

RESEARCH PAPER

Selenium nanoparticle-loaded nanofibers accelerate in vivo wound closure in a rat model

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ABSTRACT

Objective(s): The inefficiency of conventional antibiotics has led to the emergence of multidrug-resistant (MDR) pathogens, making it worthwhile to develop alternative antibacterial agents to overcome this challenge. Infection at the wound site may lead to wound dehiscence, resulting in increased patient morbidity and higher healthcare costs. Nanoparticle-based approaches offer a promising strategy for controlling wound infections by enabling targeted delivery of antimicrobial agents, disrupting bacterial cell membranes and biofilms, and supporting tissue regeneration, while potentially helping to limit the development of antimicrobial resistance. Interestingly, it has been revealed that drug-resistant pathogens can be limited by Selenium nanoparticles (SeNPs).

Materials and Methods: This study aimed to synthesize and evaluate selenium nanoparticle-loaded nanofibers for wound dressing. SeNPs were green-synthesized and characterized by DLS, TEM, and EDX. The nanoparticle-loaded nanofibers were fabricated by electrospinning technique and characterized morphologically and chemically by SEM and ATR-FTIR. The in vitro safety of these nanoparticle-loaded nanofibers was demonstrated in the MTT assay on HU-02 fibroblast cells, while the bacterial viable cell count was used to investigate the antibacterial assessment against two strains of *Staphylococcus aureus* and *Escherichia coli*. In vivo efficiency was performed on Wistar Rats.

Results: Selenium-loaded nanofibers (129 ± 30.21 nm) showed antibacterial activity with no in vitro toxicity. An in vivo full-thickness wound model confirmed enhanced wound healing in histological evaluations.

Conclusion: This study revealed the potential of SeNPs-loaded nanofibers for an efficient antibacterial wound dressing.

Keywords: Nanoparticles; Selenium; Wound dressing; Electrospun nanofibers; Chitosan

How to cite this article

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INTRODUCTION

The wound healing process is dynamic which involves tightly regulated cellular events, inflammation, tissue regeneration, and remodeling. Chronic and acute wounds, particularly those associated with infections or impaired healing, especially in diabetic patients, continue to present significant clinical challenges (1-3). In addition, resistant bacteria have become an important challenge for the global healthcare system (4-6). Consequently, developing advanced wound dressings that promote tissue repair assiduously

while preventing infection simultaneously has become a central focus in regenerative medicine.

Nanotechnology-based approaches have demonstrated promising potential in accelerating wound healing through controlled and sustained release of therapeutic agents, thereby enhancing the process of wound healing (7). Among various nanomaterials, selenium nanoparticles (SeNPs) have attracted attention for their potential as antioxidants, anti-inflammatories, and antimicrobials. Selenium plays a pivotal role in oxidative stress modulation, a crucial factor in

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delayed wound healing, through its involvement in the activity of different selenoproteins as well as glutathione peroxidases and thioredoxin reductases (8, 9). More importantly, the multi-targeted antimicrobial properties of SeNPs against multidrug-resistant pathogens propose them as a potent candidate to overcome this issue (4, 5). However, the direct application of Selenium nanoparticles challenges the non-lasting effects at the site of action.

Electrospun nanofibers, with structural similarity to the extracellular matrix (ECM), along with their high surface area and porosity, provide an ideal platform for the incorporation and sustained release of therapeutic agents (10-12). When loaded with SeNPs, such nanofiber-based dressings offer the potential to act as an air-permeable physical barrier and provide therapeutic benefits directly at the wound site.

In this study, we reported the fabrication and in vivo evaluation of selenium nanoparticle-loaded electrospun nanofibers as a novel wound dressing. We investigated their morphological and biocompatibility properties along with wound healing efficacy using a rat full-thickness wound model. Our results provided insight into the therapeutic potential of SeNP-based nanofiber systems in enhancing cutaneous wound repair.

MATERIALS AND METHODS

DMEM/HG (Dulbecco's Modified Eagle Medium, high Glucose) (Gibco, USA), MTT powder, fetal bovine serum (FBS), trypsin-EDTA, Dimethyl sulfoxide (DMSO), penicillin/streptomycin (P/S) (Sigma-Aldrich, Sweden), Polyurethane (PU), Chitosan (Cs), Polyvinyl alcohol (PVA), and phosphate-buffered saline (PBS) (Sigma-Aldrich, Germany) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

SeNPs synthesis and characterization

The SeNPs synthesis was performed using ascorbic acid as a reducing agent for the chemical reduction of sodium selenite (Na_2SeO_3), accompanied by Chitosan as a stabilizer and capping agent, as reported in our previous experiments (13-15). Briefly, the 50 mM

concentration of sodium selenite was prepared in a 0.1% chitosan solution on a magnetic stirrer. The reducing agent, ascorbic acid (50 mM), was added to the solution of sodium selenite and chitosan in a dropwise manner until the color changed from colorless to orange. Stirring continued for 30-60 min. Characterization tests of transmission electron microscopy (TEM, Zeiss-EM10C-100 kV, Germany), Dynamic light scattering (DLS) (KONE Ltd, South Korea), and energy-dispersive X-ray spectroscopy (EDX) (EX-250 system; Horiba, Kyoto, Japan) were performed for size and morphology, average hydrodynamic diameter, and elemental composition of SeNPs.

Two-layer scaffold fabrication and characterization

Initially, the first layer of a two-layer scaffold was created using a 12% Polyurethane (PU) synthetic polymer to enhance the strength and physical resistance of the wound dressing. 0.72 g of PU was dissolved in 6 mL of THF/DMF solution in a ratio of 3:1. It was placed on a stirrer at room temperature until completely dissolved. The solution was drawn with a 5 mL syringe fitted with a 20-gauge needle, then connected to a high-voltage source and electrospun onto aluminum foil. The electrospinning process was performed with the parameters which are noted in Table 1. For the second layer, a dual-nozzle electrospinning technique was used to create a dichotomous nanofiber layer consisting of Chitosan/PVA/SeNPs, along with PU.

The Chitosan/PVA/SeNPs layer was prepared at a concentration of 2% Chitosan, 10% PVA, and 50 ppm SeNPs. To prepare the chitosan solution, 0.08 g of chitosan was added to 2 mL of acetic acid (40%) and stirred until completely dissolved. To prepare the PVA solution, 0.4 g of PVA was added to 2 mL of deionized water and gently heated to dissolve the polymer completely. Chitosan and PVA solutions were mixed in a 1:1 ratio to prepare a chitosan/PVA mixture at a concentration of 2% chitosan and 10% PVA, and stirred for 30 minutes. After reaching room temperature, 100 $\mu\text{L}/\text{mL}$ of SeNPs was added, and stirring continued for another 30 minutes.

Table 1. The concentration of polymers and the combination of solvents used to form the two-layer scaffold, as well as the parameters of electrospinning.

	Concentration (wt% %)	Solvents	Ratio (W/W)	Voltage (KV)	Distance (cm)	Flow rate (ml/h)
Layer 1	PU (12%)	THF/DMF (3.1)	(3.1)	16	12	0.2
Layer 2	First nozzle: PU (12%)	THF/DMF (3.1) for PU	(3.1)	16	12	0.2
Dual-nozzle Electrospinning	Second nozzle: Chitosan (2%) PVA (10%) SeNPs	Deionized water for PVA Acetic acid (40%) for Chitosan		16	12	0.2

The mixture of Chitosan/PVA/SeNPs was drawn into the syringe and launched through one nozzle, while the PU solution was placed in the other nozzle, which was then simultaneously electrospun onto the first layer with the same parameters as the first layer. The electrospinning process was performed in a well-ventilated room with a temperature of $22 \pm 5^\circ\text{C}$ and a relative humidity of $40 \pm 5\%$ (Table 1).

Characterization of SeNPs-loaded nanofibers

ATR-FTIR and SEM

To identify and confirm the functional groups in each sample of SeNP-based scaffold components, Fourier transform infrared (FTIR) analysis in Attenuated total reflectance (ATR) mode was performed using a Magna 560 spectrometer. The principles of FTIR are based on changes in the vibrational energy of bonds in the sample. Since each bond type in a molecule absorbs radiation at a specific frequency and has its own infrared spectrum, the functional groups in each molecule can be determined. A scanning electron microscope (SEM) was used to observe and analyze the morphology of nanofibers after fixing the nanofibers and coating them with gold. Photographs were taken at the same magnification in all scaffolds. The diameter of the electrospun fibers, the porosity percentage, and the size of the pores between the nanofibers forming the scaffold were characterized by ImageJ software.

Cytotoxicity assay

The scaffolds' toxicity and their effect on the cell viability of human fibroblast cells were investigated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. First, we punched out pieces from the Chitosan/PVA and Chitosan/PVA/SeNs two-layered scaffolds to match the dimensions of the 96-well plate. To sterilize the scaffold pieces for cell implantation, both sides of the scaffold were exposed to UV light for 20 minutes. Subsequently, 5×10^3 HU-O2 cells (human fibroblast cells) were seeded on each scaffold. For the tissue culture plate (TCP) group, cells were cultured in wells without scaffolds and maintained in an incubator under standard culture conditions. Cell viability was assessed on days 1 and 3. At each time point, the cell culture medium was replaced with fresh medium containing MTT solution, following incubation for 3-4 hours. The medium was discarded, 100 μl of dimethyl sulfoxide was added, and the plate was kept at room temperature for 10 to 15 minutes. Finally, an ELISA reader was used to read the absorbance at a wavelength of 570 nm.

Antibacterial assay

The antibacterial properties of the nanofibrous scaffolds were evaluated against both Gram-negative (*Escherichia coli* ATCC 25922) and Gram-positive (*Staphylococcus aureus* ATCC 25923) bacteria. Each strain was initially cultured on Trypticase Soy Agar (TSA), and a fresh colony was inoculated into Mueller–Hinton Broth (MHB) and incubated for 18 hours to obtain an active culture. A bacterial suspension was prepared to the 0.5 McFarland standard ($\approx 1.5 \times 10^8$ CFU/mL) using sterile saline, then diluted to obtain a working concentration of 1×10^6 CFU/mL for the antibacterial assay. The antibacterial activity was investigated by the viable cell count method under direct contact conditions (17). Samples were placed in MHB, inoculated with bacterial suspension to yield a final concentration of 1×10^6 CFU/mL, and incubated in a shaking incubator at $30 \pm 2^\circ\text{C}$. At predetermined contact times (1, 6, and 24 h), aliquots were collected, serially diluted, and 0.1 mL of dilutions were spread onto TSA plates. The plates were incubated for 24 h at $30 \pm 2^\circ\text{C}$, and the formed colonies were counted using a colony counter.

The number of viable bacteria was reported as colony-forming units per milliliter (CFU/mL) according to:

$$\text{CFU/mL} = (\text{number of colonies} \times \text{dilution factor}) / \text{plated volume}$$

The bacterial reduction percentage and log reduction were compared in treated samples to those of the untreated control, by the viable cell count method.

In vivo wound model

The adult male Wistar rats weighing 200-250 g were used to establish a full-thickness excision wound model. The standard animal laboratory conditions consisted of a 12-hour light/dark cycle, $22 \pm 2^\circ\text{C}$, and 30%-70% humidity, along with free access to food and water. Animals were purchased from the Royan Institute and acclimatized for at least one week before the experiment. The institutional guidelines for animal care were considered for the animal modeling procedure, which was approved by the local Ethical Committee.

Ketamine (80 mg/kg) and xylazine (10 mg/kg) were applied intraperitoneally to three rats for anesthetization. After shaving and sterilizing the dorsal surface with 70% ethanol, three circular full-thickness skin wounds were incised on the dorsal region, with a diameter of 0.8 cm, using a sterile biopsy punch. Sterile gauze was used to manage hemostasis. Excisional wounds on animals were evaluated in three groups: control with no treatment, Chitosan/PVA, and

Chitosan/PVA/SeNPs, with a nanofiber dressing. Animals were observed daily for signs of pain, infection, or distress. Macroscopic evaluation of wound closure was performed on the 2nd, 7th, and 14th days. Rats were sacrificed for histopathological examination on the 14th day.

Histopathological evaluation

At predetermined time points, wound tissues (including a margin of surrounding normal skin) were harvested, fixed in neutral-buffered formalin (10%) for 24–48 h, and embedded in paraffin. Sections of 4–5 μm thickness were prepared and stained with hematoxylin and eosin (H&E) for histopathological evaluation. The collagen deposition and organization were evaluated by Masson's trichrome staining.

Statistical analysis

GraphPad Prism software (v. 8) was used for statistical analyses, and two-way ANOVA was used to compare differences between control and experimental groups. Data are presented as mean $\pm$ standard error of the mean. All experiments were performed in triplicate to ensure reproducibility. A p -value < 0.05 was considered statistically significant.

RESULTS

SeNPs synthesis and characterization

Selenium nanoparticles (SeNPs) were synthesized by the chemical reduction method with slight modifications from previously reported protocols (13-15). Briefly, sodium selenite (Na_2SeO_3) was used as the selenium precursor, and ascorbic acid served as a reducing agent. In a typical procedure, an aqueous solution of Na_2SeO_3 (e.g., 50 mM) was prepared in 0.1% chitosan and stirred at room temperature. Freshly prepared ascorbic acid solution (e.g., 50 mM) was added dropwise under constant stirring until a color change from colorless to orange, indicating SeNPs formation. The mixture was further stirred for 30-60 min toward complete reduction. The morphology analysis by TEM revealed the round shape of SeNPs with a size distribution of 38.32 ± 1.87 nm. The DLS reported a size of about 185 nm for SeNPs, as measured by the mean hydrodynamic diameter of the nanoparticles in Brownian motion. DLS reports the hydrodynamic diameter in liquid, which is larger than the TEM-reported size, which is the physical size of nanoparticles in solid structure. This discrepancy depends on capping agents and synthesis method (14). The EDX confirmed the elemental composition of 86.19% Se and 13.81% C in SeNPs (Figure 1).

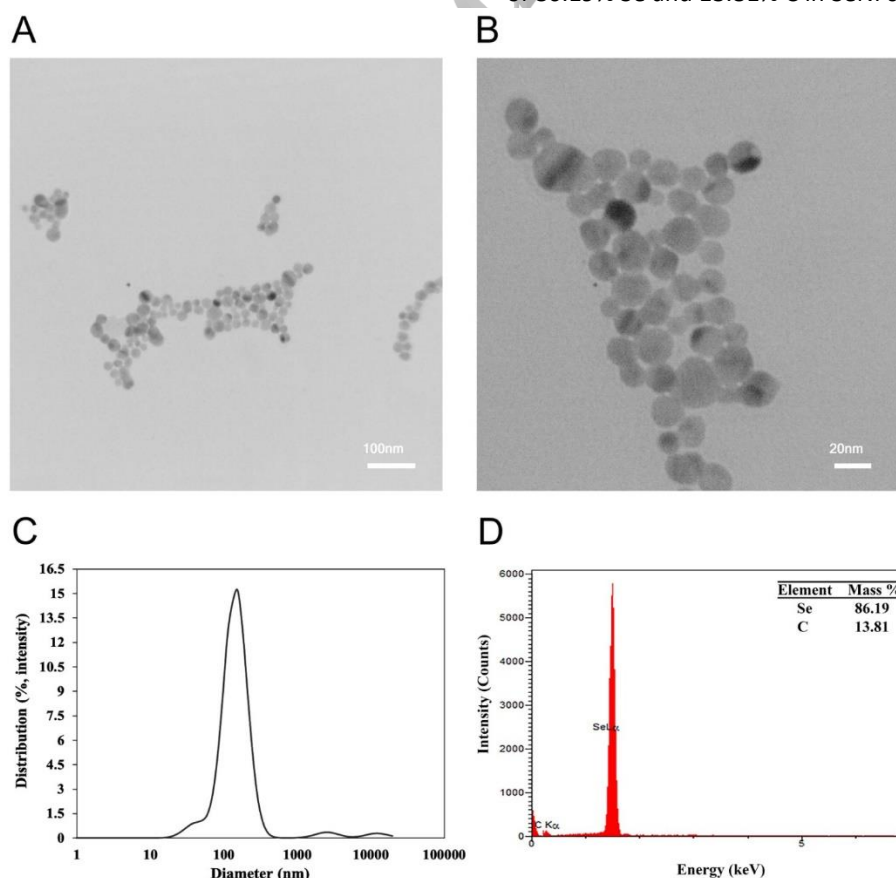


Fig. 1. SeNPs characteristics: (A, B) TEM, (C) DLS, (D) EDX.

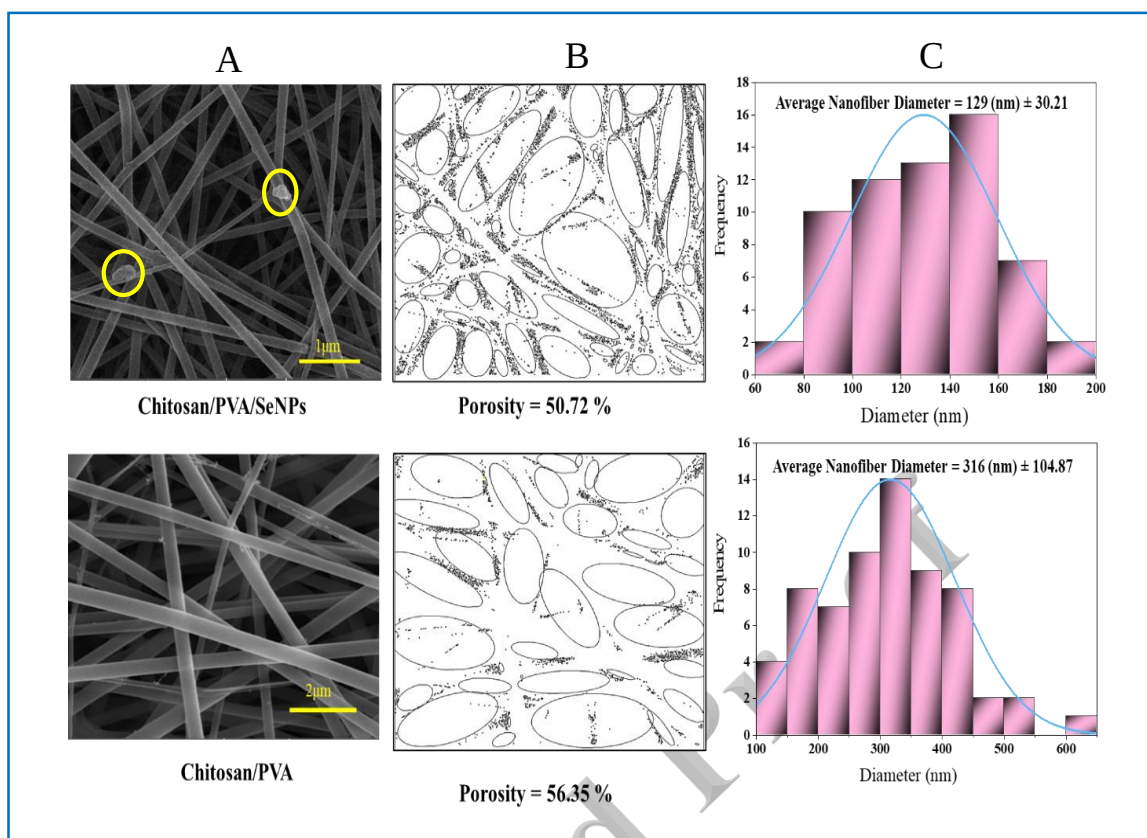


Fig. 2. (A) SEM images of Chitosan/PVA/SeNPs and Chitosan/PVA nanofibers. Yellow circles indicate the presence of SeNPs. (B) Nanofiber architecture and porosity percentage between constituent nanofibers in each group. (C) Diagram of the calculated diameter of nanofibers using ImageJ software.

Synthesis and characterization of Chitosan/PVA and Chitosan/PVA/SeNPs nanofibers

In this study, a two-layer scaffold was electrospun using a sequential, two-nozzle method on the first layer (12% polyurethane). SEM results showed that the presence of SeNPs in the Chitosan/PVA/SeNPs nanofiber scaffold had no effect on the appearance of the constituent nanofibers, and the fibers in both groups had a smooth, porous structure with homogeneous, interconnected pores. Figure 2A shows the presence of SeNPs on the surface and between the constituent fibers. As shown in Figure 2 B, the percentage of porosity calculated with ImageJ software was shown to be 56.35% in Chitosan/PVA nanofibers and 50.72% for Chitosan/PVA/SeNPs nanofibers. The presence of SeNPs on and between the nanofibers in the Chitosan/PVA/SeNPs nanofibers has reduced the porosity percentage and the number of pores between the constituent fibers. The diameter of the nanofibers in both groups was calculated using ImageJ software. Hence, approximately 50 fibers were selected from each electron microscope image, and after

determining the diameter of each fiber, the average diameter of the fibers constituting each layer was reported. Accordingly, the average nanofiber diameter in the Chitosan/PVA nanofiber was 316 ± 104.87 nm, and the majority of the fibers forming this layer were in the 300-350 nm range. The presence of selenium nanoparticles in the Chitosan/PVA/SeNPs nanofiber caused a reduction in average diameter of the nanofibers to 129 ± 30.21 nm by reducing the viscosity and increasing the electrical conductivity of the constituent polymer mixture (Fig.2C). The nanofiber network architecture, including diameter, the presence of interconnected pores, and the porosity percentage between the nanofibers forming the scaffold, affects some cellular processes, including cell attachment, proliferation, and migration. Interconnected pores in fibers allow cells to easily penetrate deep within them. Research has shown that pores less than one micrometer in size, in addition to inhibiting bacterial penetration, help allow essential gases to enter, deliver nutrients, and control secretions (16).

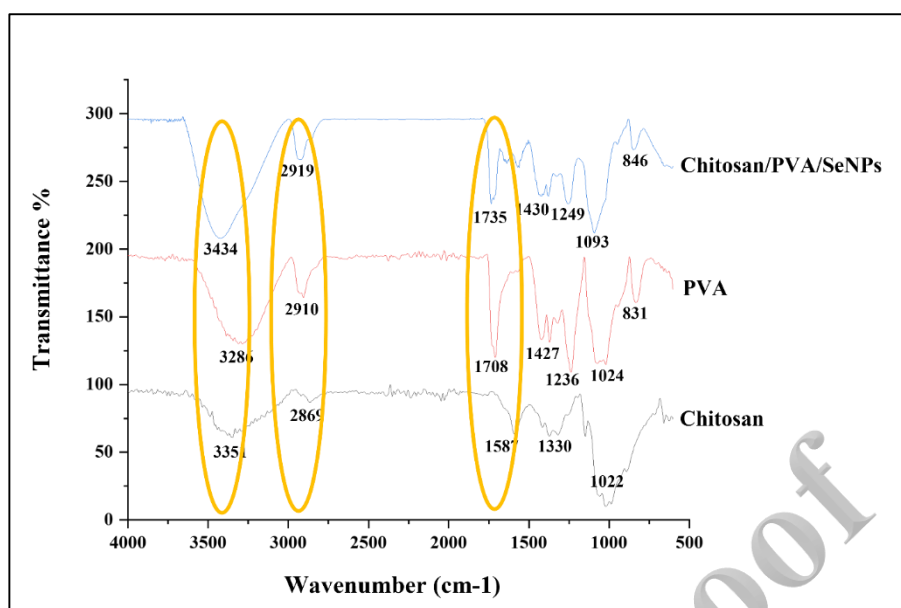
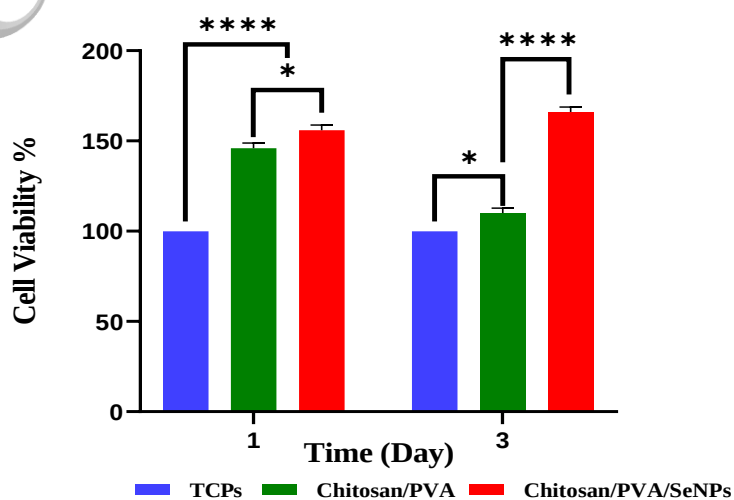


Fig. 3. Spectroscopic results of Chitosan/PVA/SeNPs, Chitosan, and PVA.

ATR-FTIR

ATR-FTIR was performed to identify functional groups and to investigate interactions between the constituent polymers and SeNPs in Chitosan/PVA/SeNPs nanofibers. Figure 3 shows the distinct peaks of each component in nanofibers Chitosan, PVA, and Chitosan/PVA/SeNPs nanofibers. The peaks observed in Chitosan at wavelengths of 800 cm^{-1} to 1200 cm^{-1} indicate the saccharide groups, and the peaks around 1330 cm^{-1} indicate the functional group of C-H, a single peak at wavelength 1587 cm^{-1} indicates the -NH stretching of Amide I, and finally the peaks at 2869 cm^{-1} and 3351 cm^{-1} indicate the OH and -NH stretching of the N-groups of the primary amino groups in chitosan polymer. The results of infrared

spectroscopy for PVA confirmed the presence of several strong peaks at wavelengths of 3286 cm^{-1} , 2910 cm^{-1} , 1708 cm^{-1} , 1427 cm^{-1} , and 1236 cm^{-1} , which represent the functional groups O-H, alkyl functional group C-H, C=O, and C-O, respectively. The spectroscopic results on the Chitosan/PVA/SeNPs nanofibers indicate several peaks at wavelengths of 3434 cm^{-1} , 2919 cm^{-1} , 1735 cm^{-1} , 1430 cm^{-1} , 1249 cm^{-1} , 1093 cm^{-1} , and 846 cm^{-1} . Peaks observed in the range of 3400 cm^{-1} to 2900 cm^{-1} were identified in both Chitosan and PVA polymers. The peak at wavelength 1735 cm^{-1} was also observed in PVA polymer. Other peaks observed at wavelengths of 1430 cm^{-1} , 1249 cm^{-1} , and 1093 cm^{-1} also confirm the presence of chitosan, PVA.

Fig. 4. Survival percentage of HU-02 cells in Chitosan/PVA/SeNPs and Chitosan/PVA nanofibers on days 1 and 3, MTT assay. $n \geq 3$, * $P < 0.05$, **** $P < 0.0001$.

Cytotoxicity assay by MTT

HU-02 cells were used to evaluate the cytotoxicity of Chitosan/PVA/SeNPs and Chitosan/PVA nanofibers on cell viability and proliferation characteristics. One of the key parameters in tissue engineering that supports tissue regeneration and repair is cell adhesion and proliferation on the scaffold. As shown in Figure 4, the survival percentage of cells implanted on both Chitosan/PVA/SeNPs and Chitosan/PVA nanofiber scaffolds was higher than that of the TCP group and revealed a statistically significant difference ($P < 0.05$). However, the presence of SeNPs significantly increased the HU-02 cell survival and proliferation by about 156%, compared to 146% in the Chitosan/PVA nanofiber scaffold ($P < 0.05$). After 3 days of cell seeding on the test scaffold, the results showed that the release of selenium nanoparticles entrapped between Chitosan and PVA nanofibers had a synergistic effect on cell growth and proliferation. Although the percentage of cell viability in the Chitosan/PVA nanofiber scaffold decreased on day 3 compared to day 1, the cell viability in this group was still higher than that in TCPs, with a significant difference ($P < 0.05$).

Antibacterial test

To evaluate the antibacterial activity of the prepared nanofibrous scaffolds for wound healing applications, Chitosan/PVA/SeNPs and Chitosan/PVA nanofibers were tested against *Staphylococcus aureus* and *Escherichia coli* using the viable cell count method under direct contact conditions, with an initial bacterial concentration of 1×10^6 CFU/mL and incubation times of 1, 6, and 24 h. The results are presented in Fig. 5A and 5B, respectively. Compared with the control group, the Chitosan/PVA/SeNPs scaffold exhibited approximately 1 log reduction against *S. aureus* after 6 h and reached a 4 log reduction after 24 h, corresponding to 99.99% bacterial reduction (Fig. 5C). In contrast, the Chitosan/PVA nanofibers showed less than 0.6 log reduction even after 24 h. Similarly, for *E. coli*, nearly 0.5 log reduction was observed at 6 h and a 4 log reduction at 24 h (99.99% bacterial reduction) in the Chitosan/PVA/SeNPs scaffold. In contrast, negligible log reduction was detected in the Chitosan/PVA nanofibers throughout the incubation period (Fig. 5D).

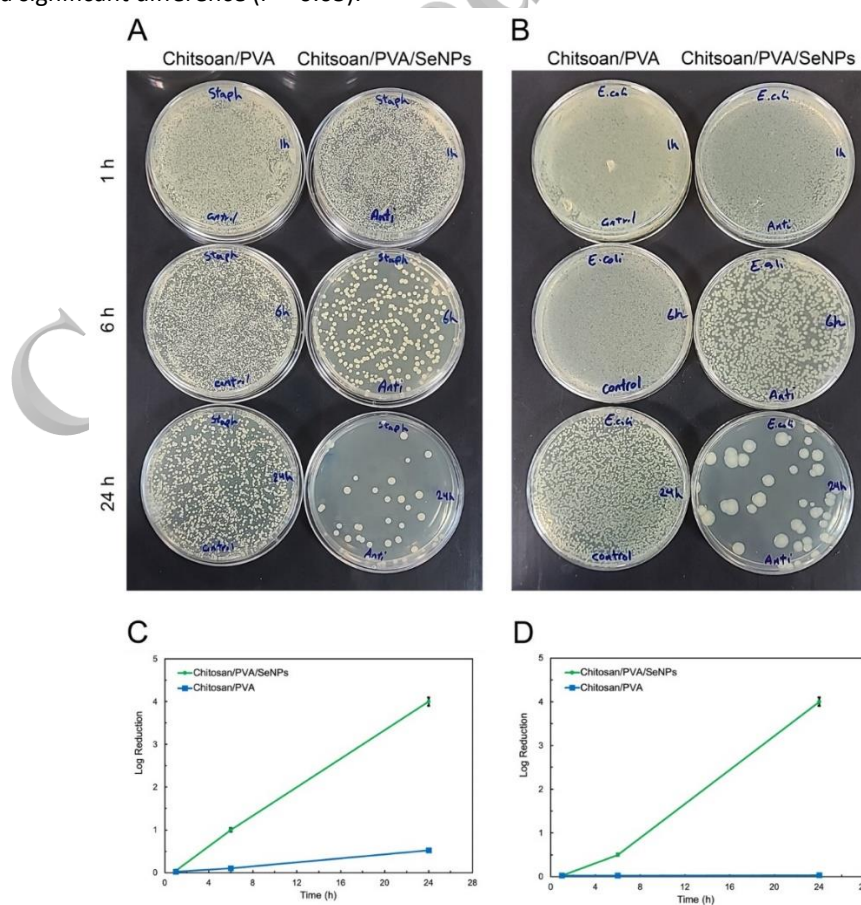


Fig. 5. Antibacterial assay of Chitosan/PVA/SeNPs and Chitosan/PVA nanofibers against *Staphylococcus aureus* (A, C) and *Escherichia coli* (B, D).

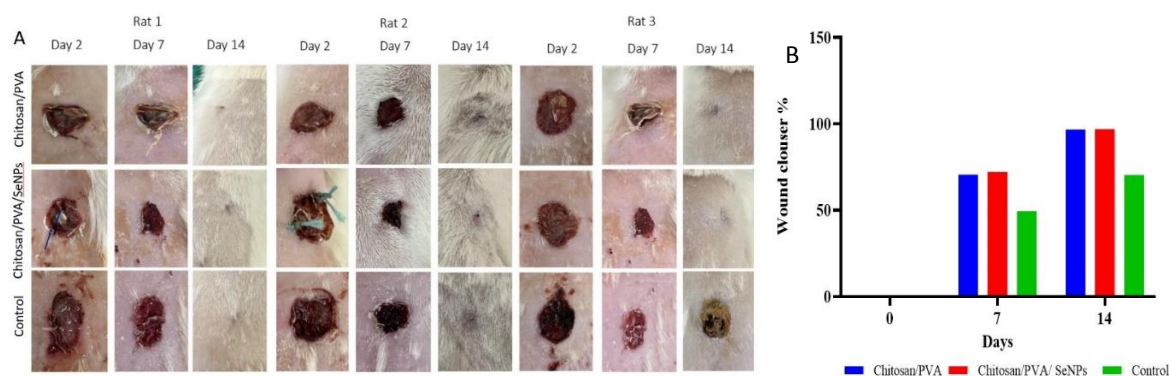


Fig. 6. Representative wound photographs showing closure over time (A), Wound closure percentage (B).

The Chitosan/PVA/SeNPs scaffold demonstrated time-dependent antibacterial activity, achieving 4-log reduction (99.99%) against both *S. aureus* and *E. coli* after 24 h. SeNPs markedly enhanced the bactericidal activity of the scaffold, which is primarily attributed to the excessive generation of reactive oxygen species (17), leading to disruption of cell membrane integrity, interference with amino acid production, and the blockage of DNA replication (18).

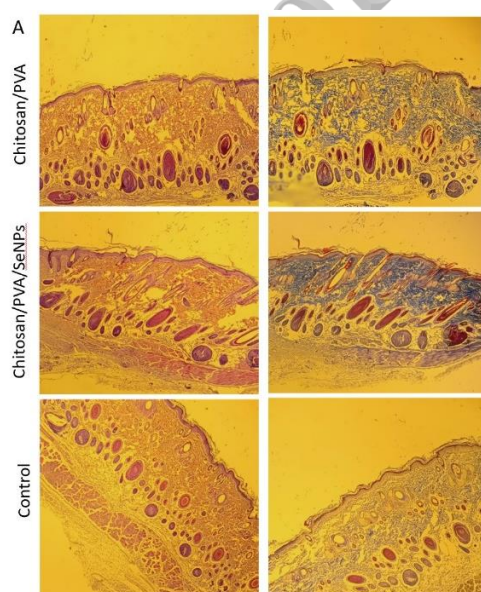
Gram-positive bacteria were more susceptible, whereas Gram-negative *E. coli* initially showed a slower reduction due to their low-permeability outer membrane and efficient efflux systems (19). These findings highlight the potential of the SeNP-loaded scaffold for wound dressing applications requiring prolonged infection control.

In vivo wound model

Macroscopic evaluation revealed progressive wound contraction in all groups throughout the study

period (Fig. 6A). Quantitative analysis showed that the Chitosan/PVA/SeNPs Nanofibers-treated group exhibited a significantly faster wound closure rate compared with both the Chitosan/PVA Nanofibers and control groups. By day 7, the mean percentage of wound closure reached 72.07 % in the Chitosan/PVA/SeNPs group, compared to 70.64 % in the Chitosan/PVA Nanofibers group and 49.40 % in the control group. However, wound closure was completed by day 14 in both Chitosan/PVA/SeNPs and Chitosan/PVA Nanofibers groups, whereas an infectious wound developed within the control group. Wound closure percentage is presented in a graph in Figure 6B.

On day 14, the *H&E staining* demonstrated persistent inflammatory cell infiltration and irregular granulation tissue in the wounds of the control group (Fig. 7A).



B

Scoring 0-3	NF	NF-SeNPs	Control
Edema	2	2	1
Leucocytes	2	2	2
Macrophage	2	2	2
Granulation tissue	2	3	2
Fibroblast	1	2	1
Collagen	2	3	2
Re-epithelization	2	3	2
Total score	13	17	12

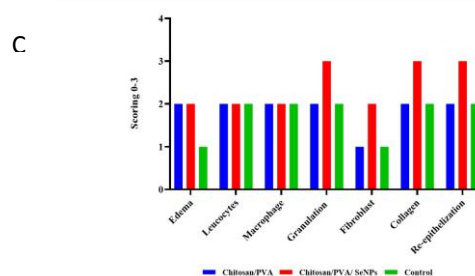


Fig. 7. The H&E and Masson's trichrome images on the 14th day (A), Semi-quantitative histological scoring system (B), and histological scoring presented in the graph (C).

The Chitosan/PVA Nanofibers group showed moderate epithelialization with reduced inflammatory infiltrates, but immature granulation tissue persisted. In contrast, the Chitosan/PVA/SeNPs Nanofibers group displayed almost complete epithelial coverage, well-organized granulation tissue, and minimal inflammatory cells. Collagen deposition and remodeling were more pronounced in the Chitosan/PVA/SeNPs Nanofibers group compared with the other groups, as revealed in Masson's Trichrome Staining. Collagen fibers appeared dense, thick, and well-aligned, indicating advanced maturation of the extracellular matrix. In the Chitosan/PVA Nanofibers group, collagen bundles were moderately organized, whereas the control group exhibited sparse, loosely arranged collagen fibers.

Semi-quantitative histological scoring confirmed these observations. The Chitosan/PVA/SeNPs Nanofibers group achieved higher scores for epithelialization, granulation tissue formation, and collagen maturation compared with both Chitosan/PVA Nanofibers and control groups (Figure 7B, C).

DISCUSSION

The bacterial infection at the wound site leads to bacterial colonisation and biofilm formation that intercept phagocytes and antimicrobial agents, resulting in inadequate wound healing (20). The wound care era faces challenges in developing wound dressings that are mechanically strong enough while also incorporating bioactive ingredients (21). SeNPs exhibit different desired bioactivities for wound healing, such as antibacterial, antioxidant, and anti-inflammatory properties along with immune regulation effects. SeNPs mediate their antibacterial activity via direct interaction to bacterial cell membrane, which is more pronounced in more positively charged nanoparticles. Direct interaction of SeNPs with the bacterial cell membrane leads to membrane potential imbalance and permeability, resulting in leakage of cellular contents and bacterial death. In addition, excessive production of ROS by SeNPs inside the bacteria is harmful to biomolecules and genetic materials, interfering with bacterial function and leading to bacterial death. Additionally, inhibiting inflammation at the site of the wound can prevent bacterial biofilm formation, which is one of the critical issues in wound healing (20, 22). In addition, the biological effects of SeNPs in regulating oxidative stress, through increased

glutathione levels and reduced lipid peroxidation (23), regulating immune responses through balancing between anti-inflammatory and proinflammatory cytokines (20), enhancing tissue remodeling and angiogenesis (20) contribute to creating a favorable microenvironment for tissue regeneration.

Different strategies have been applied toward the fabrication of SeNPs-based wound dressings, as well as incorporating SeNPs into nanofibers, hydrogels, or composites (21, 24, 25). Recent investigations highlighted the potential of SeNPs-loaded nanofiber dressings as multifunctional wound-healing materials (26, 27). The improved structural features of nanofibers after incorporating SeNPs have been investigated, as well as their antibacterial and antioxidant effects, which are highly desirable for wound healing (17, 26-30). Doostmohammadi et al. reported that the incorporation of SeNPs into polycaprolactone/gelatin (PCL/GEL) nanofibers improved fibroblast attachment and antioxidant activity, as revealed in *in vitro* experiments (26). Their *in vitro* cytotoxicity assay showed that SeNPs-loaded nanofibers outperformed PCL/GEL nanofibers alone in mouse fibroblast 3T3 cell proliferation. Similarly, Chung et al. reported that the presence of SeNPs led to enhanced antibacterial properties of silk fibroin nanofibers along with augmented human dermal fibroblast viability and cell growth (27). Another study by Chen et al. revealed that SeNPs loaded into ICG@LNT enhanced gap closure in a scratch wound-healing assay for fibroblasts, as well as elevated migration in a Transwell migration assay for HUVEC cells (31). These results were supported by Mirza et al. that cell proliferation and migration were enhanced in HaCaT keratinocyte cell lines by SeNPs (32). In another recent study, SeNPs were introduced into a composite hydrogel of chitosan and cellulose nanofibers, carrying adipose-derived mesenchymal stem cells (ADSCs), for diabetic wound healing (28). The antibacterial, antioxidant, and regenerative characteristics of this multifunctional system effectively protect the viability of ADSCs in the hyperglycemic environment of diabetic wounds from oxidative damage. The wound closure was accelerated by applying this hydrogel via enhanced angiogenesis and collagen deposition, along with reduced inflammation, as demonstrated in diabetic rat wound models. Another recent study doped SeNPs

into a protein-based electrospun nanofiber composed of pullulan blended with gelatin or gliadin to improve the wound repair process (17). Incorporating SeNPs led to improved antioxidant capacity of the scaffold, which also resulted in upregulated favorable extracellular matrix gene expression for fibroblast proliferation without compromising fiber morphology. In another investigation, selenium nanoparticles incorporated into a chitosan-zinc oxide nanostructure demonstrated a synergistic effect of SeNPs and ZnO in enhancing wound tissue regeneration via an in vivo animal model. Furthermore, in vitro investigations supported the strong antibacterial and antioxidant effects of this nanocomposite against *E. coli* and *S. aureus*, while enhancing the proliferation of keratinocytes and fibroblasts (30). SeNPs in various dressing forms are summarized in Table 2.

In line with previous studies, the cell viability assay and in vivo wound healing experiment in our results showed similar findings, indicating higher cell viability in Chitosan/PVA/SeNPs than in Chitosan/PVA nanofibers, with improved wound healing in vivo. The antioxidant property of SeNPs is one of the most important features in supporting cell proliferation and viability, eliminating harmful effects of reactive oxygen species on cells (34). The in vitro assessments for antibacterial effects of Chitosan/PVA/SeNPs demonstrated that SeNPs can exhibit their antibacterial effects even when incorporated into a nanofiber structure, which is

supported by our previous experiments (14, 15) and others' reports (27).

Taken together, current evidence suggests that SeNPs-incorporated nanofiber dressings accelerate wound healing by combining the structural support of electrospun scaffolds with the therapeutic bioactivity of selenium. Future studies should focus on standardized wound models, dose-response evaluation, and comprehensive biocompatibility testing to facilitate translation into clinical practice.

CONCLUSION

In summary, we successfully green-synthesized SeNPs and incorporated these nanoparticles into Chitosan/PVA nanofibers to introduce antibacterial properties to the nanofibers. Compared to Chitosan/PVA nanofibers, the Chitosan/PVA/SeNPs nanofibers exhibited antibacterial effects against *Staphylococcus aureus* and *Escherichia coli*, while the MTT assay showed no cytotoxicity. The possibility of antibacterial SeNPs-loaded nanofibers for wound dressing was demonstrated by an in vivo full-thickness wound model. This study strongly supports the possibility of Chitosan/PVA/SeNPs nanofibers for improved wound healing process.

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Table 2. Literature review for SeNPs in wound healing.

Nanoparticles	Hydrogel/nanofibers/dual mechanisms	In vivo	Ref.
SeNPs	CMC, PUN, GLY, PEDOT: PSS	-	(21)
ICG@LNT-SeNPs	Photothermal therapy	Diabetic infected wound model	(31)
CV-SeNPs	-	-	(33)
GL-SeNPs	-	-	(32)
SCA-SeNPs	Citric acid/gelatin/hydroxysuccinimide grafted polyacrylic acid nanocomposite hydrogel adhesive	Full-thickness cutaneous defects of rats	(24)
SeNPs	Chitosan, propylene glycol, PRP	Full-thickness cutaneous defects of rats	(25)
SeNPs	PRP	Diabetic wound model	(34)
SeNPs	Chitosan biofilm	Infected wound model	(35)
SeNPs	-	Full-thickness cutaneous defects of mice	(36)
Ch-SeND	Chitosan/doxycycline	Full-thickness cutaneous defects of rats	(37)
R-SeNPs	Red blood cell membrane as encapsulation agent	Infected wound model of mice	(38)
SeNPs/Cs/ZnO	Chitosan/ZnO nanoparticles	Full-thickness cutaneous defects of rats	(30)
SeNPs@PEI@CGI	PEI/ICG (photoacoustic)	Infected wound model	(39)
SeNPs/ChNPs	Selenium-Chitosan nanoconjugate	-	(6)
SeNPs	PCL/GEL/Vitamin E		

CMC: carboxymethylcellulose, PUN: punicalagin, GLY: glycerol, PEDOT:PSS: poly(3,4-ethylenedioxythiophene) polystyrene sulfonate, ICG@LNT-SeNPs: indocyanine green (ICG)-loaded lentinan (LNT)-functionalized selenium nanoparticles, CV: Chara vulgaris, GL: Guava leaf, SCA: multifunctional selenium nanoparticles (SeNPs)/citric acid/gelatin/hydroxysuccinimide grafted polyacrylic acid nanocomposite hydrogel adhesive, PRP: Platelet-Rich Plasma, PEI: polyethylenimine, ICG: indocyanine green, PCL: polycaprolactone, Gel: gelatin,

AUTHORS' CONTRIBUTIONS

R. Gh.: Writing, Investigation ; S. B.: Writing, Conceptualization, Investigation, Methodology ; R. F. M.: Formal analysis, Software, Investigation ; S. M. A. T.: Resources ; A. M.: Methodology, Formal analysis ; M. M. Kh.: Supervision, Resources, Project administration.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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ETHICAL CONSIDERATIONS

The study protocol was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (ethical code: IR.SBMU.RETECH.REC.1403.256).

AI STATEMENT

No AI assistant was used; only Grammarly was used for grammar editing.

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