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Electrospun Poly Vinyl Alcohol Fiber Containing *Lawsonia inermis* L.: A Promising Effect on Burn Wound

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Abstract

Henna plant (*Lawsonia inermis* L.) has unique properties and has long been used as a herbal remedy. In this study, polyvinyl alcohol (PVA) nanofibers containing henna extract in two different concentrations were studied to prepare a wound dressing using electrospinning. PVA polymer nanofibers using henna extract with two different concentrations of 3% and 6% were prepared by the electrospinning method. Human fibroblasts were cultured on these fibers and the mechanical and survival properties of the cells, as well as the antibacterial properties of henna were compared and evaluated. The results showed that by using of henna extract in PVA nanofibers, cell viability was significantly reduced ($P \leq 0.05$). However, the ability of nanofibers to water uptake and mechanical properties of fibers significantly increased with increasing concentrations of henna extract ($P \leq 0.05$). Nanofiber wound dressing with antibacterial properties of henna extract provides relatively fast and rapid wound healing. The resulting fibers are encouraging candidates for the development of improved bandaging materials.

Keywords: Poly Vinyl Alcohol; *L. inermis*; Wound dressing; Burn, Electrospinning

Introduction

The skin is the largest organ of our body that has numerous functions such as providing a first-line defense against exogenous factors, maintaining homeostasis, resorption, thermoregulation, and involvement in metabolic processes. There are many factors affecting the condition of this large epidermal barrier including skin pH, hydration, and sebum secretion [1]. The skin has a complex anatomical structure. Dermis, epidermis, and the subcutaneous tissue are the main skin layers that have different functions and structures [2]. The stratum corneum, stratum lucidum, stratum granulosum, dendritic cells, stratum spinosum, melanocytes, and stratum basale together form the epidermis that prevents excess evaporative fluid loss and passage of external substances to inside the body. The papillary and the reticular layer both form the fibrous dermis layer that aids in thermoregulation and sensation. Finally, the subcutaneous fat tissue protects our muscles and internal organs from external shocks and sudden temperature changes [3]. Thermal, electrical, or chemical skin burn injuries form major skin problems occurring worldwide. In general, there are four types of burn based on the depth of the injury; first-degree, second-degree, third-degree, and fourth-degree burns [4]. First-degree burn is usually a

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superficial burn that affects the epidermis only, thus it is often painful, non-blistering, and non-scaring. Second-degree burn has two subtypes both of which form weeps and blisters with increased risk of infection depending on its depth. The first subtype is 2A (superficial partial-thickness) burn that is usually painful and scarring. The second subtype is 2B (deep partial-thickness) burn that is generally less painful and more scarring, hence it may require surgery [5]. Full-thickness third-degree burns affect the whole dermis and are rarely painful due to the damage to the nerve endings. Fourth-degree burns involve the muscle or bone resulting in loss of the burned part [6,7].

Medicinal plants form a growing class of medications that have a very long tradition. In ancient as well as recent times, populations in both developing and developed countries have been using natural and herbal remedies for the prevention, diagnosis, management, and treatment of many health-related problems owing to their chemical composition [8]. Recently, much research has been done to develop antimicrobial compounds in wound healing to reduce the antibacterial resistance of microorganisms [9]. However, the consumption of medicinal plants should be under the supervision of a pharmacist or physician to ensure the use of herbs that have sufficient scientific evidence of benefit over the risk [10].

Skin burn is one of the problems that is being treated nowadays by herbs such as henna, pomegranate, myrrh, and many others due to their suggestive antibacterial and anti-inflammatory mechanisms [11]. Therefore, studying and reviewing the roles of these remedies in the treatment of burn-associated wounds is of great concern and value. The use of *Lawsonia inermis* L. (Henna) from the family Lythraceae is mostly linked to the culture of North Africa and Asia [12]. Populations from these regions used to prepare a paste from henna leaves to be applied on the skin for preventing topical skin infections and ulcers [13].

Lawsone (2-hydroxy-1,4-naphthoquinone) is one of the principal antibacterial secondary metabolites of *L. inermis*. The antibacterial activity of this compound is mainly due to its free hydroxyl groups that can interact with the components of the bacterial cell wall including carbohydrates and proteins, resulting in their destruction [14]. This paste is also applied to the scalp and hair to prevent or treat lice, as well as dandruff [15]. The results of the in vitro and in vivo studies have documented the antioxidant, antimicrobial and anti-inflammatory action of *L. inermis* which can be directly related to its phytochemistry [16]. Naphthaquinones, alkaloids, volatile and non-volatile terpenes are known to be linked to many of these pharmacological activities [17].

Polyvinyl alcohol (PVA) is a synthetic hydrophilic polymer matrix that can be used to prepare fibers using the wet spinning technique [18]. This polymer is yielded artificially by saponification of polyvinyl acetate and is known to have a unique biodegradable property which makes it an important agent in the food, drug, and tissue engineering industry [19,20]. Biodegradability of PVA occurs through hydrolysis due to the hydroxyl group on its carbon atom [21]. Electrospinning, being an advanced technology for producing ultrafine nanofibers has been widely used in the last decade [22]. This process is highly dependent on the created electrical field. When a polymer solution is pumped to the needle of the equipment, force in high voltage is applied in the system to create an electrical field between the needle and the collector plate to overcome the surface tension of the liquid droplet. Following this, the droplet is distorted leading to an electrically charged jet ejection that moves towards the rotating collector forming thin fibers [23]. These fibers are scaffold for tissue engineering because they are known to improve cell adhesion, growth, and direct migration [24]. The high surface area of nanofibers, abundant pores in electrospun nanofibers and the ability to be designed in different forms allow nanofibers to be used in a variety of applications, including tissue engineering, drug delivery and biomedicine, and wound healing. Adaptation to the wound environment, as well as control of the amount of humidity and oxygen is a vital issue for rapid healing and prevention of dryness for cases of skin burn. This study aimed to produce a suitable antibacterial bandage and wound dressing of electrospun biodegradable polymer nanofibers and to investigate the operating conditions to minimize the average diameter of nanofibers containing the active substance in order to have desirable properties in wound healing such as minimal contamination for cases of skin burn.

Materials and Methods

Chemicals

All the reagents and chemicals utilized in this study were analytically graded and purchased from Merck (Darmstadt, Germany). Human fibroblast cells (HDF) were obtained from the National Center for Genetic Resources of Iran.

Preparation of the Plant Extract

L. inermis were gathered from Shiraz, Fars Province, Iran (Herbariam code: HSHVO17113). Leaves were dried in the laboratory at an optimal temperature (26-28° centigrade). After complete drying, the hydroalcoholic extract of *L. inermis* leaves was prepared using the maceration technique in which the powdered plant material was soaked and shaken in the solvent. After collecting the leaves and drying them for a week at normal temperatures in the shade, the plant mate-

rial was grounded by electric mortar. Following that, 1000 g of the powder was weighed and transferred to a 2000 mL Erlenmeyer flask that contained 2000 mL of 50% ethanol. The flask was then covered with aluminum foil and kept at room temperature for 72 h with shaking in between. The yielded solution was filtered using a Buchner funnel and then concentrated by a rotary evaporator to get two solutions in concentrations of 3% and 6%. The solutions were then labeled and stored at 4°C [25].

Preparation of the fiber

10% by weight solution of PVA in 70% acetic acid were prepared. The feed rate of the solutions was done by a syringe pump of 0.1 mm per hour. The solutions were electrospun through 5 ml syringes with a 20-gauge needle. The distance from the tip of the syringe to the collecting plate was 12-13 cm. The flow rate of the solutions from the syringes was 0.5 ml per hour with 12 cm of distance from a tip-to collector at a rotation speed of 800 rpm selected. By applying a voltage of about 18 kV, the desired solution was placed in an electrospinning device (Spinner 3X-Advanced, Iran) for 3–4 h to spin the desired nanofibers on aluminum foil. To increase the strength and mechanical strength of nanofibers, crosslinking by glutaraldehyde vapor was performed.

Characterization of Electrospun Fibers

Mechanical Properties

Tensile test was performed for four groups of nanofibers at a constant tensile speed of 1 mm/min. The length, width, and thickness of the samples were measured in micrometers, the final size was repeated three times and the results were averaged. Tensile test continued from the start to the rupture of the specimens and Young's moduli, as well as ultimate tensile strength, were determined. The tensile strength and elongation at break for each sample was expressed in terms of average ± standard deviation by testing at least three replicates.

Cell Seeding and Culture

Human fibroblast cells (HDF) obtained from the Institute Pasteur in Iran were cultured in a DMEM medium with 10% fetal bovine serum, penicillin 100 µg/mL and streptomycin 100 µg/mL at 37 °C, 5% CO2, and 95% humidity [26]. The third passage of cells was used for culture on nanofibers in different groups.

Cell Viability Assay

Thiazolyl Blue Tetrazolium Bromide (MTT) colorimetric technique was performed to assess the viability of cells. In this method, dehydrogenase enzymes in living cells with their enzymatic activity led to the conversion of tetrazolium salts to formazan crystals. Therefore, the amount of formazan produced will be proportional to the number of living cells.

For MTT assay, the supernatant was discarded and the cells were washed twice with phosphate buffered saline (PBS) following the incubation period. Thereafter, 100 µL of 0.5 mg/mL MTT solution was added to each well. The cell plates were then incubated at 37 °C for 4 h. After the desired time, the wells were drained and 100 µL of dimethyl sulfoxide (DMSO) was added to each. DMSO acts as a solvent for formazan crystals and produces a purple color with varying intensities depending on whether the cells are alive or dead. The light absorption of each well at 570 nm was read by enzyme-linked immunosorbent assay (ELISA) reader and the percentage of viability of cells in different groups was studied on the first, third and sixth day. The obtained data were analyzed and plotted on a graph [27].

Determination of the Water Uptake of the Scaffold

The water uptake of the scaffold in the three experimental groups was obtained by floating 60 mg of the test scaffold in 500 mL of phosphate-buffered saline at 37 oC for 24 h. Thereafter, samples were filtered and weighed. The degree of swelling in percentage is then calculated using the following formula [28].

$$\text{Degree of swelling (\%)} = [(W_{St} - W_{dt}) / W_{dt}] \times 100$$

Where WSt and Wdt represent the wet and the dry weight of the test scaffold, respectively.

Determination of the Antibacterial Action of the Scaffold

Standard strains of Gram-negative bacteria including Escherichia coli and Gram-positive Staphylococcus aureus were used for culture in agar medium. At this stage, some bacteria were first removed by an unsterilized ounce and drawn on the surface of the environment in parallel lines in several directions. Three wells were made in each vessel to which 0.5 mL of each suspension was injected. Thereafter, the Petri dishes were incubated at 37 ° C for 48 h [29]. After incubation, the zone of inhibition was measured and compared with antibiotics using ImageJ software.

Statistical analysis

Presented results were shown as mean values ± SD. The statistical analysis of the obtained data was performed using SPSS (version 22). One-way analysis of variance (ANOVA) followed by the LSD post hoc test was performed to compare the means of samples by considering the statistical significance of P values <0.05.

Results

Mechanical Measurement

A proper wound dressing must possess acceptable mechanical strength to be adapted with body movements. Fiber mats must be strong enough to withstand the mechanical pressures and load applied by the surrounding cells and environment. The mechanical properties of the scaffold might change following extension. The assessment of the mechanical properties of fibers, which had included Young's modulus, elongation percentage at breakpoint, and tensile strength were displayed in table 1. According to the findings, the ultimate tensile strength of *L. inermis* (*LI*) treatment groups was higher compared to PVA fibers. The Young's modulus of *LI* 3% and 6% fibers has been measured to be 0.57 ± 0.14 and 0.62 ± 0.11 MPa, respectively. These amounts were significantly higher compared to PVA fibers. Furthermore, the elongations (%) at breakpoint have been detected to be 10.203±0.15 and 14.06±0.04 %, respectively, which had been indicative of the lower percentage of 6.712±0.08 in comparison to that of the PVA fiber. In fact, the presence of *LI* in the samples increased the modulus and elongation. *LI* has a lower molecular weight than PVA used in this project; thus, it decreases the average molecular weight of the mixture and acts like a plasticizer resulting in the increase of the elongation at break. These results are displayed in table 1.

Table 1. The mechanical properties of the prepared nanofibers in different concentrations.

Samples	Young's modulus (MPa)	UTS* (MPa)	Elongation (%)
PVA	0.53 ± 0.11	0.71± 0.7	6.712±0.08
PVA/3% *LI*	0.57 ± 0.14	1.12 ± 0.12	10.203±0.15
PVA/6% *LI*	0.62 ± 0.11	1.02 ± 0.16	14.06±0.04

Cell Viability

Evaluation of cytotoxicity of prepared fibers and their adhesion are important aspects to evaluate the effectiveness of fibers for skin applications, which was evaluated by MTT test (Figure 1). The MTT assay measures the metabolic activity of the cells and it can be related to the viability and proliferation of the cells. Cells viability on the surface of fibers was higher compared to the control group and contained *LI* with an increasing incubation time of survival and growth of fibroblasts. On the other hand, with the increase in the weight percentage of *LI* in the structure of fibers, the rate of cell proliferation has also increased compared to PVA and control group. However, in 0.6 % extract the cell viability was decrease compared to 0.3 % due to decreasing of the porosity of the nanofibers. In day 6 cell viability was decreased in treatment group. Due to the confluency of fibroblast cells. However, this decreasing was not significant compared to day 3. Also, PVA and *LI* 6 % had increasing effect on cell viability and growth without significantly in comparison with control group which were cells without scaffold. These results indicate that *LI* has a safe and non-toxic substance that is well present in the structure of fibers. The presence of hydroxyl groups in the structure of *LI* is responsible for its antioxidant properties.

Water Uptake of the Scaffold

The results of water uptake of the nanofiber scaffold indicate that PVA scaffold has the least percentage of water uptakes. The water uptake ability of PVA was increased by the addition of *LI* extract. This relationship was shown to be directly proportional when the concentration of the added *LI* extract increased; the percentage of water uptake was increased. The increase in the swelling ratio from PVA to PVA containing 3% *LI* extract was significant ($P < 0.05$). However, this increase was not statistically significant when comparing the two groups of PVA containing 3% *LI* extract and PVA containing 6% *LI* extract. These results were shown in (Figure 2).

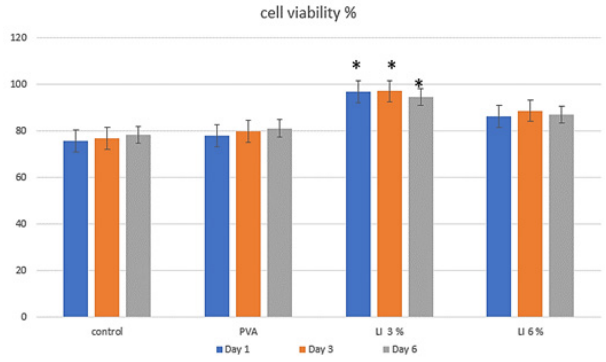

Figure 1. Cell viability was assessed by MTT test as described in Section 2. Results are expressed as growth percentage of each fibroblast (in the concentration of 3% and 6% *LI*) compared with that of PVA and represent the mean (± SD) of triplicate cultures performed in three days. In group 3% cell viability increased significantly compared to all group $P \leq 0.05$. the increasing of viability in group 6 % was not significant.

Antibacterial Analysis

The results of the antibacterial analysis of the PVA scaffold indicate that PVA scaffold is more sensitive to the Gram-negative bacteria Escherichia coli. The addition of *LI* extract to PVA has increased the antibacterial activity of the PVA scaffold. This increase was more significant ($P<0.05$) in PVA containing 6% *LI* extract compared to PVA containing 3% *LI* extract and PVA alone. The antibacterial activity of PVA containing *LI* extract in increasing concentrations

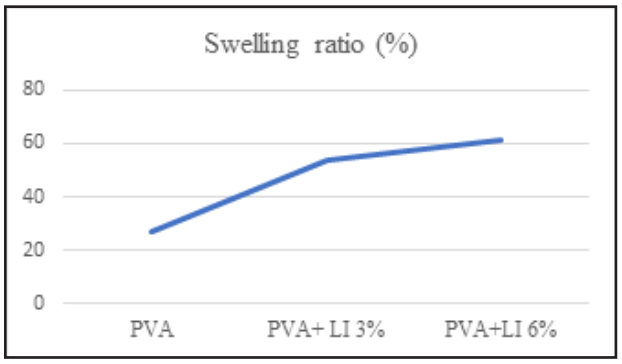

Figure 2. The change in the percentage of water uptake of PVA scaffold compared to PVA containing LI extract in the concentration of 3% and 6%

Table 2. The diameter of the zone of inhibition around the tested fibers on cultures containing gram-positive and gram-negative bacteria

Bacterial Index	PVA (mm)	PVA+ *LI* 3% (mm)	PVA+ *LI* 6% (mm)
Escherichia coli (G-)	10±0.08	11±0.05	14±0.19*
Staphylococcus aureus (G+)	8±0.21	9±0.67	11±0.09

against the Gram-positive bacteria Staphylococcus aureus was also observed. This proves that the active ingredients of *LI* can destruct both Gram-positive and Gram-negative bacterial cell walls. However, the difference in the activity between the three tested groups was not significant. Results are displayed in table 2.

Discussion and Conclusion

Wound healing is a multistep process that becomes longer and more complicated in absence of treatment. Therefore, sophisticated wound healing/dressing materials are required to promote the healing process. Today, the best option in medical application, especially in treatment defects or injuries in the body such as wounds and skin injuries is the nanofibers [30]. The electrospinning method is very adaptable and a wide range of polymeric materials with a wide range of fiber diameters can be produced on an industrial scale [31]. In the study of Sadri et al. (2015), the results showed that the addition of henna in the structure of nanofibers can be a good choice to facilitate the wound healing process [32]. In many studies, it has been shown that a hybrid blend of synthetic and natural polymers provides sufficient mechanical traits and tissue-engineered patches for skin wound healing [33]. Previous studies mentioned that the ideal tensile strength range is from 0.8-18 MPa for dermal cell culture and wound dressing [34].

Moreover, the addition of *LI* extract to PVA scaffold increases its swelling or water uptake capacity. This result is supported by another study conducted by *Li* Q et al. in which *LI* extract-loaded chitosan nanoparticles had a greater swelling capacity when compared to plain chitosan nanoparticles [35].

In addition, due to the low molecular weight of *LI*, it acts as plasticizers in PVA scaffold which weaken intermolecular forces in PVA polymer thus increasing its flexibility and moisture. Keeping the wound surface moist prevents the attachment of the PVA polymer-based bandage to the surface of the wound; thus, allowing the exchange of oxygen to its surface to speed the wound healing process [16]. This also prevents any inconvenience during the removal of the bandage. Similar to the results of a study conducted by Malekzadeh [36], our study showed that the antimicrobial activity of *LI* extract-containing PVA against Gram-positive and Gram-negative microorganisms increases with increased *LI* extract concentration. Our results indicate that *LI*-containing PVA scaffold forms a suitable environment for the growth of fibroblasts. In addition, the increase in the concentration of the *LI* extract was not associated with serious drawbacks. Furthermore, the outcomes of this study indicate that the use of higher concentrations of *LI* extract in PVA nanofibers decreased cell viability which is probably caused by the reduction in the scaffold porosity. As observed, the cell viability of all samples compared to the control is above 70%. These results confirm that the produced samples are biocompatible and non-toxic, suitable for use as wound dressing or skin tissue engineering scaffold. Our result is supported by previous reports on the proliferative effect of henna leaves extract-loaded chitosan nanofibers on human normal foreskin fibroblast cells [37].

These results are aligned with previously conducted studies in which the use of Punica granatum extract was associated with a reduced scaffold porosity [38,39]. Nonetheless, there is no evidence of toxicity associated with highly concentrated scaffolds.

The *in vitro* studies in the field of regenerative medicine have highlighted the efficacy of electrospun scaffolds based on plant extracts in stimulating cell proliferation, controlling inflammatory responses, and preventing bacteria colonization [40]. This antibacterial activity may be owing to the ketone function groups of the lawsone molecule [41]. Previously conducted studies also showed that henna leaf extract does not reduce the epidermal cells including fibroblasts. In addition, these studies showed a post-healing increase in the collagen filaments and skin appendages [42]. The improvement in the mechanical properties of PVA fibers in presence of *LI* extract of increasing

concentrations suggests its use as a bandage suitable for burn-associated wounds.

Conflict of Interests

The authors declare no conflict of interest.

Acknowledgements

None.

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