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Application of Chitosan/PVA Nano fiber as a potential wound dressing for streptozotocin-induced diabetic rats

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ABSTRACT

Diabetes mellitus is a worldwide health problem affecting 1–2% of the population of world with noticeable morbidity and mortality. Vascular events such as hypertension, nephropathy, neuropathy and retinopathy are happened in diabetic patients. Decline in tissue blood circulation may causes hypoxia and finally may leads to slow wound healing and amputation. PVA/Chitosan Nano fiber wound dressings have high moisture vapor transmission rate and good antimicrobial activity[1] PCNWD substrate does not have any recognized cytotoxicity effects and has excellent odor absorbing capability. In the present study, Streptozotocin (STZ) is used to induce diabetes in rats, Skin ulcers are produced experimentally in the experimentally induced diabetic and non-diabetic rats. Then PCNWD used as wound dressing for 2 weeks period to evaluate its macroscopic and microscopic effects on wound healing in comparison with untreated diabetic and non-diabetic rats experimental ulcers. The findings of current study indicate significant acceleration in diabetes wound healing on the rats treated by PVA/Chitosan Nano fiber.

1. Background

There were 131 million diabetic people in 2000, worldwide, and it is estimated to be increased to 366 million people by 2030 [1]. Diabetes mellitus is characterized by a dysfunction in glucose metabolism associated with consequently considerable negative effects on lipid and protein metabolisms. Diabetes should be controlled, otherwise, diabetic patients may face complications in both short-term and long-term periods [2]. In general, diabetes ketoacidosis and hyperosmolar hyperglycaemic nonketotic coma (HONK) are considered as acute complications of diabetes [3,4]. Chronic complications of diabetes can be categorized into three general groups: nerve damage (neuropathy), macrovascular disease (disease of the large blood vessels), damage to small blood vessels such as capillaries (microvascular diseases) [5].

Diabetic wounds are among the most prevalent incapacitating complications of the diabetes. [6] In the diabetic patients, the high level of glucose in the blood can result in the impairments in small blood vessels and peripheral nerves leading to damages to the skin [7]. As a result, different types of infection can affect the patients' body. Generally, the lower limbs such as foot are more vulnerable. Accordingly, more than 25% of the untreated or improperly treated cases may face amputation. The mortality rate of diabetes is increased due to these types of infections [8].

The wound dressings is also regarded as an important factor in diabetic wound care management [9]. Today, several dressing types are available for wounds. Recently, nano-sized wound dressing materials from biopolymers by Electrospinning method are significantly considered [10]. Electrospinning is a technique for generating nanofibers from micrometers to nanometers. Recently, electrospinning technique has been widely regarded and used. The

Abbreviations: PCNWD, PVA/Chitosan Nano fiber wound dressings; PVA, polyvinyl alcohol; STZ, Streptozotocin; SEM, scanning electron microscopy.

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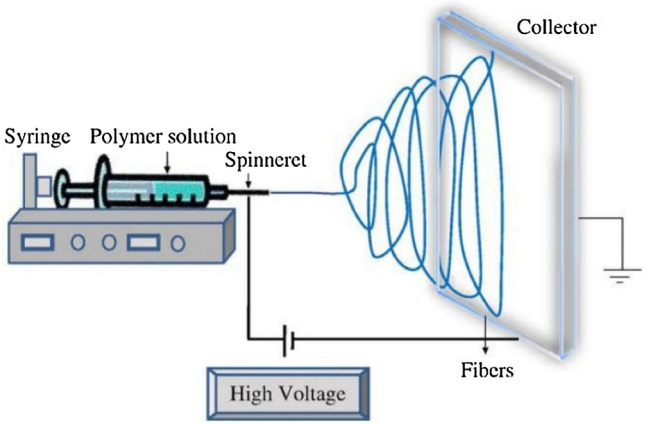

Fig. 1. Overview of electrospinning process.

great results have been achieved using electrospun fiber for wound dressings due to its nano-sized structure (Fig. 1). Absorptivity and oxygen permeability are two main factors of the wound dressing to heal the wounds. Chitosan is a polysaccharide used to blend with Poly vinyl alcohol (PVA) polymer, because of its good biocompatibility, biodegradability, cellular binding capability, antimicrobial activity and wound healing effect [11].

In this study, PVA polymer blended with chitosan in different proportions and the electrospinning parameters analyzed using scanning electron microscope (SEM), as well. Then obtained nanofiber webs were used for wound dressing in order to evaluate the effect of PVA/chitosan nanofibre on wound healing in the streptozotocin–induced diabetic rats.

2. Methods

2.1. Preparation of chitosan/PVA Nano fiber

To provide Web Nano fibers Chitosan/PVA, a solvent with a density of 2.7% weight by volume, and the ratio (75:25) provided in solvent acetic acid and water were mixed, then, the resulting solvent was stirred for 12 h at the temperature of the room, next, the obtained solvent was electro spinned which was done under the processing condition voltage 18 kV, feeding rate 0.35 ml/h and the distance from collector to tip is 15 cm.

2.2. Measuring electro spun fiber diameters

After preparing chitosan/polyvinylalcohol nanofiber from Nano-Scale Technology Company, and preparing SEM images for confirmation that fiber dimensions were Nano-scale, through using manual method, the diameter of 20 Nanofiber was measured randomly which was mean diameter. The conventional way of measuring electro spun fiber diameter is to analyze the micrograph manually. The manual method normally consists of the following steps: determining the length of a pixel in the image (setting the scale), measuring the number of pixels between two edges of a fiber perpendicular to its axis, converting the number of pixels to nm using the scale and recording the result. Typically 20 diameters are measured through a histogram of fiber.

2.3. Measuring electro spun fiber thickness

The mats were cut into dumbbell shaped strips with 40 mm length and 5 mm inner width. The thicknesses of the mats were measured using the electronic digital micrometer screw gauge. The thickness of the Chitosan/PVA nanofiber is at the scale of 0.3 mm.

2.4. Animal models

Wistar rats weighting between 250 and 300 g were used in the study. The rats were maintained in a clean room with 12 h lightdark cycle, controlled temperature environment with between 20 and 23 °C, free access to water and commercial rodent chow throughout the study. The animals were randomly divided into three experimental groups (n = 6), including: nondiabetic control, diabetic control and treated with Chitosan/PVA Nanofiber.

2.5. Induction of diabetes by streptozotocin in rats

Eighteen adult wistar rats weighting 250–300 g were used for the induction of diabetes. The animals were injected intraperitoneally by streptozotocin at the dose of 55 mg/kg of the body weight.

2.6. Creation of excisional wounds in rats

The animals of all groups were shaved on the dorsal between the shoulders. The wound was made to the dimensions of $1 \times 1\ cm^2$ (Fig. 2).

2.7. Macroscopic evaluation of the wounds

The wounds evaluated macroscopically on the days: 0, 4, 7 and 14 to assess the healing process and measurement the wounds area. Wound area on day 7 and 14 are regarded as important indicator of wound healing and calculated using software Adobe Photoshop Cs6.

2.8. Microscopic evaluation of wounds healing

Biopsies of the wounded areas were performed for histological evaluation tissue on the days 7th and 14th, transferred inside the container containing10% buffered formalin, and were sent to the laboratory. These samples were prepared as sections with 5 micrometer diameters and after hematoxylin and eosin (H & E) staining, studied with an optical microscope (4Nikon, Japan) with magnifications of, 10 and 40.

2.9. Biopsy of normal and diabetic rats pancreas tissue

For the study and comparison of pancreas Langerhans islet beta cells in streptozotocin-induced diabetic rats, and normal rats, pancreatic biopsy of normal and diabetic rats were done and tissue samples were fixed in 10% formalin, then were stained by Hematoxylin & Eosin and finally were evaluated by Leitz microscope with 400 times enlargement.

3. Results

3.1. Physical characterization of Nano fibers

SEM image of chitosan/Polyvinyl alcohol Nano fibers prepared by electro spinning of Chitosan/PVA by volume percent 75:25 in aqueous acetic acid is shown in Fig. 3.

SEM images confirmed that fiber dimensions were Nano-scale (the mean diameter of 20 Nano fibers were measured randomly which 279.843 nm).

3.2. Induction of diabetes by streptozotocin

Streptozotocin at dose 60 mg/kg, resulted in high mortality in test group (11death per 25 rats), Therefore the dose: 55 mg/kg was replaced it and diabetes developed within 3 days. The mortality

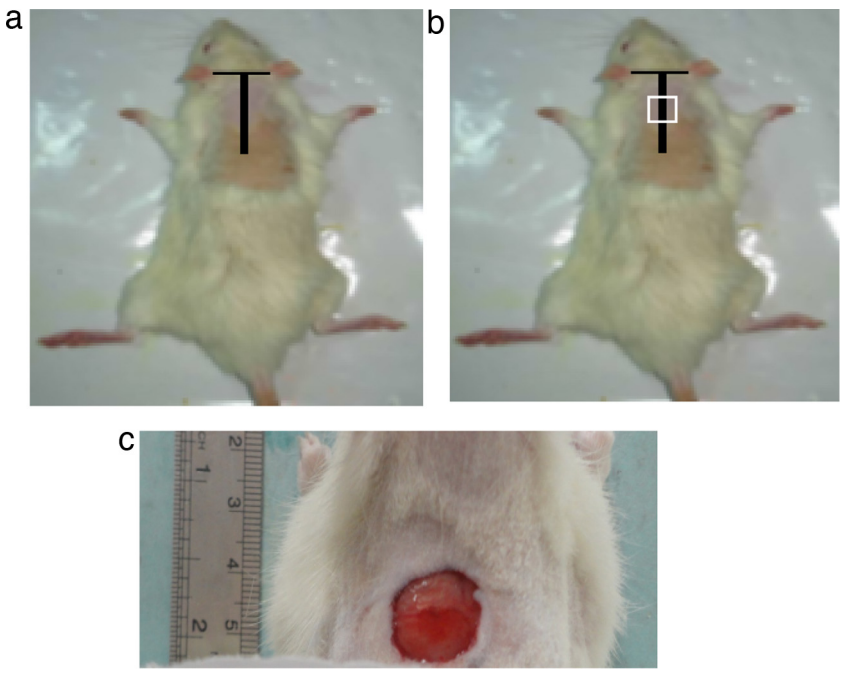

Fig. 2. Steps of excisional wounds creation in rats; a: Shaved back of the rats b: Determine the range of lesions c: create of lesions with scalpel (dimension $1 \times 1\ cm^2$).

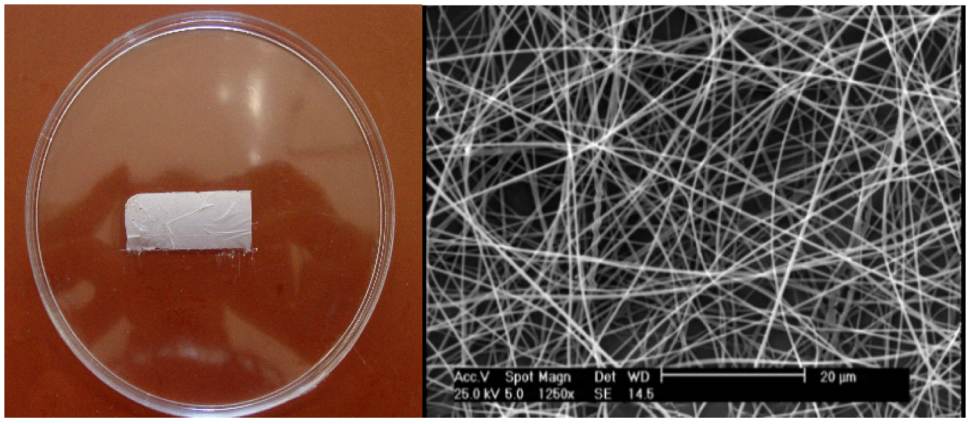

Fig. 3. SEM image of chitosan/Polyvinyl alcohol Nano fibers prepared by electro spinning from Chitosan/PVA by volume percent 75:25.

rate significantly reduced (3 death per 25 rats). The blood glucose measurement before and after diabetes revealed that: the levels of glucose in healthy adult rats was 101 ± 5 mg/dl, But in diabetic rats was measured as 374 ± 10 mg/dl (Fig. 4). The results of measuring of rats body weights indicated that average of body weight in diabetic rats reveals loss of weight and thinness in diabetic adult rats (Fig. 5). Pancreatic biopsy of normal and diabetic rats confirmed that the islet and cells were destroyed due to the effect of Streptozotocin in diabetic rats. The comparison of these pictures shows that the tissue of pancreatic Langerhans and the beta cells of diabetic rats have been degenerated irreversibly (Figs. 6 and 7).

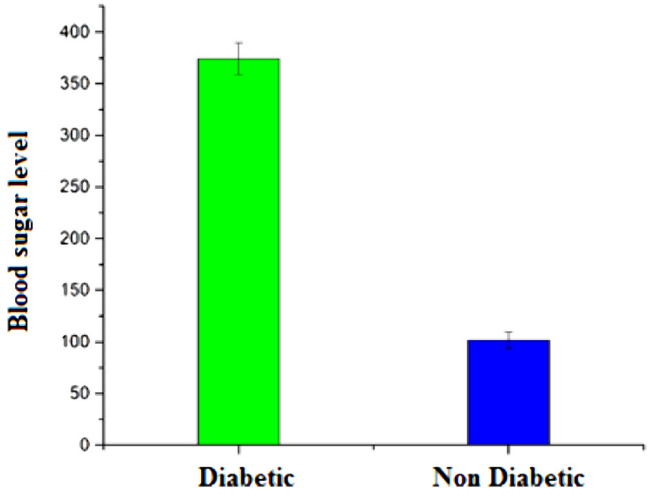

Fig. 4. Shows the changes of average level of glucose in serum of diabetic and Non diabetic rats.

3.3. The investigation of macroscopic wound

The average wound area in the diabetic group respectively was, $96 \pm 7/78$ and $22/078 \pm 3/18\ mm^2$ square at the day 7 and 14. The average of wound area in the treatment group with polymer Nano fibers respectively was, $76 \pm 3/42$ and $0\ mm^2$ square at the day 7 and 14. From comparing the two groups of the study, it is resulted that the average of wound area between the treatment group and

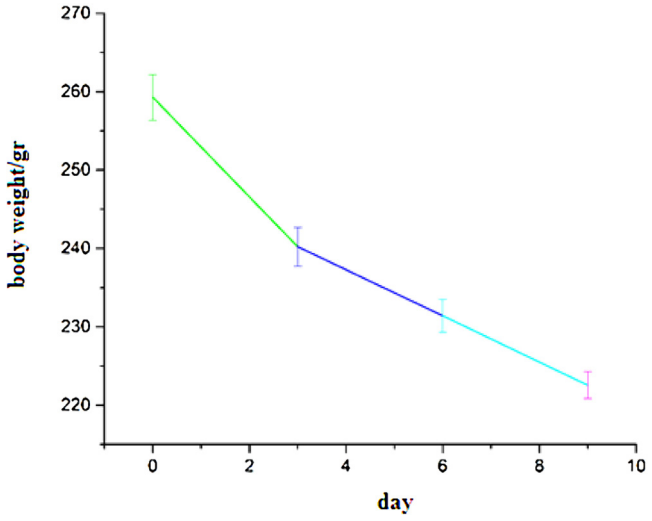

Fig. 5. Shows continuous changes in average of body weight in diabetic rats in days 0, 3, 6 and 9.

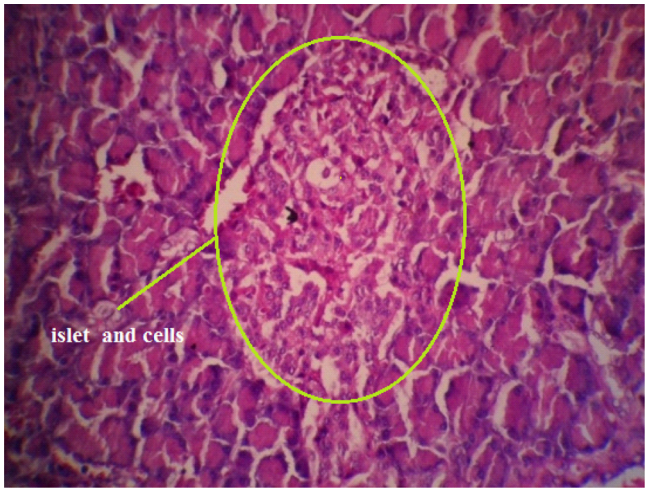

Fig. 6. Pancreatic biopsy of normal rats.

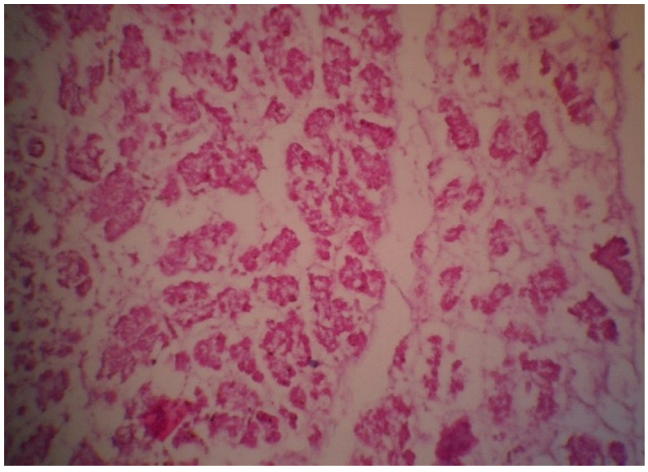

Fig. 7. Pancreatic biopsy of diabetic rats that confirms the necrosis of islets and cells due to the effect of Streptozotocin.

the control group of diabetic ulcers shows a significant difference ($P < 0.05$) (Fig. 8).

3.4. Microscopic evaluation of wounds healing

In the experiment group (chitosan Nano fiber), histological findings indicate the reduction in the wound size and epidermis gap and dermis lesion surface length in the 7th and 14th days compared to the diabetic and healthy control groups (Fig. 9). This issue indicates its positive effect on the process of wound healing (Figs. 10–12).

3.5. The observations of the microscopic in the seventh day

3.5.1. The diabetic control group

In the investigation of tissue sections stained with, H & E gap (the gap), the epidermal layer is not restored and the epithelial lining is progressing and the wound surface of blood vessels is seen (Figs. 10–12).

3.5.2. The experimental group

In the investigation of tissue sections stained with, H & E gap (the gap the epidermal layer is not restored and the epithelial lining is progressing and the length of gap epidermal and dermal area in the wound area is lower than the control group. It has been seen that in granulation tissue, the cell density is more and the blood vessels are more frequent than in the control group (Figs. 10–12).

3.6. Microscopic observation in the fourteenth day

3.6.1. Diabetic control group

In the investigation of tissue sections stained with, H & E gap (the gap) epidermal layer still has not been restored, it has been seen the randy epithelial cells and the remaining scar on the wound. In some cases it has been seen a sample of granulation tissue as a thin layer under epithelial and the scar tissue is forming under the granulation tissue (Figs. 10–12).

3.6.2. The experimental group

In the investigation of tissue sections stained with, H & E gap (the gap) epidermal layer has been restored, and it has not been seen the remaining scar on the wound. The length of gap epidermal in the wound has been reached to zero and epidermal area in the wound area is lower than the control group (Figs. 10–12).

4. Discussion

In the present study, streptozotocin was used to induce experimental diabetes in the rats. Streptozotocin can prevent DNA synthesis in the mammals and bacterial cells. In the bacterial cells, it can lead to a special reaction with cytosine groups, resulting in degeneration and destruction of DNA. Mammalian cell death is owing to its biochemical mechanism. Streptozotocin can prevent cellular reproduction with the less doses needed for inhibiting the substrate connection to the DNA or inhibiting several enzymes involved in DNA synthesis [12].

The level of blood sugar before and after diabetes induction and body weight one day before, at the day o, and one day after diabetes induction were measured. There was an increase in blood sugar and the decrease in the rat's weight. Comparing the pancreas of the diabetic and normal rats, the Langerhans islets and beta cells of the diabetic rats were clearly destructed.

In this research, PVA/chitosan nanofibers used as the wound dressing.In spite of investigating the effect of different nanofibers particularly PVA/chitosan nanofibers on wound healing, the present study is the first study evaluating diabetic wound healing.

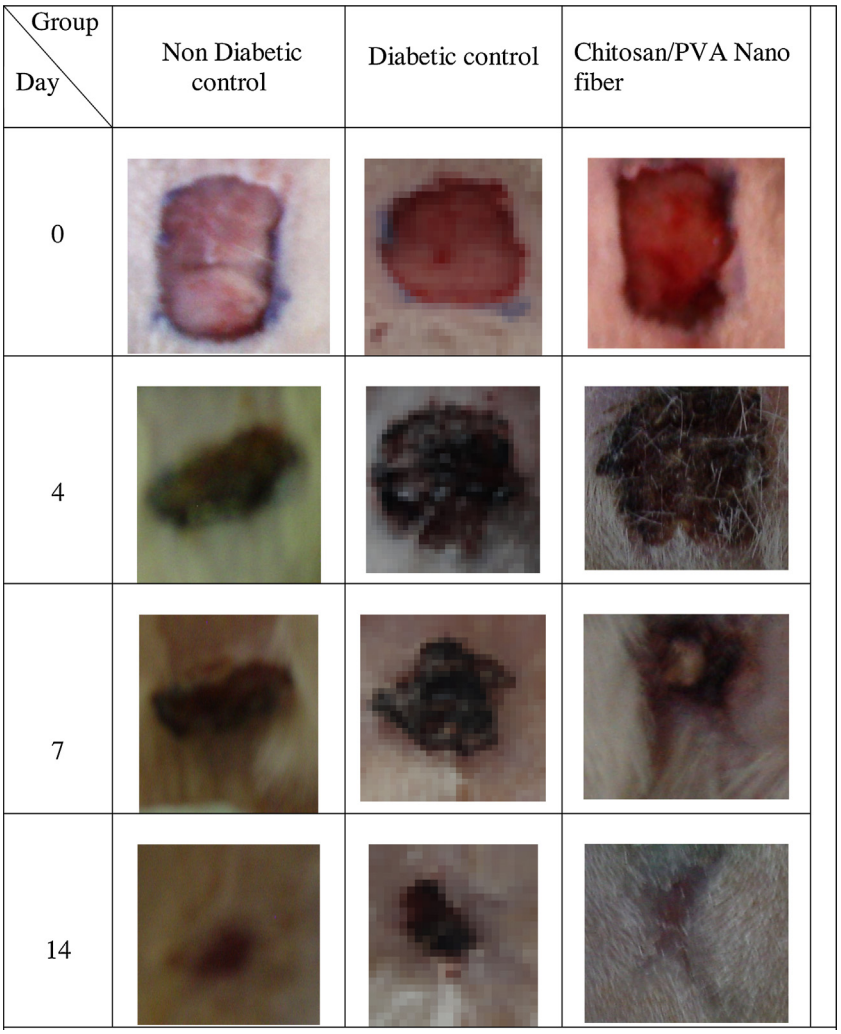

Fig. 8. Photographs of macroscopic appearances of wound excised from untreated diabetic and non-diabetic rats (control groups) and experimentally diabetic rats treated with Chitosan/PVA in days 7 and 14.

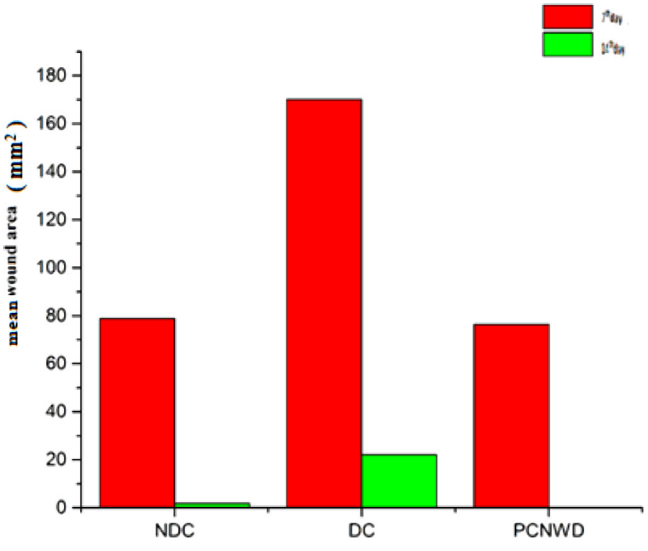

Fig. 9. Wound areas in different groups on days 7 and 14. NDC, Non diabetic control; DC, diabetic control; PCNWD, PVA/Chitosan Nano fiber wound dressings.

PVA/chitosan nanofibers reduced the length of epidermis and dermis area compared to the normal and diabetic control groups in the 7th day of treatment. In the 14th day of treatment, a significant reduction was observed in the length of epidermis gap and dermis area in the treated group compared to the normal and diabetic control groups.

To prevent the infiltration from external infectious effects, preventing the passage of oxygen and water, biocompatibility, biodegradability, accelerating wound healing, preventing bleeding to some extent, are not needed to be replaced for several times. Chitosan is greatly used in food and medicinal industries, since these features can be found in the Chitosan. This solution, due to high viscosity, cannot be snipped by its own. Therefore, the polymers such as PEO and PVA are used to reduce viscosity .Chitosan is solvable in the water in a slow speed, which can be accelerated by the acetic acid.

In the present study, 0.5 M acetic acid was used. The positive effects of this polymer and even the materials obtained from its decomposition in wound healing have been confirmed in different studies. The coverage coated by c can appropriately be used instead of previous methods for wound coverage, due to the antibacterial and healing factors. Electrospinning technique allows us to cover the wound without any need for dressing by gauze and band aid. As a result, wound dehiscence is prevented when replacing bandage and the process of wound healing is improved.

The result of the present study revealed that the chitosan/PVA nanofibers can be properly used as a wound dressing. The wound

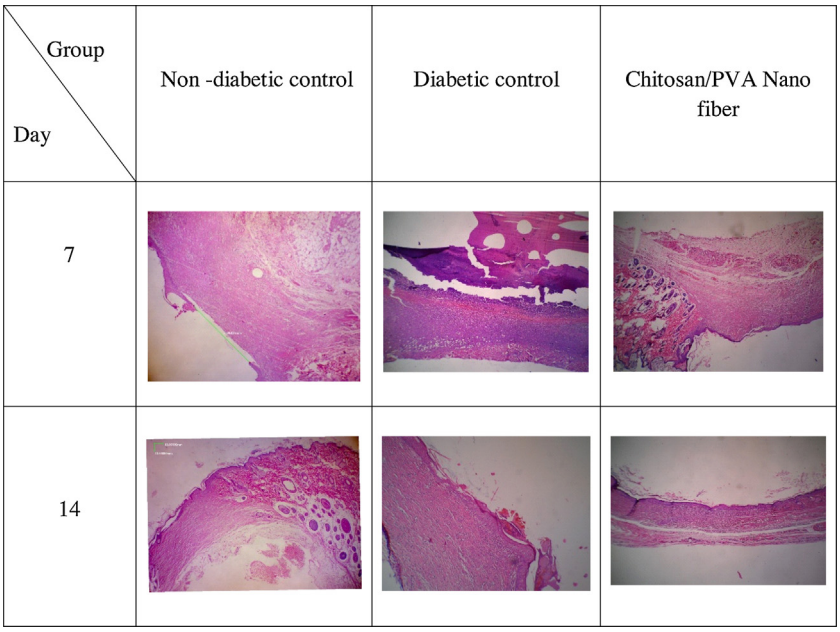

Fig. 10. Histological study of wound healing in groups Non Diabetic control, Diabetic control and Chitosan/PVA Nano fiber.

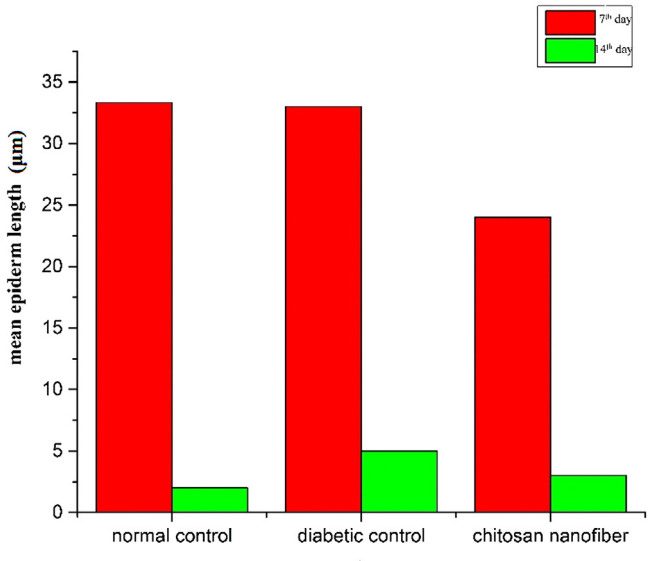

Fig. 11. Length of epidermis gap in the study groups at day 7 and 14.

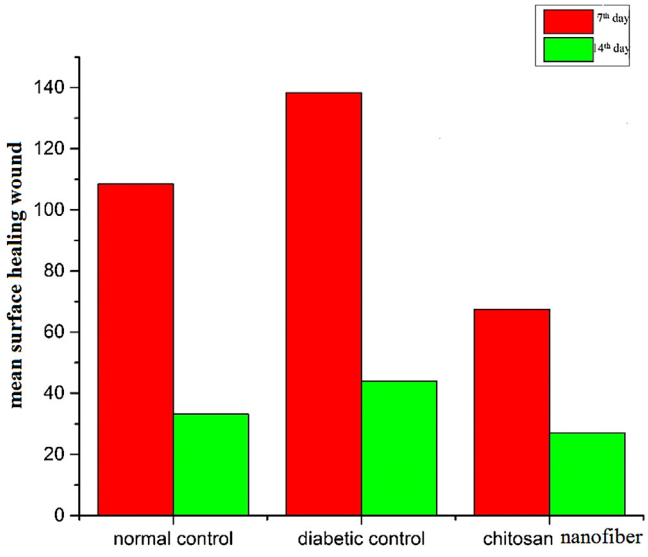

Fig. 12. Healing wound Surface of dermis in the study groups at day 7 and 14.

healing was accelerated and the nanofibers sheet was tightly adhered to the wound. This result is in agreement with that of Sathirakul et al. [13] and Khan et al. [14] reporting a good adherence, reducing contamination and promoting tissue bonding.

The following results correspond with present study:

In a research conducted by Kang et al. Nano fiber scaffolds were prepared from polyvinyl alcohol by electro spinning covered by chitosan solution. Then, polyvinyl alcohol-chitosan Nano fiber scaffolds were experimented on rats as wound covering for investigating the process of wound healing. The results indicated it's more accelerating effect on wound healing in the treatment group than in the control group [15].

Yan et al. using the polyvinyl alcohol-chitosan compound, prepared a biocompatible and non-toxic Nano fiber in Tissue Engineering. In this study, the new electro spinning process was used for preparing controllable electro spinning Nano fibers [16].

In another research, Nano fiber scaffolds were prepared based on polycaprolactone (PCL)-chitosan- polyvinyl alcohol for the Tissue Engineering. In this study, Nano fiber scaffolds were electro spinned by the composite of polycaprolactone (PCL)-chitosan- polyvinyl alcohol with ratio of 1.2 and 1.5. Fibroblast cells were properly indicted necessary broadening and adherence on scaffolds. Due to random orientation of Nano fibers, cells expanded as appropriately as possible in all directions. Macroscopic evaluation of clinical results indicates the favorable effect of Nano fiber scaffolds on healing incisional wound in such a way that scaffolds with cells, in the fifteenth day after the operation, heals skin as properly as possible and without leaving no scars [17].

The pore size within the electro-spinned nanofibers makes the coated layer as a favorable coverage for wound healing and burn treatment. The similarity between electro-spinned nanofibers and the natural extracellular matrix can lead to the growth of a new healthy tissue in the injured area. It can also reduce the required length for healing by decreasing the formation of tissue, wound or

burn. The nano-sized pores can protect the injured tissue against the bacteria which can infect the damaged scar tissue. The high porosity and area can increase the process of fluid absorption and leading to the wound healing [18].

In general, the causes of wound healing with Nano fiber chitosan can be mentioned due to two main reasons: 1. the three-dimensional, network and porous structure of the Nano fiber scaffold which causes the pus and blood moisture absorption and high passage of oxygen over wounds and facilitating wound healing. 2. The chemical structure of chitosan and its favorable biological properties such as its biocompatibility and antibacterial nature cause the assimilation of the biochemical environment of the natural tissue and absorption of fibroblasts as well as facilitating wound healing. The instances of wounds are healed after 10 days [19].

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