Wound healing activities of polyurethane modified chitosan nanofibers loaded with different concentrations of linezolid in an experimental model of diabetes

Mahmoud H. Teaima\textsuperscript{a,\textsuperscript{*}}, Mohamed K. Elasaly\textsuperscript{a}, Samia A. Omar\textsuperscript{b}, Mohamed A. El-Nabarawi\textsuperscript{a}, Kamel R. Shoueir\textsuperscript{c,d,\textsuperscript{**}}

\textsuperscript{a} Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt
\textsuperscript{b} Department of Pharmaceutics, Faculty of Pharmacy, Sinai University-AlQantara Campus, AlQantara, Egypt
\textsuperscript{c} Institute of Nanoscience & Nanotechnology, Kafrelsheikh University, 33516, Kafrelsheikh, Egypt
\textsuperscript{d} Institut de Chimie et Procédés pour l’Énergie, l’Environnement et la Santé (ICPEES), CNRS UMR 7515-Université de Strasbourg, 25 rue Becquerel, 67087, Strasbourg, France

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ABSTRACT

Diabetes is associated with impaired wound healing, making patients susceptible to chronic non-healing wounds. Nanofibers have proven to possess potential wound healing properties when nanofibers are loaded with other wound dressing materials. This study aimed to investigate the potential role of nanofibers loaded with the linezolid as a model drug in enhancing wound healing in streptozotocin (STZ) induced diabetic rats. Additionally, the current research aimed to increase the effectiveness of the linezolid and increase its absorption through the skin cells by preparing it through the electrospinning technique. Thus, an admixture of polyurethane (TPU) blended with the prepared modified chitosan was utilized for nanofibers formation. The latter mixture solution was incorporated with different concentrations of the linezolid. The prepared nanofibers loaded with linezolid were extensively investigated using advanced tools in terms of morphological features and mechanical properties. The resultant data illustrated that the linezolid had no negative impact on TPU/modified chitosan (CS) morphology. The nanofibers are formed with homogeneous filament and with a small diameter. The research work extended to the use of these prepared materials as a dressing for diabetic wounds. Thus, rats were divided into four wounded skin animal’s groups were uniformly dressed with an experimental sterile dressing made of nanofibers based on polyurethane (PU) blended with modified chitosan and different concentrations of linezolid: 1 mg (Group II), 3 mg (Group III) and 5 mg (Group IV) per kg b.w. The negative control group was maintained with only polyurethane modified chitosan nanofibers dressing without any treatment (control group). The data obtained that the linezolid promoted the healing of diabetic wounds and effectively controlled the growth of microorganisms at the wound site. This strategy plays an essential role in the treatment of both acute and chronic wounds. Wound healing was accelerated by the ability to infuse epidermal cells and growth factors. As a result of the investigated properties for the synthesized nanofibers in this research, the electrospun nanofibers are helpful and crucial in wound treatment. From these data above, it can be demonstrated that the nanofibers comprising polyurethane/modified chitosan loaded with linezolid can be considered synergetic material that can heal diabetic wounds and be used instead of traditional gauze.

1. Introduction

Wound healing is a motivated reaction to various factors that may damage the skin or organs [1]. A simultaneous cascade of events occurs in tissue or skin damage, inevitably leading to normal tissue regeneration [2]. Cell angiogenesis, inflammation proliferation, collagen deposition, and re-epithelization are the different stages of wound healing [3, 4]. Impaired wound healing and chronic skin ulcers are signs of several
human diseases. Chronic wounds are a common problem in diabetic patients, and they often necessitate resection [5]. Diabetes is directly linked to slow wound healing, increasing the risk of chronic non-healing wounds, and about 84% of all diabetic lower extremity amputations are preceded by such wounds [6].

Chronic diabetic wounds are trapped in a chronic inflammatory condition, with excessive concentrations of pro-inflammatory cytokines and proteases, as well as compromised growth factor function and a rise in oxidative stress, which results once the production of reactive oxygen species (ROS) meets the anti-oxidant potential in people with diabetes [5,7]. In diabetic, the emergence of effective glycation end-products (AGEs) and their association with their receptors (RAGE) are indeed related to impaired wound healing [8]. Neutrophils are the most common cell type in the early stages of inflammation and begin to shrink after 24–36 h due to apoptosis as circulating monocytes invade the wound and develop into tissue macrophages, which play a vital role in tissue regeneration [9]. The chemokine IL-8, which is generated by neutrophils and attracts macrophages and other cells to the wound site, also plays a role in the development process [10]. Despite the widespread use of multiple wound healing agents, it has recently been demonstrated that topical drug delivery systems based on specific nanofibers are efficient for transporting antibiotics to skin tissue [11]. As a result, nanofibers fabrication is a promising trend, with various nanoscale materials used in biomedical applications to avoid multiple diseases [3,12-17].

Wound dressings have evolved from natural materials that merely protected and enclosed the wound to materials concentrated on moisture conservation. Most lately, to materials that distribute the active ingredients into the cells or specific chemicals in the local wound environment [18,19]. Developments of dressing technologies have resulted in a recent number of recent topical drugs that do more than just protect and cover the wounds; they can also help in the healing process and address unique challenges in non-healing wounds [20]. The primary goal is to achieve the fastest degree of wound healing and the finest cosmetic wound recovery possible with the utilization of an efficient carrier such as nanofibers loaded with the efficient drug. Linezolid is one of these valuable drugs and is described as a potent antibiotic being used to treat lung infections and complicated soft tissue infections induced by gram-positive bacteria, S. aureus [18,21]. Linezolid belongs to the oxazolidinones class of synthetic antimicrobial agents [22]. Linezolid can inhibit different strains of multidrug-resistant species and have bacteriostatic activity [23]. Linezolid is available exclusively in tablet, oral, and injectable delivery formulations [24]. The development of new technologies to maximize the benefit of such a drug opens up new possibilities for improving a pharmaceutical product’s efficiency and increasing the likelihood of receiving a particular version profile. The efficiency of linezolid is expected to be enhanced via encapsulation or in-situ loading onto electrospun nanofibers [25]. In addition to utilizing a synthetic polymer to facilitate the high-throughput of scalable preparation of nanofibers [26].

Because of its barrier properties and oxygen permeability, polyurethane (PU) is a biocompatible hydrophilic synthetic polymer frequently used for wound dressings [27]. PU is also a thermoplastic polymer with robust mechanical properties and water insolubility, making it suitable for various applications, including biomaterials, biosensors, protective fabrics, and so on [28].

Herein, a nanofibrous scaffold based on polyurethane (PU)/modified chitosan/linezolid was fabricated via an electrospinning process to be used as wound dressing material. The surface structure of electrospun nanofibers and their mechanical features were assessed. The characterized nanofibrinous scaffold incorporated with different content of linezolid was used to heal diabetic wounds. In light of these findings, we aimed to investigate the potential role of linezolid loaded polyurethane/modified chitosan nanofibers in enhancing wound healing in an experimental model of diabetes.

2. Materials and methods

2.1. Chemicals and materials

Chitosan (Mw 160 kDa, 99%) and Polyurethane (Selectrophore™), and linezolid (C_{16}H_{20}F_{3}N_{4}O_{4}) were supported by Sigma Aldrich Co. North Carolina state, USA. Modified chitosan was synthesized according to published elsewhere by our group [18]. Tablets of Phosphate buffer saline were purchased from Sigma Co. (USA). With no further purification, all other chemicals were used as received. Hepatocellular carcinoma (HePG2), Colorectal adenocarcinoma (Caco-2), Epithelioid carcinoma (HeLa), and Mammary gland (MCF7). The cell line was acquired from ATCC via VACSER, a Cairo, Egypt-based holding firm for biological products and vaccines. Doxorubicin was a common anticancer drug. The reagents used were RPMI-1640 medium, MTT and DMSO (Sigma Co. Louis, USA), and Fetal Bovine serum (GIBCO, UK).

A total of forty male Sprague-Dawley rats weighing 180 ± 10 g was used here in this current study. For one week, all rats were kept separately in clean and sterile stainless-steel housing under normal temperature and light conditions and fed standard rodent chow. Animal research was undertaken in conjunction with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals [29].

2.2. Methods

2.2.1. Electrospinning of polyurethane linezolid combination

The experimental setting of electrospinning consists of a syringe pump with a 10 mL metal needle, a high voltage power supply connected directly to the needle, and the other side connected to the metal collector plate, which is far a 15 cm distance from the needle. The viscous polymeric solution was inserted into the syringe, then attached to the digital syringe pump with a 21-gauge blunt-end needle. To capture the nanofibers, an aluminum film was applied to a cylinder-shaped metal detector. For 4 h, electrospinning was carried out at 27 °C and 35% relative humidity (RH). The process parameters are a voltage of 17 kV, a tip-to-collector gap of 15 cm, and a flow rate of 1.5 mL/h. The TPU viscous polymer solution (7 gm) was first prepared by dissolving in 10 mL of DMF solution with continuous stirring until a homogenous solution was formed. Three falcon tubes were used to prepare three different polymer solutions mixed with the same ratio from modified chitosan. In each tube, 10 mL of TPU solution was physically mixed with 0.3 mg of modified chitosan, and 1, 3, and 5 mg of linezolid were subsequently added to the mixtures.

They were stirred for 1.5 h before electrospinning. The electrospinning was performed at ambient temperature and pressure. The electrospun nanofibers were vacuum dried at room temperature for 48 h. The amount of linezolid-loaded nanofibers was determined by ultracentrifuge [30], and the absorbance is measured by UV spectrophotometer at λ_{max} = 250 after scanning the standard solution between 200 and 400 nm.

2.2.2. Induction of diabetes

Streptozotocin was dissolved in a mixture containing sodium citrate solution (50 mM) and sodium chloride (150 mM) and administered intravenously to rats as 6 mg per 100 g body weight. After three days, fasting blood sugar was measured to affirm the progression of diabetes mellitus. If the animals’ fasting glucose level was more than or equal to 200 mg/dl, they were classified as diabetic [31].

2.2.3. Incision wound operation

Diethyl ether (40%) was utilized to anesthetize the diabetic rats, and their dorsal surface was cut with a sterile razor. Diluted ethanol (75%) was used to sterilize the shaved area. On the shaved patch, a single longitudinal skin incision was made.
2.2.4. Experimental design

The wounded skin animals were uniformly dressed with an experimental sterile dressing made of polyurethane modified chitosan nanofibers loaded with different concentrations of linezolid as an antibiotic; 1 μg linezolid (Group II), 3 μg linezolid (Group III), and 5 μg linezolid (Group IV) per kg b.w. The negative control group was maintained with only polyurethane-modified chitosan nanofibers without antibiotics (control group). This experiment was conducted for seven days. Every day, the animals were given a new nanofiber impregnated with the appropriate concentration of linezolid. On the first and last days of the study, fasting blood sugar was measured.

2.3. Characterization of modified chitosan and linezolid loaded PU/modified chitosan nanofibers

The samples powder and nanofibers were scanned using a field emission scanning electron microscope (FE-SEM; model: QUANTA-FEG250, Netherlands). A transmission electron microscope (TEM, JEOL1400, 120 kV, Japan) was used to recognize of shape and size of linezolid at 5 mg incorporated PU/modified chitosan nanofibers.

The mechanical parameters have also been assessed using LLOYD INSTRUMENTS, UK. The samples were supplied in rectangular strips with dimensions of (100200.1) mm for this reason. The stress/strain test was then carried out by dragging the nanofibrous up to the fracture point at a 30 mm/min rate, complying with ASTM D882.

2.4. Assay in vitro drug cytotoxicity

By using MTT assay, the inhibitory effects of nanofibers loaded with medication on cell proliferation were assessed using the four cell lines [32].

\[
\text{Viability} (\%) = \frac{A \text{ of treated sample}}{A \text{ of untreated sample}} \times 100
\]

(1)

\[
\text{Inhibition} (\%) = 100 - (\text{viability} \%)
\]

(2)

The annexin V kit was used to dye MCF-7 cells treated with the IC50 of nanofibers using a BD FACS Calibur flow cytometer (Becton Dickinson, Sunnyvale, CA, USA).

2.5. In vitro linezolid release study

In-vitro linezolid release analyses of linezolid-loaded nanofibers were conducted twice. Shortly, 20 mg of the examined nanofibers were adequately weighted and put in 100 mL of phosphate buffer (pH 7.4). The temperature was set to 37 °C, and the glass beaker was placed in a mechanical shaking bath, 2 ml of solution was removed and replaced with freshly buffer solution at predetermined time intervals, and the volume of linezolid released was measured using a single beam UV spectrophotometer at 253 nm wavelength.

2.6. Measurement of the wound area

The progressive changes in wound area (mm) were reported on the first and seventh days of the experiment. Photographs of the wound area were taken, and the percentage of wound contraction was measured from the equation described previously [33].

\[
\text{Wound Contraction} (\%) = \frac{\text{Wound area day (0) } - \text{ wound area day (n)}}{\text{Wound area day (0)}} \times 100
\]

(3)

At the end of the experiment for the animal rats, blood was taken from the eye’s retro-orbital venous plexus using heparinized capillary tubing and stored in a test tube containing sodium fluoride for fasting blood sugar assessment, and the other dry clean tube without anticoagulant for serum separation. A cooling centrifuge (Laborzentrifugen, 2K15, Sigma, Germany) was used to centrifuge all tubes for 10 min at 5000 rpm. The serum was separated and held at 80 °C till the biochemical parameters were assessed.

2.7. Biochemical assays

Blood glucose levels were determined using a procedure listed elsewhere [34]. According to Moshage et al., process [35], nitric oxide was calculated as nitrite and determined using Griess reagent. The amount of malondialdehyde was measured using the Ruiz-Larrea et al. procedure to assess lipid peroxidation [36]. Hcy was calculated using Agilent technologies 1100 series high-performance liquid chromatography (HPLC) with a quaternary pump.

3. Results and discussion

3.1. Morphological properties of the prepared linezolid loaded PU nanofibers

Before using nanofibers to heal diabetic wounds, the prepared nanofibers produced from an admixture of PU, chitosan, and different concentrations of linezolid should be first characterized to outline the morphology nanofibers also the cell viability should be examined. Regarding this, the morphological structure of the produced environmentally nanofibers is illustrated below. Firstly, studying the morphology of linezolid and modified chitosan is necessary before the electrospinning process with the supporting electrospun formation synthetic polymer (PU). Thus, Fig. 1a illustrates the surface of linezolid under the SEM technique. It is seen that linezolid has a well-defined appearance of long particles with a large diameter. The linezolid morphological is following previously published work [18].

Meanwhile, modified chitosan (Fig. 1b) displays small spherical particles due to the interaction of chitosan with reactants that destroyed the edges of pure chitosan. The appearance of these tiny particles of modified chitosan agrees with the work documented by Shoueir K.R. et al., [37]. Meanwhile, the electrospun PU (Fig. 1c) displays an ideal nanofiber formed with a smooth surface and beads free. The nanofibers have homogenous fibers. Thus, it can be noted that the thin filaments are sized with a size less than 2 μm. The surface appearance of PU/modified chitosan nanofibers is displayed in Fig. 1d. It is worth noting that the nanofibers created have a well-defined, three-dimensional formation of fiber filaments, with smooth surfaces and edges sparkling brighter than flat surfaces implying that the modified chitosan is entirely compatible with PU compound, leading to the formation of fibers with no noticeable beads. In addition to the porosity of the formed nanofibers binds to the host tissue and gives nutrients to cells [38].

Moving to the scanned samples of PU/modified chitosan loaded with different concentrations of linezolid; 1 mg, 3 mg, and 5 mg as demonstrated in Fig. 1 (e, f & g) respectively, It can be concluded that the incorporating of linezolid does not provide any noticeable change to the fiber diameter and structural morphology. This observed phenomenon may be attributed to the complete encapsulation of linezolid into the pores of the formed nanofibers and also in-between the fibers filament [18,39]. In addition, the non-change in the fiber structure depicts the compatibility between all components that are used for nanofibers formation. Moreover, it can be expected from the full linezolid encapsulation that it will be sustained-release during in vitro release and hence the application for wound healing.

Fig. 2. With respect to SEM morphology, the incorporation of linezolid into nanofibers was tested using TEM analysis. PU/modified chitosan nanofiber before and after loading of linezolid was collected on the carbon-coated copper grid before the examination. Fig. 2a depicted pure nanofiber without linezolid, and the formed nanofiber is relatively smooth without any distortion. In Fig. 2b, linezolid is randomly
distributed in the continuous phase of PU/modified chitosan nanofiber owing to the biocompatibility between the drug and the nanofibers. An applied electric field in the electrospinning process is higher than the viscous solution’s surface tension, which leads to higher stretched and thick fibers to some extent and gives a chance for linezolid to be distributed in the nanofiber out of the surface area of the Taylor cone [40].

3.2. Evaluation of mechanical strength

To evaluate the fabricated nanofibers’ mechanical (stress/strain) behavior based on TPU/modified CS, selected nanofibers of TPU/modified CS loaded with the low and high content of the linezolid (1 mg and 5 mg of linezolid) were assessed. Thus, Table 1 depicts the development of stress/strain conduct as drug involvement differs throughout the nanofibers of the polymeric mixture comprised of TPU and modified CS. The table presents that with the incorporation of the linezolid to the TPU/modified chitosan mixture prior electrospinning process, the tensile strength decreased from 5.670.51 to around 3.100.72 MPa, while the fracture strength ranged from 1.610.41 to 0.320.05 MPa for the

<table>
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<tr>
<th>Composition</th>
<th>Young’s modulus (MPa)</th>
<th>Tensile strength (MPa)</th>
<th>Fracture strength (MPa)</th>
<th>Maximum strain at break (%)</th>
<th>Toughness (MJ/m³)</th>
</tr>
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<tbody>
<tr>
<td>TPU/modified CS</td>
<td>0.73 ± 0.08</td>
<td>5.67 ± 0.51</td>
<td>1.61 ± 0.41</td>
<td>68.72 ± 5.45</td>
<td>3.17 ± 0.43</td>
</tr>
<tr>
<td>TPU/modified CS 1 mg linezolid</td>
<td>0.39 ± 0.06</td>
<td>4.72 ± 0.43</td>
<td>4.88 ± 0.84</td>
<td>66.22 ± 4.84</td>
<td>2.74 ± 0.65</td>
</tr>
<tr>
<td>TPU/modified CS 3 mg linezolid</td>
<td>0.55 ± 0.08</td>
<td>4.14 ± 0.81</td>
<td>4.22 ± 0.21</td>
<td>67.04 ± 4.41</td>
<td>2.42 ± 0.53</td>
</tr>
<tr>
<td>TPU/modified CS 5 mg linezolid</td>
<td>0.66 ± 0.09</td>
<td>3.10 ± 0.72</td>
<td>0.32 ± 0.05</td>
<td>66.99 ± 4.13</td>
<td>0.93 ± 0.31</td>
</tr>
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Fig. 1. SEM of (a) linezolid, (b) modified chitosan, (c) PU nanofibers, (d) PU/modified chitosan nanofibers, (e) 1 mg of linezolid PU/modified chitosan nanofibers, (f) 3 mg of linezolid PU/modified chitosan nanofibers, and (g) 5 mg of linezolid PU/modified chitosan nanofibers.

Fig. 2. TEM of PU/modified chitosan nanofiber (a) before and (b) after loading of linezolid.
minimum and maximum content of the linezolid.

In addition, the maximum strain at the break was not less than 66.224.84%, and the elasticity modulus dropped from 0.730.08 to 0.930.31 MJ/m² for x = 0.0 to x = 4.0, represents the average absorbed energy for the degradation of nanofibrous scaffolds.

The addition of modified CS to TPU solution and submitted for electrospinning step has a negative impact on the TPU/modified CS blend’s overall mechanical properties, as is being observed [41]. Besides that, TPU exhibit a much better crosslinking than pure modified CS, which is attributed to the existence of a chemical group as a stress resistance factor. Consequently, linezolid incorporation through the admixture is allowed to serve as a crumble factor, resulting in a lower ability to withstand the applied stresses [42].

3.3. Prediction of cytotoxicity

Next, the cytotoxicity of the prepared linezolid (9 mg) loaded nanofibers was assessed in detail. As seen in Table 2, the cytotoxicity IC₅₀ (µg/mL) for the evaluated samples of linezolid and that loaded nanofibers were examined against many types of human cancer cells such as Hela, Caco2, HeP2G2, MCF-7, respectively. It is observed that the cytotoxicity is correlated to the presence of linezolid. Besides, cell viability (%) directly relates to the concentration of the evaluated linezolid loaded PU/modified chitosan nanofibers.

As shown in Fig. 3 that the cell viability potential is correlated with the concentration of nanofibers. Overall, the increasing pattern reflects the well potential for cell proliferation and wound healing features. It is depicted that the nanofibrous scaffold achieved more than 90% for all the utilized human cancer cells while using 10 µg of Linezolid loaded nanofibers. The value of cell viability was reduced to above 80% as a result of using 20 µg. Then, the values against the human cancer cells are sharply decreased to about 50% when they are in contact with 30 µg of drug-loaded nanofibers, followed by marginal decrease even using 100 µg of Linezolid loaded nanofibrous scaffold. A sample of TPU modified CS 5 mg drug was selected to examine the cell’s death and compared with that of MCF-7 cell.

As seen in Fig. 4, cell death is inevitable by apoptosis or necrosis. Fig. 4 depicts the apoptotic percent of MCF-7 cells treated with IC50 Linezolid loaded nanofibers compared with untreated MCF-7 cells. As known, when a cell loses its ions, organelles, and the whole-cell swell, and the membrane permeability increases as the cell’s intracellular material is released. Apoptosis (cell death) allows the cell to shrink while maintaining plasma membrane stability and nuclear degradation [43]. The annexin V and PI assays are considered precise and reliable to identify apoptosis in heart and kidney cells.

3.4. In-vitro release of linezolid from PU/CS nanofibers

The in-vitro release of the loaded linezolid release is a critical parameter in predicting drug bioavailability in various formulations. Firstly, the release profiles of all the studied formulations were identical. As shown from Fig. 5, all nanofibers loaded with multiple concentrations of linezolid have a fast initial release step accompanied by a slower and more steady release. Desorption of the surface-bound or adsorbed compound induced the burst release process during the first 60 min.

Linezolid propagation through the pores of PU/modified CS nanofibers caused the second step to be relatively long, lasting up to 5, 7, and 9 h for 1, 3, and 5 mg linezolid nanofibers, respectively. By and large, it can be observed that the in vitro release of linezolid (1 mg and 3 mg) loaded PU/modified CS is more released than nanofibers included 5 mg linezolid.

The favorability can be attributed to the complete encapsulation while using 1 mg and 3 mg of linezolid, which, in turn, impedes its release, working on its stability for a more extended period inside the pores of the fiber, which increases its effectiveness in the long term [44]. On the contrary, loading the nanofibers with 5 mg of linezolid is easily entrapped into the pores of the nanofibers. At the same time, the rest can be adsorbed onto the surface of the nanofibers, which accelerate the release of linezolid, particularly at the beginning of the in-vitro release (30 min–90 min) [45].

In diabetic subjects, the wound healing process takes a long time and is impaired rather than prevented. A non-healing wound is prone to produce many complications, which is considered an essential factor in delaying the healing process. These complications include functional limitations, including alteration in gait and difficulty in walking; infections like cellulitis, abscess, osteomyelititis; gangrene and septicaemia and possible malignant changes in some cases. Chronic wounds are at risk of developing malignant changes, known as a Marjolin’s ulcer, an aggressive form of squamous cell carcinoma.

It is also accompanied by the current study that the percentages of wound contraction in the diabetic group were maintained with only nanofibers without any treatment (control group) were only 50%, as shown in Fig. 6.

Thus, diabetes is a disease wherein glucose homeostasis is disrupted, and chronic hyperglycemia causes the advanced glycation end product (AGE), which is necessary mainly for cell damage and has a poor turnover rate [25,46]. Hyperglycemia causes an increase in reactive oxygen species (ROS) output and the release of cytochrome C, which contributes to caspase-3 activation inducing myocardial cell apoptosis [47]. Myocardial cell death is now almost entirely prevented by partial inhibition of elevated glucose levels via insulin; therefore, it may be proposed that hyperglycemia causes a considerable rise in apoptosis. Apoptosis dysregulation in response to hyperglycemia spreads across the body, impairing the healing of wounds and causing participation of many other target organs [48].

Under hyperglycemia, the formation of advanced glycation end-products (AGEs) elicited the development of ROS by linking to its
receptor (RAGE), which is expressed in keratinocytes, fibroblasts, dendrocytes, and to a lesser degree in endothelial cells and mononuclear cells, and activates NF-κB, resulting in expression of pathological genes and further obstructs these cells’ natural function throughout tissue regeneration, resulting in poor healing for rat wounds.

Elevated pro-inflammatory cytokines, including IL-6, TNF-α, NF-κB, and uncontrolled diabetes, causes wound infections and inflammation. Increased levels of these cytokines have been attributed to impaired recovery, which results in fewer fibroblasts and keratinocytes migrating and proliferating, less collagen aggregation, and delayed re-epithelialization (Fig. 7).

The current study was in agreement with these studies; hence the inflammatory biomarkers in this study presented as homocysteine,

Fig. 4. MCF-7 cells were treated with (a) nanofibers and (b) untreated cells.

Fig. 5. In-vitro release of linezolid-loaded PU/modified CS nanofibers.

Fig. 6. Wounded skin contraction in experimental rats at the end of the experiment.

Fig. 7. Wound healing in different studied groups in 1 and 7 days.
and NF-κB in group I represent inflammatory chronicity, whereas a lesser degree of inflammation was seen in wounds treated with linezolid-loaded nanofibers. Groups I, II, and III (Table 3). Hcy levels can also be affected by active inflammatory processes.

The obtained data for diabetic wound healing can be discussed in the following manner. To begin with, nanotechnology is an interdisciplinary technological area that has piqued the interest of scientists and industry professionals all over the world [15,49]. Nanofibers allow for the rapid growth of biocompatible nanomaterials that can be used in various medical and biological fields. Nanofibers provide a wide variety of uses and, in particular, have a novel wound care remedy.

In the current study, the percentages of wound contraction in treated groups (group II, III, and IV) were significantly higher as recorded (79.9%, 86.6%, and 95.5%), respectively; that is mean the high concentration of polyurethane modified chitosan nanofibers loaded with different concentrations of linezolid gave the best effect and showed high wound contraction rates than the lower concentrations; thus, the healing of these wounds in our study appeared as a dose-dependent.

Drug-loaded nanofibers have a high degree of biocompatibility and biodegradability in the range of health problems, making them an essential material for bandages in diagnosing various wounds. As a result, polyurethane-modified chitosan nanofibers loaded with various concentrations of linezolid have piqued the interest of wound-healing researchers because of their physicochemical and biological characteristics. In our work of wound care, the as-prepared linezolid-loaded nanofibers are non-cytotoxic and safe for patients. Nanofibers’ unique intrinsic properties facilitate wound healing and efficiently regulate microorganism development at the wound site. Such nanofibrous materials are helpful and urgently needed for treating both wounds, acute and chronic.

In the present study, the treated groups displayed a substantial improvement in the activity of antioxidant enzymes as assessed by GSH levels and a dramatic reduction in oxidant free radicals as measured by MDA and NO levels, as opposed to the control group (Table 4). There is also an improvement in cellular protection mechanisms; this improvement was confined to lower doses of linezolid on secondary skin cell lines, although it is interesting that the non-toxic dosage spectrum is similar to that found in topical domains of nanofibers drugs, offering support for the safety of linezolid at those doses [50].

Inflammatory biomarkers, including neutrophil, elastase, homocysteine, and NF-κB in group I, indicate inflammatory chronicity, while wounds treated with polyurethane modified chitosan nanofibers filled with linezolid showed a lower degree of inflammation in all treated categories. Also, there are significant differences between treated groups (Table 3), indicating the apparent effect of the high concentration of nanoparticles in group 4 compared to other treated groups (group III and IV). Linezolid-loaded nanofibers are thought to reduce the time it takes for fibroblasts to penetrate wound tissue and have anti-inflammatory features. A protease inactivator, polyurethane modified chitosan nanofibers loaded with different concentrations of linezolid, is reported to minimize inflammation and the time it takes for granulation tissue to develop.

### 4. Conclusion

In summary, this current research has been designed to demonstrate how electrospin nanofibers can improve skin cell processes and aid wound healing. Electrospin nanofibers of polyurethane (TPU)/modified chitosan (modified CS) have positive health benefits, including such good mechanical features, high porosity, and a high surface area to volume ratio, which facilitate cell attachment, differentiation, and proliferation while still enabling water, oxygen, and nutrient exchange. The resultant data depicted that Hcy is an increase in wound contraction, MDA, NF-κB, Hcy, and elastase in a blank group compared to treated groups concomitant with a decrease in plasma nitric oxide and reduced glutathione activities, while treatment with linezolid significantly ameliorated these parameters in the treated group compared to the blank group. Linezolid-loaded nanofibers have been shown to have high wound contracting thresholds, and their physicochemical and biological properties have piqued the interest of researchers working on wound healing applications.

### Ethical approval

The methods in this study were validated according to the relevant guidelines and regulations and applied to animals following the recommendations (Approval no. 2019-520-PM5).

### Declaration of competing interest

Wound healing activities of polyurethane modified chitosan nanofibers loaded with different concentrations of linezolid in experimental model of diabetes. All authors are listed in the manuscript and there is no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers’ bureau; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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