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An investigation of electrospun Henna leaves extract-loaded chitosan based nanofibrous mats for skin tissue engineering

Iman Yousefi [a,e,*], Mehdi Pakravan [b], Hoda Rahimi [c], Abbas Bahador [d], Zahra Farshadzadeh [d], Ismael Haririan [e,f,g,**]

[a] School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
[b] Department of Chemical Engineering, Ecole Polytechnique de Montreal, Montreal, Quebec, Canada
[c] Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[d] Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[e] Medical Biomaterials Research Center (MBRC), Tehran University of Medical Sciences, Tehran, Iran
[f] Department of Pharmaceutical Biomaterial, Tehran University of Medical Sciences, Tehran, Iran
[g] Department of Pharmaceutics, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Wound healing characteristics of some plant extracts have been well known for many years, and they have been utilized for such applications in traditional way. Recently electrospun nanofibrous mats showed promising properties for tissue engineering and especially for skin repair. It is expected that incorporation of plant extracts into such structures could provide higher performance and synergistic effect for biomedical and wound healing applications. The final purpose of this study is to fabricate chitosan based nanofiber mats loaded with a traditional plant extract of *Lawsonia inermis* (Henna) leaves to enhance the antibacterial efficacy and wound healing of the precursor nanofibers. The morphology, structure, mechanical properties and swelling and weight loss degree of the electrospun nanofibers have been investigated in this study. Antibacterial activity, cell biocompatibility evaluations and in vivo wound healing activity of the abovementioned mats were also studied. The FESEM images of Henna leaves extract-loaded nanofibers proved that homogeneous, smooth and defect free nanofibers of 64–87 nm in diameter have been prepared. Presence of Henna extract in the electrospun fibers was approved by Fourier Transform Infrared spectroscopy. Incorporation of Henna extract into the nanofiber mats exhibited significant synergistic antibacterial activity against bacterial cells. It was well supported by the results of cell viability and proliferation of human foreskin fibroblast cells on the prepared scaffolds. Therefore, the results of this work showed that Henna leaves extract incorporated chitosan nonwoven mats have a great potential to be used as the biodegradable, biobased and antibacterial wound healing dressings.

1. Introduction

Skin is the largest organ of the human body, serving primarily as a protective barrier against the pathogens and excessive water loss. Skin damage is mainly caused by burn injuries, chronic wounds, excision of skin and other severe dermatological conditions [1,2]. Recent studies estimated that nearly six million patients worldwide suffer from severe burns; among them, 300,000 die ultimately [3]. Traditional herbal remedies based on plant extracts have been well known to accelerate wound healing process, and have been used in many countries [4,5]. Reports about medicinal plant and their role in improving various phases of wound healing process such as coagulation, inflammation, fibroplasia, epithelialization, collagenation, and wound contraction, have been widely demonstrated in the scientific literature [6,7]. Recently, the use of plant extracts, as well as other alternative forms of medical treatments, has been revised to be combined with updated technologies [8,9].

Henna, [*Lawsonia inermis* (*L. inermis*) Linn,] is a glabrous highly branched shrub with a greyish brown dark color, which has been used for medicinal purposes for centuries [10,11]. Henna extracts possess diverse pharmacological ingredient for healing of wounds and burn injuries where they exhibit, antioxidant, analgesic, anti-inflammatory, antibacterial, antifungal, and anticancer activities [12–15]. The active coloring and biologically active ingredient of Henna Leaves is found to be Lawsone (2-hydroxy-1, 4-naphthoquinone) which can serve as a starting material for synthesizing large number of therapeutically useful

* Correspondence to: I. Yousefi, Biomaterials Research Center (MBRC), Tehran University of Medical Science, Tehran, Iran.
** Correspondence to: I. Haririan, Medical Biomaterials Research Center (MBRC), Tehran University of Medical Sciences, Tehran, Iran.
E-mail addresses: iman_yousefi@ut.ac.ir (I. Yousefi), haririan@tums.ac.ir (I. Haririan).

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compounds [16]. It has been approved that these compounds have potent wound healing characteristics [17,18]. Previous studies demonstrated that Henna leaf extracts were capable of inhibiting the growth of microorganisms which are involved in causing burn wound infections [19]. Moreover, it was reported that extracts of *Lawsonia inermis* leaves have significant wound healing activity in excision, incision, and dead space wound models [20–22]. Henna extracts enhanced the rate of wound contraction, skin breaking strength, granulation of tissue, hydroxyproline content, collagen organization, and fibroblast proliferation [17,23,24]. Henna extracts have been used in conventional forms of ointment and aqueous solutions for treatment of burns, skin inflammations, wound and ulcers [25]. However, the physical form of these products might restrain cell migration, limit cell-to-cell commutation and permanency of these materials in the chronic wound for acceptable clinical periods. These limitations lead to a slow wound healing process and an increased risk of wound infection. In order to avoid such complications, several new approaches such as electrospinning, electrospraying, solution blow spinning and film casting have been applied to combine the advantages of utilizing plant extracts in the form of suitable products to serve as wound dressing materials [26–28].

Electrospinning is a simple, versatile, an efficient process to produce continuous ultra-fine fibers, which is also applicable to different polymers [29–31]. The large surface area to volume ratio of electrospun nanofibers as well as their porous structure favors cell adhesion, proliferation and differentiation [32,33]. Therefore, electrospun nanofibrous nonwoven mats attracted a great attention in the last decade to be used for various biomedical applications especially for wound dressings [34–36]. Functional nanofibers with antibacterial and healing effects are being increasingly required. There is a fast growing interest in applying electrospinning technique to produce nanofibers containing active medicinal ingredients such as plants extracts. Recent studies have been focused on incorporating of remedial plant extracts with polymer-based electrospun nanofibers for various biomedical applications [26]. For example, the potential of electrospinning was investigated by using four different plant extracts of Indigofera aspalathoides, *Azadirachta indica*, Memecylonedule (ME) and Myristica andamanica with a biodegradable polycaprolactone (PCL) polymer for skin tissue engineering. The results of this research showed that PCL/ME nanofibers had the least cytotoxicity among the other loaded scaffolds [37]. Chitosan nanofibrous structures showed promising results to be used as a carrier of plant extracts for skin graft substitutes [38–43]. Chitosan based nanofibers are biodegradable, biocompatible and antibacterial with proliferation properties and morphological similarity to the natural extracellular matrix (ECM) in the skin [44,45]. These characteristics make them appropriate to be utilized as an excellent wound healing materials. All these useful properties of chitosan based nanofibers can promote cell adhesion, migration and proliferation [46–48]. However, chitosan is a challenging polymer to electrospin into fibrous structure because of its low solubility in common organic solvent, rigid chemical structure and the strong molecular interactions. This obstacle was overcome by several techniques [34,49]. The most successful and easiest method to improve the electrospinnability of chitosan is blending it with other polymers such as PEO. Blends of chitosan and PEO can combine two desired characteristics, i.e., excellent fiber forming ability and highly hydrophilic properties of a PEO, and the specific cell affinity of chitosan. Electrospun scaffolds based on blends of chitosan and PEO can enhance both physical properties and biological functionality [50,51]. Blending of Chitosan/PEO nanofibers with plant extracts may provide a straightforward pathway to combine different bioactivities for biomedical applications and benefitting from both physical characteristics of the nanofibrous structure and combined chemical and antibacterial properties of chitosan and the natural extracts.

These outstanding and promising structures recently got the attention of researchers, for example, curcumin loaded chitosan/poly (lactic acid) (PLA) nanofibers were produced using electrospinning. The in vivo wound healing capability of this nanofibrous structure was demonstrated by an increased rate of wound closure on a rat model [52]. Charernsriwilaiwat and coworkers prepared nanofibrous structures from chitosan-ethylene diamine tetra acetic acid–polyvinyl alcohol (CS-EDTA–PVA) incorporated with assorted concentrations of *Garcinia mangostana* extract. The extract-loaded mat was non-toxic and the predominant active ingredient, α-mangostin, was rapidly released, which retains the antioxidant and antibacterial activity, that accelerated the wound healing process [40]. Rieger and coworkers also demonstrated the ability to incorporate essential oils into chitosan/poly (ethylene oxide) (PEO) nanofiber mats. They reported that the intrinsic antibacterial activity of chitosan along with the quick release of cinnamaldehyde from the nanofibers showed high inactivation rates against *E. coli* and *P. aeruginosa* [38].

However, to the best of our knowledge the capability of chitosan based nanofibers loaded with Henna extract aimed for wound healing applications has not been reported yet. In this study, chitosan-based nanofibrous mats loaded with Henna extract were fabricated to enhance antimicrobial efficacy and biocompatibility of nanofibrous mats for wound healing acceleration. The morphology, mechanical and swelling characteristics of the electrospun nanofibers, along with their antibacterial activity, cell biocompatibility and wound healing activity were also investigated to support the core idea.

2. Experiment

2.1. Materials

Chitosan (molecular weight of 200 kDa, degree of deacetylation: 75–85%) and polyethylene oxide (PEO) with average molecular weight of 900 kDa were purchased from Sigma-Aldrich Co, USA. The leaves of *Lawsonia inermis* (Henna) were collected from Mazandaran Province, north of Iran and authenticated by Herbarium of the department of pharmacognosy, Shahid Beheshti University of Medical Sciences. Reagent grade acetic acid (99.7%, Merck) was used to prepare the aqueous solutions. Lysogeny broth (LB), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Dulbecco's Modified Eagle's Medium (DMEM), Fetal bovine serum (FBS) and antibiotics (Penicillin, Streptomycin P/S), ketamine and xylazine were purchased from Sigma (St. Louis, MO, USA). The normal human foreskin fibroblast (NHF) was obtained from the National Cell Bank of Iran (NCBI). Dimethyl sulfoxide (DMSO) and Phosphate-Buffered Saline (PBS) were purchased from Gibco Life Technologies (Grand Island, NY, USA). Ethanol and glutaraldehyde were supplied by Merck KGaA (Darmstadt, Germany). Commercial Henna ointment was purchased from Barij Essence Pharmaceutical Company, Iran. All the chemicals were of analytical reagent grade and used without further purification.

2.2. Preparation of Henna leaves extract

The fresh leaves were collected and dried in an oven at a temperature of 60 °C. The dried leaves were then ground into a fine powder using a mixer grinder. Then 10 g of dried leaves powder was suspended in 100 ml of 10% (*v*/*v*) ethanol solution and was stirred overnight. The extract solution was filtered then after through a Whatman No. 1 filter paper under suction. The resulting orange filtrate solution was then evaporated to remove the solvent using a vacuum rotary evaporator at 40 °C. The prepared Henna leaves extract was kept in vacuum desiccators to fully eliminate the moisture content. In this study, we selected hydro-alcoholic extract since it is known to haul out a variety of chemical constituents (both polar and non-polar). Thus, it was considered that most of active components have been extracted out in the hydro-alcoholic extract [53].