

The Effects of Chitosan-based nanofibers /PEO/ henna extract on recovery of superficial second-degree burn in rat

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Abstract

In the present study, electrospinning of chitosan was evaluated by adding henna extract and producing nanofibers with restorative properties. Based on SEM images, it is confirmed that nanofibers with appreciate diameter and size distribution was produced by electrospinning of chitosan/ polyethylene oxide polymeric solution and henna extract. Superficial second-degree burn in the anesthetized rat was created by placing 80°C plate for a second in the back of a rat. The result of the study showed that adding henna extract in nanofibers lead to an effective response in the recovery of grade II burning.

Keyword: electrospinning, chitosan, Henna, burning, restorative properties.

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1. Introduction

Some ulcers, for example, superficial ulcers, are not properly treated or need a long period of treatment due to a defect in one or more stages of treatment (1). All process of wound dressing and drug administration are planned to promote recovery time. A good wound dressing must be chosen based on the type of ulcer and healing process. Maintaining the most suitable environment between ulcer and dressing, absorb extra secretions without any rebounding to the dressing surface, make thermal insulation, bacterial and mechanical protection, Permeability of liquids and gases, decrease ulcer odor, and anti-allergic properties are some of the main characteristics of the new generation of dressing.

In recent years, increasing trend of World Health Organization (W.H.O) toward the evaluation of the physiological and pharmacological effect of plants extract was observed due to the fewer side effects,

low toxicity, cost-effectiveness and various effective materials in herbal plants. According to the WHO estimation, nearly 25% of new drugs directly or indirectly derived from plants (2). However, in some cases, particularly in antibiotic and anti-cancer drugs, 60% of drugs made from herbal plants (3). It is also noteworthy that if herbal plants used as the whole plant (contain several compounds) or combination of several plants synergistic effect observed and the efficacy of drugs increased and in the other hand, the side effect of drugs decreased, which it contrasts with conventional treatments with other drugs (4).

In traditional medicine herbal plants extract was used for skin lesions healing. Henna, *Lawsonia inermis* is one of these plants used from 9000 years ago. Henna belongs to class magnoliopsida which is grown in southern part of Iran, like Baluchestan and Bam. Antibacterial, antifungal, exfoliating skin cracking, antitumor and anti-inflammatory effect of Henna were reported. It's leaves are egg shape or

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oblong in green color and contain Lawson or 2-hydroxy-1,4-naphthoquinone in addition to tannin, mucilage and resin compounds. In traditional medicine, henna leaf powder was tropically used for treatment of joint's pain, acne, ulcer, burn and fungal infection (5, 6).

Electrospinning is one of the best and simple methods for nanofibers production. In this method, polymeric fiber formation on Target object controlled by electric charge. Electrospinning process is created by applying high voltage and electric charge between nozzle containing polymeric solution and collector. In next step, under the influence of electrical field, electric charge induced in drop surface. The solution is thrown rapidly into the collector and the solvent is evaporated. Finally, solid polymer fibers are placed on the collector (7, 8).

Chitosan, is biopolymer of glucosamine and N-acetyl glucosamine which produced by chitosan deacetylation. Chitin and its derivative chitosan have several physical and chemical properties include high strength, biocompatibility and biodegradability and non-toxicity. Inhibitory effects on wide range of fungi, yeast and bacteria cause widespread use in the medical field (9).

2. Material and Method

2.1 Material

Chitosan polymer powder (molecular mass: 190 kDa) and 75-80% degree of deacetylation (Sigma-Aldrich, Iceland), polyethylene oxide (PEO) polymer powder with 900,000 g/mol molecular mass (Sigma-Aldrich, USA), Acetic acid glacial with 99.8% purity and 60.05 g/mol molecular mass (Merck, Germany), alcoholic extract of henna leaf (Barij Essence, Iran) and male Wistar rat with weight range of 200-250g.

2.2 Polymeric solution preparation

Chitosan 3wt% and PEO 3wt% solution in acetic acid 0.5M prepared separately and after that mixed with 90:10 ratio (v:v; chitosan: PEO), tightly closed, and mixed with a magnetic stirrer (RET basic, IKA Inc, Germany) in 500rpm and 37° C. After that, 1% henna extract was added to CS/PEO solution and mixed with magnetic stirrer with the aforementioned condition. At the end of the process, solution rest in room temperature for 2h.

2.3 Solution electrospinning

Solution electrospinning was measured with Electrospinning Machine (Electroris ES1000; Fanavaran Nano-Meghyas). CS/PEO (90:10 ratio) and CS/PEO containing the henna polymeric solution, transferred to 5ml plastic syringe (13.6 mm internal diameter) and attached to stainless steel nozzle with flap tip and 18-gauge size. The

Distance between needle to collector and Injection speed was considered 10 cm and 0.3 $\mu\text{L/h}$, respectively. Electrospinning was performed in 20kV for 4h. In the final step, for the elimination of remained solvent, aluminum foil containing nanofibers rest at room temperature for 2h

2.4 SEM imaging

Shape and size of fibers produced by electrospinning method were determined by scanning electron microscope (SEM)(Cam Scan MV2300). Coater instrument (E5200 Autosputter) was used for the preparation of nanofibers surface for SEM imaging. Size and mean diameter of fibers were determined by Image J and Digimizer software.

2.5 Animal experiment

Male Wistar rat (n=6; 200-250g) were fed with pellets and water and kept in the separate cage in a room with 20-24°C temperature. After weighing, rats anesthetized with Nesdonal (40mg/kg; I.P). After shaving the back hair, superficial grade II burn was developed by 1.75 cm² and 80°C iron plate for a second (10). In the next step, CS/PEO and CS/PEO/LI nanofibers, henna extract (positive control) and sterile gauze (negative control) are placed in each ulcer. The difference in the level of ulcers on the tenth day compared to the first day, multiply 100, determined the wound recovery percentage.

3. Results

Electrospinning of CS/PEO/LI solution was persistent, homogenous and without loops with mean diameter 80 nm and SD=18 (figure 1).

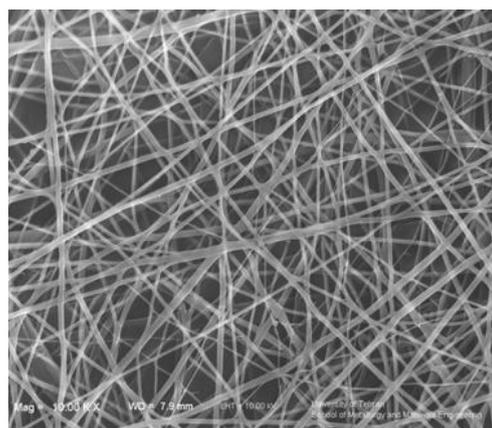


Figure 1. SEM image of nanofiber of CS/PEO/LI solution

CS/PEO nanofiber cause wound drying and preventing the secretion of lymph. Nanofibers which produced by adding henna extract to polymeric solution lead to increase antimicrobial properties and promote the wound healing process.

Faster drying of wound causes a faster fall of the damaged layer of the wound (figure 2).

4. Discussion

Importance of burn ulcer and pathogen resistant to the antibiotic, leading to the development of new material for wound dressing. In recent years, a medical instrument containing chitosan, like CELOXTM and bleeding homeostasis and

HemCon® or QuikClot bandage and ChitoFlex wound dressing for bleeding control approved by FDA and introduced in Europe and USA markets (11). Microbial activity spectrum of chitosan includes fungi, yeast, and bacteria. Antimicrobial activity of chitosan, control the bacterial growth in wound area. Chitosan ability in the stimulation of the immune system, promote cell growth and bacterial growth is a good factor for using in the wound healing process (12).

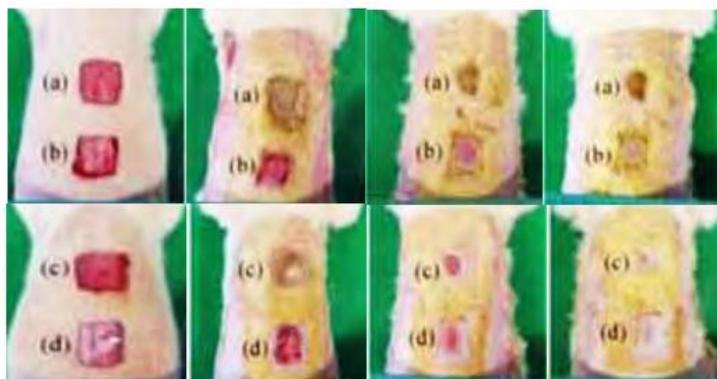


Figure 2. Animal experiment images left to right: first day, 3rd day, 7th day and 10th day. a) positive control (extract); b) negative control (sterile gauze); c) CS/PEO/LI 1% nanopolymer; d) CS/PE polymer

Polymeric nanofibers can directly Electrospined in the wound or burning area in human skin, which causes dressing with nanoscale cavities. In addition to air exchange, these dressings protect from bacterial penetration and cause normal growth of skin. The other hand, with control of drug release, eliminate wound inflammation (13).

In the present study, CS/PEP/LI nanofibers containing 1% henna extract with mean diameter 80nm and suitable morphology and diameter produced by electrospinning. Adding of henna extract to CS/PEO/LI nanofibers, promote restorative effects of chitosan and have the potential to use in the medical area as a wound dressing.

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