly lower than the control group. However, in the treatment groups, the level of catalase enzyme activity was not significant compared to the control group. It is possible that trauma and production of free radicals in the spinal cord (injury site) were less in the 2 treatment groups of the present study. Therefore, by reducing the amount of oxidative stress in the area of damage, chitosan hydrogel loaded with selenium nanoparticles probably has positive effects on the SCI.

## Introduction

In traumatic spinal cord injury (SCI), there is a possibility of involvement of the patient's consciousness and physical activity due to damage or destruction of nerve cells. In addition to causing pain, discomfort and distress to the patient, these injuries have notable treatment and maintenance costs for the patient and the community.[1] In SCI, bleeding can last for 12 to 24 hours and develop. In the vicinity of the injury site, the white and gray bodies become softer due to edema. The result of SCI after 8 days is the presence of large tissue containing debris, and about 2 weeks after the injury, cavities are seen in the spinal cord that can merge to form a spinal cyst. Secondary injuries due to SCI involve both apoptotic and necrotic processes. In fact, the process of nerve cell necrosis occurs following impaired blood flow. In addition to the presence of inflammation, the invocation of inflammatory cells in situ also accelerates and intensifies the cycle of destruction in the spinal cord.[2] In fact, the pathological responses of SCI are multiple and complex and include inflammation, peroxidation, apoptosis, necrosis and

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stress response.[3] In fact, high production of free radicals, low concentration of antioxidants, and high metabolism in patients with SCI increase oxidative stress. The oxidative stress response plays an important role in SCI. In the early stages of SCI, denaturation and decomposition of fatty acids at the site of injury lead to the formation of reactive oxygen species (ROS). Free radicals significantly aggravate the clinical condition of patients with SCI. Free radicals are formed by superoxide and hydrogen peroxide, hydroxide radicals, peroxynitrite, nitric oxide, lipoproxyl.[4] In addition, the oxidative stress response further promotes the release of inflammatory factors (TNF-α and IL-6) and aggravating neuronal apoptosis.[5] However, any therapeutic intervention that can control oxidative damage and to reduce or eliminate reactive oxygen species can be a significant approach in the treatment of SCI.

Selenium nanoparticles have been introduced as a micronutrient as an important source of selenium with minimal toxic effects for humans and animals. Positive and therapeutic effects of selenium nanoparticles on acute spinal cord injuries have been reported.[6] The antioxidant effect of selenium nanoparticles seems to play a role in the healing properties of spinal cord injuries. It has been reported that selenium nanoparticles can increase the activity of reactive oxygen species scavenger enzymes, including glutathione peroxidase (GPx), superoxide dismutase, and catalase in the brains of rats and mice and have neuroprotective effects.[7] Therefore, if these nanoparticles can be administered in appropriate doses and for a certain period of time, interesting therapeutic and antioxidant effects can be achieved in SCI. In fact, in this study, it is intended to load these nanoparticles in a suitable scaffold that can be administered in a controlled manner. Over the past decade, efforts have been made to load nanoparticles made into substrates of various scaffolds, including polysaccharides, monosaccharides, proteins, amino acids, etc., to provide a new and stable synthetic agent. Among these scaffolds we can mention chitosan; Natural polysaccharide that has biodegradability properties.[8] Chitosan hydrogel is a compound that has been extensively tested in pharmacy and has special potential in the design and fabrication of drug delivery systems.[9] The use of this hydrogel as a scaffold, due to its antioxidant effects, for loading selenium nanoparticles can provide a suitable drug delivery system.[10]

In the present study, considering the importance and benefits of administration of selenium nanoparticles with its protective potential on neural tissues, the preparation and implantation of a controlled drug delivery system of chitosan hydrogel loaded with selenium nanoparticles was the aim and antioxidant effects of this system in rats with SCI has been investigated.

## Materials and Methods

### *Materials*

Commercial Eud (Eudragit RL100) was obtained from Evonik industries (Essen, Rhine–Westphalia, Germany). Low molecular chitosan, glycerol 2-disodium hydrate (β-GP), selenium oxide, ascorbic acid and other reagents and solvents were purchased from Merck (Darmstadt, Hesse, Germany).

### *Preparation and Characterization of Selenium Nanoparticles*

At first, a 500 ml solution of selenium oxide at a concentration of 1 mM was prepared. A magnetic stirrer rotated this solution at 800 rpm. Then, drop by drop, 50 ml of 44 mM ascorbic acid solution was added to the initial solution to generate selenium nanoparticles (SeNs). A centrifuge is used to separate the produced nanoparticles, which are then dried. Then, in 5 ml of acetone, 100 mg of Eudragit RL100 was dissolved. After 12 hours of agitation in the refrigerator, the final particles as SeNs/Eud are collected by centrifugation and dried by freeze dryer. The nanoparticles that were created in the end were characterized by FESEM, FTIR and EDAX methods.

### *Preparation and Characterization of Chitosan Hydrogel Loaded with SeNs/Eud*

To make a homogenous solution, 480 mg of chitosan was dissolved in 12 ml of 0.1 M acetic acid solution and stirred for 3 hours. 27 mg produced SeNs/Eud were dispersed in 4 ml deionized water before being added to the chitosan solution. In a separate container, 2 g of glycerol 2-disodium hydrate (β-GP) was dissolved in 2 ml deionized water. The chitosan and β-GP solution containers were then placed in an ice bath at 4 °C for 10 minutes before the β-GP solution was introduced dropwise to the chitosan solution. Finally, SeNs/Eud@Cs#β-GP was mixed for 4 hours before being stored at 4 °C for future use. The final prepared hydrogel was characterized by some later methods.

### *Investigation the Morphology of Prepared Hydrogel by SEM*

Samples of chitosan hydrogel and cross-linked chitosan hydrogel were evaluated using this technique to confirm cross-linking performed on chitosan hydrogel by β-GP. The prepared samples were frozen fast in a -70 °C freezer before being dried in a freezer drier. The acquired samples were sliced into thin layers and their morphological structure was investigated using the SEM method.

### Investigation of the Structure of Prepared Hydrogel by FTIR

To confirm the cross-linked performance, the FTIR method was employed to investigate the absorption spectra produced in the final hydrogel sample. Chitosan, β-GP, and β-GP cross-linked chitosan samples were used in this assay.

### Investigate the Duration of Gelation Time of Prepared Hydrogel

To eliminate bubbles, 2 ml of the produced β-GP cross-linked chitosan sample was placed into a 5 ml vial and maintained at 4 °C for 12 hours. The vial was then placed in the bath (at 24 and 37 °C). Every minute, the vial was put horizontally and the gelling time of the hydrogel was measured. The moment when the solution was entirely fixed was designated the gelling time.

### Standard Calibration Curve of Selenium

1000 mg of SeNs were dissolved in 1000 ml of water to make a standard stock solution of selenium. Dissolving 2.0 g of potassium iodide in 100 ml of water yielded the potassium iodide solution. 1 g of soluble starch was dissolved in 100 ml hot water while constantly stirring. The solution containing 2–12 mg of selenium was transferred into a series of 10 ml calibrated flasks in this operation. 1 ml of 2% potassium iodide solution was added to it, followed by 1 ml of 2 M hydrochloric acid. The contents were topped up to the mark with distilled water after the combination was gently agitated until a yellow tint developed, signifying the liberation of iodine. At 570 nm, the absorbance of the colored solution was compared to that of a reagent blank.

### Determination of in Vitro Release of SeNs/Eud from Hydrogel

At 37 ° C, 1 g of the finished hydrogel was placed in 500 ml of phosphate buffer with a pH of 7.4 and shacked at 100 rpm. 1 ml of sample was taken and 1 ml of fresh buffer was added at 1, 2, 3, 6, 12, 18, 24, and every 24 hours for 7 days. A UV spectrophotometer was used to examine the gathered samples. By comparing its absorbance at 265 nm, the amount of released SeNs was estimated. All measurements were carried out three times. The data is presented as means standard deviations.[11]

### Animals and Experiment Groups

A total of 60 female Wistar rats weighing 200-250 g were used for the present study. The animals were fed with free access to water and standard commercial food while kept in standard environmental conditions. Rats were randomly assigned to 3 equal groups: (A) Control group who did not receive any drug intervention; (B) the chitosan group that received the chitosan hydrogel and (C) the chitosan-selenium nanoparticle (ChSeN) group, which received chitosan hydrogels loaded with selenium nanoparticles. All the stages of design and conduct of the present study were reviewed and approved by the Council of the Department of Veterinary Clinical Sciences of Shahrekord University (VF-1398-12-03).

### Experimental Surgical Procedure

To induce experimental traumatic spinal cord injury (SCI), after induction of anesthesia by intraperitoneal injection of a combination of ketamine (50 mg/kg; Ketamine 5%; Alfasan; Netherland) and xylazine (5 mg/kg; xylazine 2%; Alfasan; Netherland), the animals were kept under general anesthesia using isoflurane (Terrel Isoflurane, USA). Then, they were placed on the operating table and their dorsal region of the spine was aseptically prepared. In all animals, following dorsal laminectomy at T8-T9 level and the appearance of the spinal cord, the aneurysm clips (g force 53×; Aneurysm Clip; Codman) were placed on either side of the spinal cord for one minute to experimentally induce spinal cord compression and induce SCI. Paralysis of the hind limb and tail of rats is a clinical sign of induction of SCI and efficacy of spinal compression technique.[12,13] In the control group, after controlling the bleeding, paravertebral fascia and skin were sutured using simple continuous pattern and no drug intervention was performed. In the second group, after controlling the bleeding and before closing the surgical site, chitosan hydrogel (0.5 ml) was placed on the surgical site. Additionally, in the third group, after controlling the bleeding and before closing the surgical site, 0.5 ml of chitosan hydrogel loaded with selenium nanoparticles (0.25 mg/kg/day) was placed on the site.

In order to prevent urinary tract infections, bladder swelling, and other potential postoperative infections, all animals received intramuscular ampicillin for 5 days and daily bladder emptying by manual expression of rats' abdomen as well as the daily litter replacement was considered as postoperative management.

### Assessment of Antioxidants

Days 3, 7, 21, and 28 after surgery were considered as sampling times and 5 rats in each group were sacrificed for sampling at any time. Following euthanasia of animals (by overdose of ketamine-xylazine combination) and precise removal of the spinal cord, after complete dorsal laminectomy, the entire length of the spinal cord was harvested as a sample of tissue antioxidants and was sent to the laboratory. After weighing and homogenizing the samples, PBS was added to the tissue samples to reach a volume of 1.5 ml and stored in a micro tubes and

centrifuged. Finally, the spinal cord tissue extract was stored in the freezer at -70 °C until the antioxidants were measured. To determine the rate of lipid peroxidation, tissue MDA content was measured using thiobarbituric acid method (Nalondi-Lipid Peroxidation Assay Kit-MDA; Navandsalamat Co.). The basis of tissue catalase measurement is the preoxidative activity of this enzyme, which was measured using its kit (Nactaz-Catalase Enzyme Activity assay kit; Navandsalamat Co.). GPx enzyme activity was measured using a diagnostic kit guideline (Nagpix-GPx Assay kit; Navandsalamat Co.) based on NADPH oxidation. SOD measurements were based on the inhibition of the pyrogallol auto-oxidation reaction using a diagnostic kit (Nasdox–Superoxide Dismutase Assay Kit; Navandsalamat Co).

### *Statistical Analysis*

Values are expressed as the mean ± standard deviation (SD), and statistical analysis was performed using SPSS version 23.0 statistical software. The Kolmogorov-Smirnov test was used to evaluate data normality. For comparison of mean of parameters among different groups and comparison of the mean of each parameter within the group, analysis of variance was performed at the significant level of $p < 0.05$, followed by Tukey's honest significant difference test.

## Results

### *Study of Nanoparticles*

**Surface and Morphological Properties.** The field emission scanning electron microscopy (FE-SEM) was used to examine the morphological aspects and surface properties of produced SeNs and SeNs/Eud. The spherical shape and homogeneous distribution of nanoparticles were confirmed in Figure 1. SeNs were roughly 150 nm in size (Figure 1A). After coating SeNs with Eud, the size of the particles increased by about 20 nm. Eudragit polymers on the surface of SeNs/Eud was seen in Figure 1B. The coated layer's thickness grew SeNs/Eud size compared to the SeNs obtained in the previous step.

**Evaluation by Fourier Transform Infrared Spectroscopy (FTIR).** Figure 2 shows the results of FTIR investigations of SeNs/Eud, SeNs, and Eud. Figure 2A shows the SeNs/Eud composite sample. This diagram shows all of the index peaks of substances such as SeNs and Eud. Some peaks, such as C=O and Se-O, were discovered in figure 2B. Stretching and bending vibrations of Se–O at 1105 and 470/cm, respectively, are responsible. These findings back up the idea that SNPs like $SeO_2$ and ascorbic acid can entrap some beginning material. The existence of carboxylic acid functional groups in Eud was established by stretching O–H from 3300–2500/cm and C=O from 1760–1690/cm (Figure 2C). Eud present was the target of those stretching vibrations.

**EDAX Assay Measurement.** The elemental composition of SeNs and SeNs/Eud were determined using the energy-dispersive X-ray analysis (EDAX) method (Figure 3). The discovered elements and atomic ratios of each one were shown in Table 1. As a result of these findings and presence of chlorine (Cl) elemental in SeNs/Eud composition more than SeNs, it was confirmed that SeNs/Eud were coated on the surface by Eud.

### *3.2. Study of hydrogel*

**Evaluation of Morphology.** The SEM results of chitosan and crosslinked chitosan hydrogel were shown in Figure 1. In chitosan hydrogel, the pore size is about

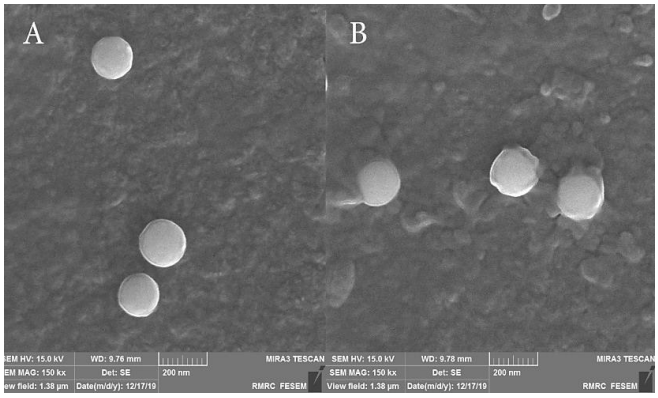


***Figure 1.*** FE-SEM images of (A) SeNs and (B) SeNs/Eud.

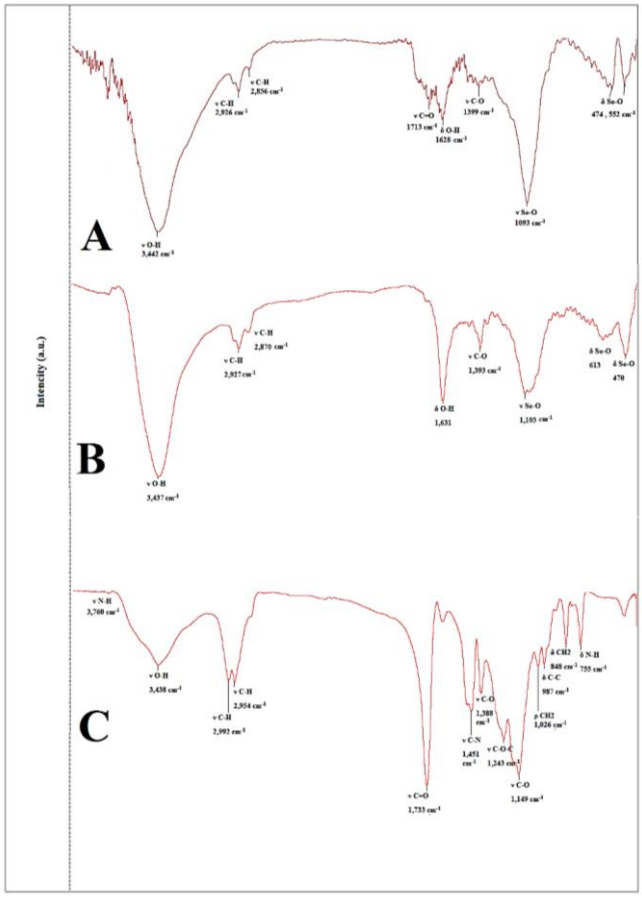


***Figure 2.*** FTIR spectra of: (A) SeNs/Eud, (B) SeNs and (C) Eud.

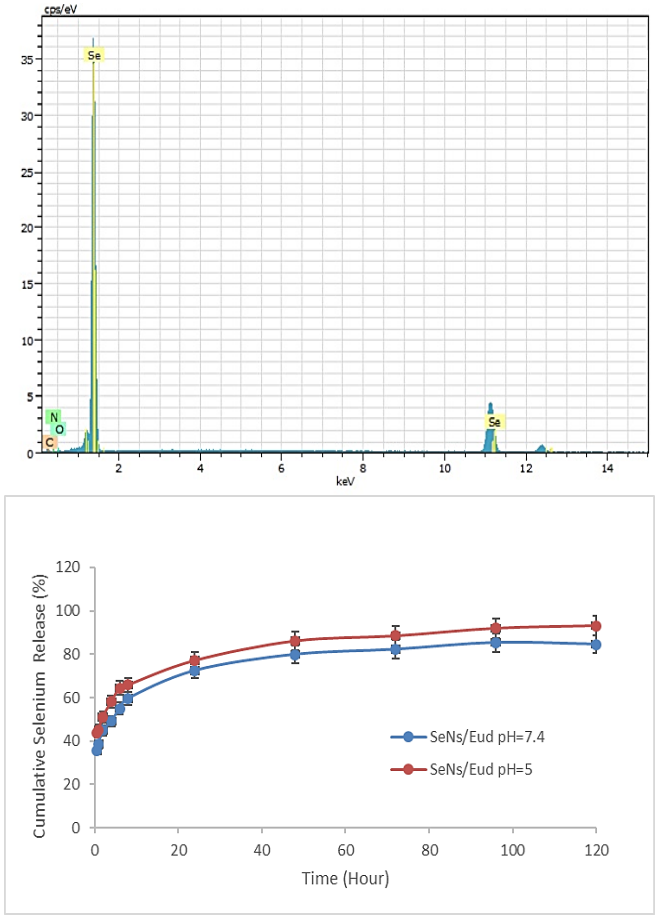


***Figure 3.*** (A) EDAX spectra obtained for SeNs and SeNs/Eud; (B) *In vitro* selenium release profile from SeNs/Eud@Cs#β-GP at pH = 5 and 7.4.

***Table 1.*** Atomic ratios from EDAX assay measurement for SeNs and SeNs/Eud.

| Element | % in SeNs | % in SeNs/Eud |
|---|---|---|
| **Se** | 62.45 | 44.27 |
| **C** | 31.74 | 43.66 |
| **N** | 3.28 | 11.48 |
| **O** | 2.53 | 0.30 |

10-20 nm compare than crosslinked chitosan hydrogel porosity size as 5-10 nm. As shown in the results, crosslinked chitosan hydrogel has more complexed network by additional β-GP chains (Figure 4).

**FTIR Studies.** The crosslinked chitosan was confirmed by FTIR as shown in Figure 5. The FTIR spectra of chitosan, crosslinked chitosan, and β-GP is shown in Figures 5A, 5B, and 5C, respectively.

The infrared spectra of chitosan samples revealed absorbance bands in the 3400/cm range for the N-H and O-H groups, and 2930/cm for the H-C group. The absorbance spectrum also emerges in the 1620-1620/cm range due to the presence of the amide group and interference with the N-H bending band. The bending variations of C-H groups can also be seen in the absorption spectrum in the range of 1200-1500/cm. In the absorption spectra of the -$CH_2$-OH group and the C-O-C group, the stretching vibration of C-O is also detected in

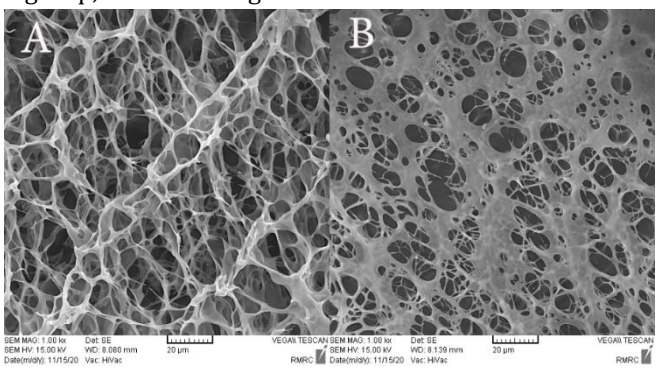


***Figure 4.*** SEM images of (A) chitosan hydrogel and (B) crosslinked chitosan hydrogel.

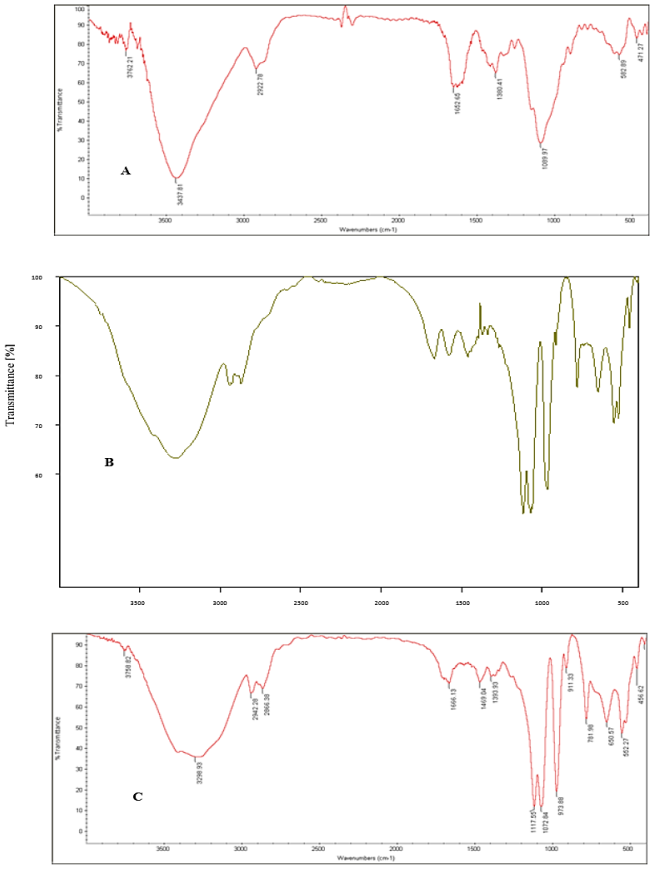


***Figure 5.*** FTIR spectra of: (A) chitosan, (B) crosslinked chitosan, and (C) β-GP.

the ranges of 1380/cm and 1530-1305/cm. Figure 5A also shows two indication absorption spectra in chitosan at 1652.65/cm and 1597.2/cm, which correspond to the stretching vibrations of the -NHCO- (type 1 amide) and -$NH_2$ bending group (type II amide), respectively. In the absorption spectra of cross-linked chitosan samples shown in Figure 5B, no extra distinctive bands form. However, these changes were observed in this system due to the electrostatic interaction between the positive charge of chitosan amino groups and the negative charge of β-GP phosphate groups: amide groups shift in the lower absorption spectrum at 1571.2/cm, and beta glycerophosphate functional groups bands shift from 973 and 1073/cm to 966.23 and 1069.65/cm, respectively.[14]

The FTIR spectrum produced from β-GP is shown in Figure 5C. The stretching vibration of the aliphatic P-O-C group is represented by the absorption spectrum in the region of 1973.88/cm, and the absorption spectrum in the region of 1072.84/cm represents the -$PO_4$- group, which are the two fundamental properties of GP. The presence of the -$HPO_4$- group can also be seen in the absorption spectra at 911.33/cm.

### 3.3. The Gelation Time of Produced Hydrogel

The gelation time of SeNs/Eud@Cs#β-GP hydrogel at 24°C and 37°C was investigated. The hydrogel was converted to gel in different time duration as 35 and 21 minutes, respectively. The gelation time was reduced about 60% by temperature rises which confirmed the adding β-GP into the chitosan hydrogel as previous study.[15]

### *In Vitro* Release of Selenium from Produced Hydrogels

The release of selenium from SeNs/Eud@Cs#β-GP follows a long-term release pattern, as seen in Figure 3. During the first 24 hours, around 72 and 77 percent of selenium was released at 7.4 and 5 pH, respectively. Also, 84 and 93 percent of selenium was released after 120 hours, respectively. Although the both 7.7 and 5 pH indicated have distinct releases, there is no significant variation between them. This lack of relevance could be explained by the small size of selenium nanoparticles, which formed cross-links in the final hydrogel but had little impact on the final release.

The optimal approach for determining the release kinetic pattern of selenium from the produced hydrogel was identified using a regression coefficient ($r^2$) close to 1. Table 2 displays the values for $r^2$. The drug release data for selenium at both 5 and 7.4 pH best suit Higuchi's kinetic model, according to the regression coefficient values.

### Assessment of Antioxidants

Figure 6 shows the comparison of the mean of SOD in various evaluated groups and different times. Intragroup comparison showed a significant change only in the control group, so that the mean of SOD on day 7 of the study was significantly increased ($p < 0.05$). Intergroup evaluation at similar times revealed that the mean of this parameter in control group, in comparison of other groups, was significantly increased on days 3 and 7 of the study ($p < 0.05$). Figure 6 shows the comparison of the mean of MDA at different times and between different evaluated groups. Comparison of this parameter within the group shows significant changes. In chitosan and ChSeN groups, a significant increase in this parameter was recorded on days 3 and 21, and 28 of the study ($p < 0.05$). Comparison of the mean of this parameter between different groups and at similar times also shows a significant increase on days 3, 7 of the study ($p < 0.05$).

***Table 2.*** Release Mathematical models' correlation coefficients of SeNs/Eud@Cs#β-GP at different pH 5 and 7.4.

| $R^2$ of release models | pH 7.4 | pH 5 |
|---|---|---|
| **Zero-order** | 0.6132 | 0.563 |
| **First-order** | 0.7492 | 0.7245 |
| **Higuchi** | 0.8365 | 0.7981 |
| **Hixon crowel** | 0.7042 | 0.6704 |
| **Krosmeyer peppas** | 0.4757 | 0.4275 |

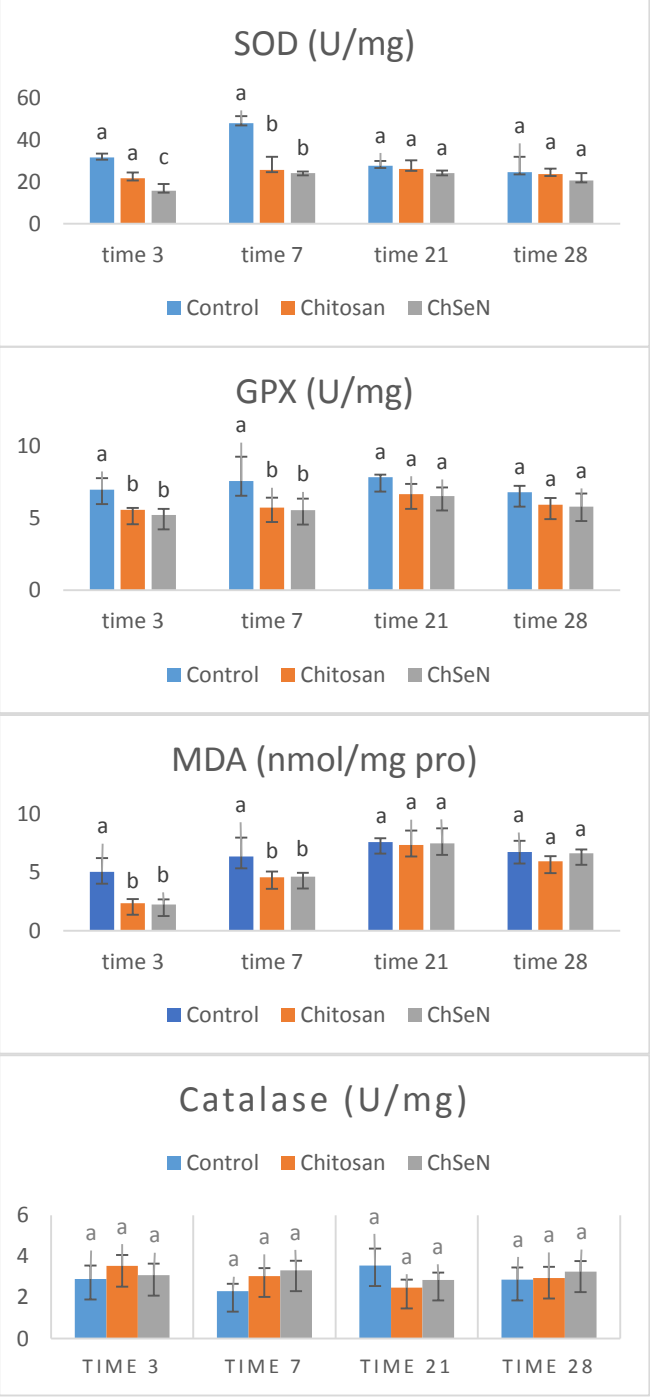


***Figure 6.*** Comparison of mean ± SD of antioxidant in different times and groups. ChSeN (Chitosan-selenium group).

Evaluation of the mean GPx parameter within the group in all three groups indicates the absence of significant changes at different times ($p > 0.05$). Results indicated that there was a significant increase in the mean of GPx on day 3 of the study in the control group in comparison with the other groups ($p > 0.05$) (Figure 6). Additionally, Figure 6 shows the comparison of the mean of catalase at different times and between different groups. Comparison within the group in all three groups indicates the absence of significant changes at different times ($p > 0.05$). Comparison of mean of catalase between groups at similar times also showed no significant difference at similar times ($p > 0.05$).

## Discussion

Oxidative stress has been shown to play a role in the progression of spinal cord injury following ischemic injury or primary injury. Therefore, in the present study, while synthesizing a controlled-released drug delivery system, selenium nanoparticles, which have antioxidant effect, were administered locally in SCI-induced rats and the level of tissue antioxidants in the damaged spinal cord was measured. Following the induction of any tissue damage and the release of free radicals, an increase in SOD activity occurs.[16,17] In fact, SOD is an important endogenous antioxidant enzyme that acts as a first line component of the defense system against ROS.[16] This enzyme converts the superoxide anion ($O_2$-) to hydrogen peroxide ($H_2O_2$), which is converted to $H_2O$ and O2 by GPx or catalase.[18] Another mechanism that may contribute to the effectiveness of SOD is the regulation of neutrophil apoptosis. To relieve inflammation, activated neutrophils must be properly removed through apoptosis. SOD may be effective as an inhibitory mediator of neutrophil inflammation.[19] Significant increase in SOD, on days 3 and 7, in the control group of the present study compared to the two groups of chitosan and ChSeN showed more oxidative damage in this group, while it seems that therapeutic intervention with chitosan and chitosan loaded with selenium nanoparticles Inhibits oxidative damage and ROS production in spinal cord tissue.

GPx can protect tissue against ROS and oxidative stress by catabolizing H2O2 and lipid peroxides.[20] It was reported that GPx levels did not change on the first day of subacute spinal cord injury, while on the fourteenth day of injury showed a significant increase. Glutathione peroxidase catalyzes the reduction of lipoperoxides and other organic hydroperoxides by reducing glutathione (as an H + donor).[21] But, it was reported that the mean level of tissue GPx increased significantly one hour after spinal cord injury.[22] In the present study, it was found that on days 3 and 7 of the study, the mean tissue GPx in the control group was higher than the chitosan and ChSeN groups. Most of the time, GPx activity depends on the selenium cofactor, so it can play an important antioxidant role.[23] Selenium has been reported to reduce NF-κB-associated apoptosis signaling and stimulate GPx activity during quinolinate-induced injury in the rat striatum.[24] In addition, selenium and vitamin E have been shown to improve brain tissue damage associated with high-dose corticosteroid injections and reduce GPx levels.[25] But, another report states that selenium does not affect GPx in the rat cortex.[26] Also, in the study of Ghodbane *et al.* (2011), a significant effect of mineral selenium on brain GPx was not observed in mice.[27] MDA is a marker of tissue damage, oxidative damage, and is formed during lipid peroxidation. Therefore, MDA assay is widely used as an indicator of lipid peroxidation.[28] Unsaturated fatty acids (arachidonic acid, docosahexaenoic acid) are targeted for free radicals; therefore, they produce highly reactive electrophilic aldehydes such as MDA, which are considered biomarkers of oxidative damage.[29] In a study by Seligman *et al.* (1977) on the effect of ethanol and trauma on MDA levels in the cat spinal cord, MDA was shown to be one of the first evidences of free radical production in SCI and increases within 5 hours after spinal cord injury.[30]

In the study of Kuyumcud and Aycan (2018), the rate of MDA in patients with spinal fractures was significantly higher than the control group ($p < 0.001$).[31] It was reported that the increase in MDA level was observed in 30 minutes after SCI in rat spinal cord contusion, peaked in 3 hours and returned to baseline after 12 hours.[28] It has been shown that following treatment of spinal cord injury with Anakinra (an interleukin receptor antagonist 1), the mean serum MDA level decreases compared to the control group (no SCI treatment).[22] In the study of Erol *et al*. (2008), the level of tissue MDA in spinal cord samples in the melatonin-treated groups was significantly lower than the laminectomy group (spinal cord injury) ($p < 0.01$).[32] Studies by Beytuta *et al*. (2018) show that high doses of prednisolone cause oxidative damage to the brain by increasing ROS, lipid peroxidation leads to increased MDA levels, and administration of selenium and vitamin E has a protective effect and leads to decreased levels of cerebral MDA.[25] Oral administration of selenium has been shown to have a tissue-protective effect in the form of reduced levels of MDA (TBARS) in the liver and kidneys.[33] Kiełczykowska *et al*. (2015) showed that in rats treated with organic selenium (diphenyl di selenide) GPx mitochondria of the cerebral cortex did not change, while MDA was significantly reduced.[34] The results of the present study showed that spinal tissue MDA in treatment groups (chitosan and ChSeN) was significantly lower than the control group on the third and seventh days after injury and the cause should be considered in the effect of selenium nanoparticles and chitosan.

Catalase, as an antioxidant enzyme, breaks down $H_2O_2$

(a product produced by free radicals that is toxic to tissues) into water and molecular oxygen, thereby reducing free radical damage.[16] It has been observed that the frequency of catalase in the spinal cord tissue and around the spinal cord cross-section was unchanged on the first day after injury and increased significantly on the 14th day after injury.[21] It has also been suggested that treatment of spinal cord injury with anakinra leads to a significant reduction in catalase levels in spinal cord tissue.[22] But, the results of the present study did not show a significant difference in the level of spinal tissue catalase between different groups. However, to determine the exact effect of chitosan and selenium nanoparticles in SCI on the tissue level of catalase, measurement of $H_2O_2$ (biomarker of oxidative damage) in each group before and after therapeutic intervention is recommended in subsequent studies. It has also been suggested that nanoparticle selenium is very effective in increasing the expression of selenoproteins and scavenging free radicals.[35] It has previously been suggested that selenium nanoparticles play an essential role in the antioxidant defense system and in reducing oxidative stress.[36] Another point that should be considered in the design of controlled-drug release systems in the present study is the use of chitosan as a scaffold for loading selenium nanoparticles. Evidence has shown that chitosan can prevent lipid oxidation in biological systems by scavenging free radicals. This effect is attributed to the presence of hydroxyl and amine groups in chitosan.[37] Therefore, chitosan and its compounds can be considered as an effective antioxidant.[38, 39]

Traumatic spinal cord injury (SCI) causes harmful injuries in sufferers. Excessive production of reactive oxygen species plays a key role in the occurrence and promotion of secondary damage caused by SCI. In the present study, the design, fabrication and local use of a controlled released drug delivery system of chitosan hydrogel loaded with selenium nanoparticle in experimental traumatic spinal cord injury in a rat model were considered. Also, antioxidant factors were measured in the spinal cord tissue on days 3, 7, 21 and 28 after induced spinal cord injury. On days 3 and 7 after surgery, the amount of SOD, MDA and GPx in the treatment groups showed a significant decrease compared to the control group. Also, the amount of SOD on day 3 in the chitosan+selenium nanoparticles group was significantly lower than the chitosan group. However, no significant difference was observed for catalase. Summarizing the results of the present study, the loading of selenium nanoparticles on the chitosan scaffold to provide a controlled-release drug system is effective on the measured antioxidant enzymes and the positive changes of these biomarkers can be considered as a positive effect of this system in SCI.

## Conflict of Interest

There is no conflict of interest to declare.

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