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# Albumin-Loaded Nanofiber for Topical Wound Healing

**Fatemeh Saadat**[1], **Anahita Fathi Azarbayjani**[2]✉

[1]Student Research Committee, Urmia University of Medical Sciences, Urmia, Iran. Email: i.saadatsogand@gmail.com
[2]Department of Pharmaceutics, School of Pharmacy, Urmia University of Medical Sciences. Email: anahita@u.nus.edu

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✉ ***Correspondence to:***
Anahita Fathi Azarbayjani

**Email:**
anahita@u.nus.edu

**A B S T R A C T**

**Objective:** Intravenous albumin administration increases blood circulation and enhances wound healing. Topical application of albumin can inhibit the growth of certain bacteria on topical wounds. Topical caffeine can induce vascularization and the formation of blood vessels on the skin. The purpose of this work is to explore for the first time the effect of topical albumin and caffeine on wound healing rate in rat models.

**Methods:** This work aimed to develop albumin and caffeine-loaded nanofibers by the electrospinning method and to evaluate their topical effect on wound healing. Nanofiber formation was assessed by SEM and considered using FT-IR spectroscopy. The therapeutic activity of topical albumin and caffeine was investigated on a full-thickness excision skin model.

**Results:** Albumin alone or in combination with caffeine effectively reduced the exposed wound area. Wound contraction at the end of week 2 was higher in albumin and caffeine loaded nanofiber group (96%) relative to the control group (79%).

**Conclusion:** Data show that daily albumin-loaded wound dressing displayed good healing properties and enhanced wound closure rate. These findings may indicate the successful application of the sauce as a promising tool in wound healing therapy.

Keywords: Albumin, caffeine, nanofiber, wound healing

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## Introduction

Malnutrition has a significant effect on albumin level and the wellbeing and hospitalization of patients. The influence of nutrition and calorie intake has been investigated to determine the level of energy needed to improve the healing of pressure ulcer. Patients with high daily protein intake of 45 g have a significantly higher plasma protein level as compared to groups with low protein intake of 20 g. High energy intake increases serum albumin level which helps to promote wound healing [1]. Measurement of serum albumin level in hospitalized patients can help assess healing rate in individuals. Plasma albumin level was found to be higher in patients with healed pressure ulcer. Intravenous albumin administration reduces edema and activates protein metabolism and help in body fluid circulation resulting in enhanced wound healing. Additionally, it was found that serum albumin correlates to CRP level and may serve as a useful indicator for disease condition and inflammation [2-4].

Topical application of albumin has been shown to inhibit the growth of certain bacteria and can act as an anti-biofilm to prevent microbial growth on wounds. Albumin coated nanoparticles can facilitate cellular uptake and can act as a potential antibacterial delivery system [5]. Albumin-based hydrogels have been developed for tissue regeneration





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engineering and wound healing application. Results have demonstrated significant growths in blood vessels in as little as 24 hr after application [6].

Topical application of caffeine has anti-aging effects, stimulates hair growth, enhances collagen production and helps promote wound healing [7]. Topical use of this molecule has shown to enhance wound healing rate in rat models. Topical application of caffeine through nanofiber formulation has demonstrated enhanced vascular proliferation in wound models [8].

Many polymer-based-drug delivery systems and wound dressings have been developed to help accelerate wound healing. Application of nanofibers in wound healing has gained increasing interest. Such scaffolds may serve as drug reservoir and help achieve sustained release of the drug at the site of action. [9,10]. High surface-to-volume ratio and porous structure of the nanofiber mat helps mimic the extracellular matrix and provides a suitable carrier for drug delivery [11].

There is no investigation on the influence of topical albumin on wound healing and closure rate. In this work, the incorporation of albumin and caffeine within nanofiber framework was carried out to help stimulate formation of complete skin layers of the wounded area.

**Table 1.** Composition of polymeric solutions used in nanofiber production

| formulation | PVA | Albumin | Caffeine |
|---|---|---|---|
| | W/V % | | |
| A | 10% | _ | _ |
| B | 10% | 12% | _ |
| C | 10% | _ | 5% |
| D | 10% | 12% | 5% |

Solutions were loaded into a syringe connected to a blunt 23 G stainless steel needle. The flow of the solution was kept constant at 1 ml/h using a syringe pump (Fanavaran Nanomeghyas, Iran). An adjustable DC power supply (Fannavaran Nanomeghyas, Iran) was employed to apply voltage of 20 kV at room temperature. The electrospun nanofibers were collected on a metal collector wrapped in aluminum foil at ambient temperature.

## Fourier Transform Infrared (FTIR) Measurements

The reaction between the polymer and active molecules were studied via FTIR. Spectras were obtained on a Perkin Elmer (Spectrum) in the wavelength region 500-4000 cm-1 at room temperature with a resolution of 1 cm-1.

## Scanning electron microscopy (SEM)

## Materials and Method

### Material

Caffeine sodium benzoate was obtained from Merk. Polyvinyl alcohol (Mw 89000~98000) was acquired from Sigma Aldrich. Albumin was obtained from Biotest Germany. Ketamine and xylazine hydrochloride were bought from Pfizer, USA and Bayer, Germany respectively. Healthy male Wistar rats (180-200 g) were purchased from Pasteur institute, Tehran, Iran.

## Electrospinning

Polymeric solutions were prepared by dissolving PVA (10% W/V) in Milli-Q water under magnetic stirrer at 80 oC until the formation of a transparent solution. Detail of each formulation is tabulated in Table 1.

To prepare ethanolic extract, 10 grams of dried plant powder with 100 ml of 96% ethanol for 24 hours at room temperature is mixed by shaker (Pars Azma; Iran) at a speed of 130 rpm, then by Whatman No. 2 paper was flattened. The solvent was separated from the extract by a rotary apparatus (Heidolph; Germany) using a vacuum pump (vacuum distillation). The weighted extract was then dissolved in DMSO solvent. The obtained extract was stored in the refrigerator at 4 ° C until use in antimicrobial experiments [29].

## In vivo studies

In vivo studies were conducted on healthy adult male Wistar rats (180-200 g). All animals were acclimatized to animal house conditions and were maintained in individual cages and housed at a temperature of 25 ± 2 oC at a 12 h light - dark cycle for 10 days prior studies. Animals were handled in accordance to welfare of experimental animal and the protocol was approved by the local ethical committee of Urmia University of Medical Sciences. A single excision-type wound 1× 1 cm2 was created in the dorsal region of each rat under xylazine (5 mg/kg) + ketamine (40 mg/kg) anesthesia. Animals were randomly allocated into 5 experimental groups and were caged individually (n=4):

Control: open wound (nothing was applied to the wound area)

Pure nanofiber wound dressing without any drug

Nanofiber wound dressing containing albumin

Nanofiber wound dressing containing caffeine

Nanofiber wound dressing containing albumin + caffeine

The dressings were applied once daily for 2 weeks. Wound contraction (%) was quantified on day 5, 10 and 15 post surgeries. Wound area (cm2) was measured precisely using a ruler. Wound contraction (%) was quantified according to [12]:

$$\text{wound contration (\%)} = \frac{\text{day 0 wound area} - \text{day n wound area}}{\text{day 0 wound area}} \times 100$$

## Tissue processes and histopathological analysis

Rats were sacrificed 15 days post-treatment and skin tissue sections were collected under anesthesia. Full thickness skin was fixed in 10% neutral buffered formalin and embedded in paraffin for slice sections (5µm thick). Histopathological changes at the wound site were evaluated using Hematoxylin and eosin (H&E) staining. Granulation formation and wound maturity were visualized using an optical microscope (Oxion) at 40×magnification.

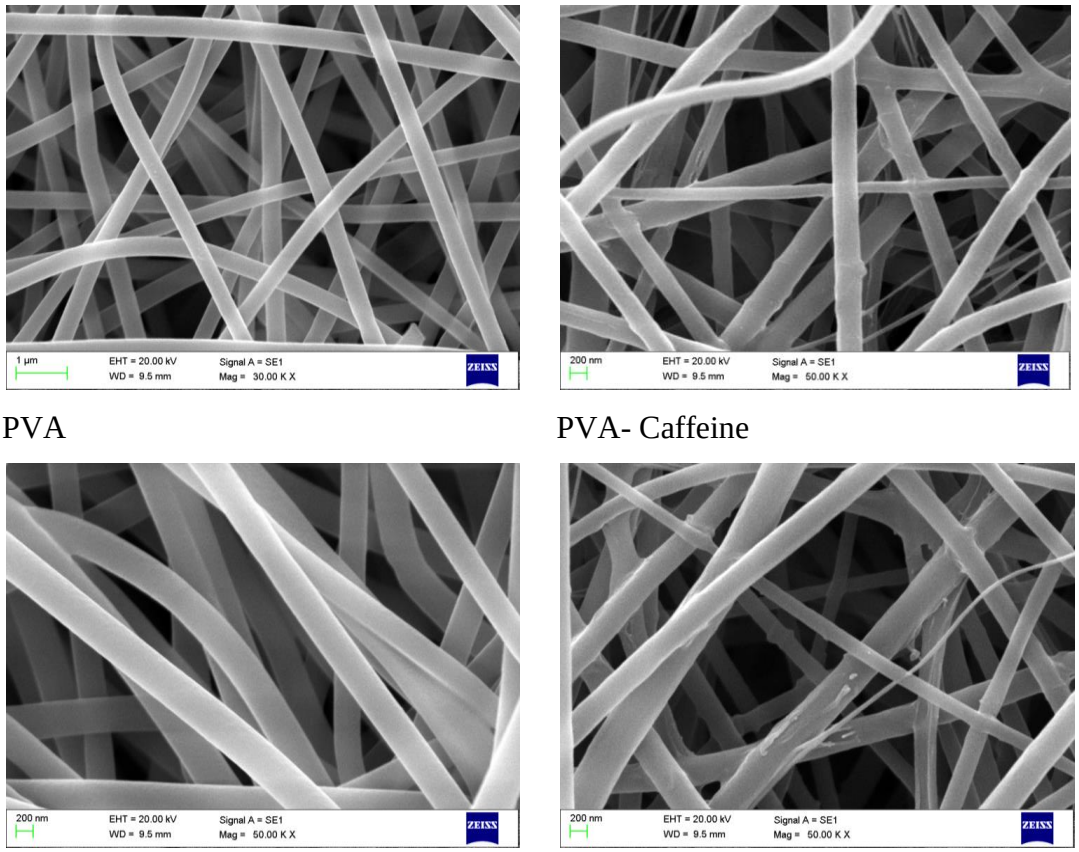
## Statistical analysis

The data are presented as mean ± SD. One way ANOVA and Tukey's post hoc was performed for all tests and $p < 0.05$ was considered statistically significant (n=4).

## Results and Discussion

## SEM imaging of nanofiber membrane

The microstructure of the nanofibers shown by SEM is illustrated in Fig. 1. Bead-free and randomly positioned uniform nanofiber network is observed. The average fiber diameter of the pure polymer solution was 187.80 ±28.5 nm, while samples containing caffeine had slightly lower diameter at 125.28 ± 12.80 nm. Some large spindle elongation is seen in samples containing albumin and average fiber diameter is 359.58 ±34.56 nm. This may be due to the stretching of the solution during electrospinning. Decrease in fiber diameter is seen in samples containing both caffeine and albumin (300.17 ± 45.40 nm). Caffeine is a surface active agent and helps to decrease bead formation and reduce fiber diameter [13,14].




PVA PVA- Caffeine

PVA-Albumin PVA-Caffeine-Albumin

**Fig. 1.** SEM images of the nanofiber formulations

## FTIR analysis

FTIR of all formulations are shown in Fig. 2. The main characteristic FTIR spectra for dried albumin are the amide I

band and the amide II band seen in the region of 1500-1750 $cm^{-1}$. The amide I band mainly consists of C=O stretching and the bending of the –NH bond of the amide II bands are seen at 1704 $cm^{-1}$ and 1549 $cm^{-1}$ respectively and the –OH Stretching band is seen in the region 2500-3500 $cm^{-1}$ [15,16].

The main characteristic peaks for caffeine is seen at 1735 cm-1 (C=O stretching) of the amide group and 1550 $cm^{-1}$ (–CH3 stretching). The vibration in the skeleton of pyrimidine ring is seen at 745 $cm^{-1}$. The PVA spectrum shows a broad band at around 3325 $cm^{-1}$ which may be due to the presence of water molecules absorbed by the PVA backbone. Bands at 1093 $cm^{-1}$ and 2938 $cm^{-1}$ correspond to the –OH stretching and –CH– stretching respectively [17,18]. The peaks of the functional groups of the molecules are present and this data suggests that there is no physical or chemical interaction between the PVA and caffeine or albumin [13,14].

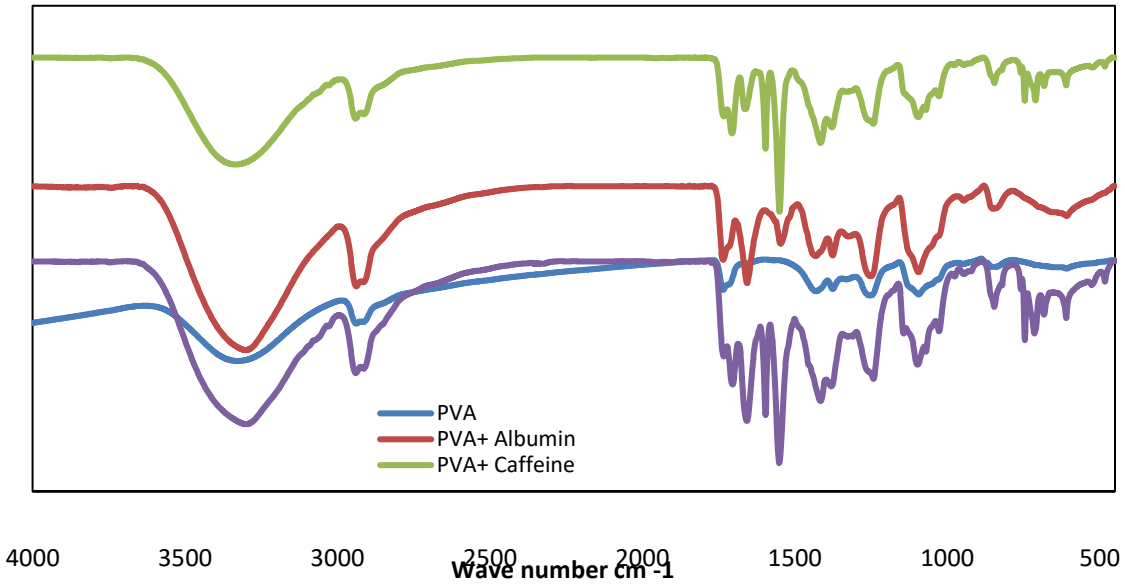



**Fig. 2.** FTIR spectra of nanofiber formulations

## Histological observation of wound healing

Fig. 3 depicts the wound closure in various treatment groups. The results clearly indicate a faster healing of wounds covered with albumin. The wound area shows obvious difference among the control and treatment groups however the results were not significant. Albumin alone or in combination with caffeine effectively reduced the exposed wound area; however the results were not significant. This is while caffeine treatment alone did not show any visible effect on wound closer rate.

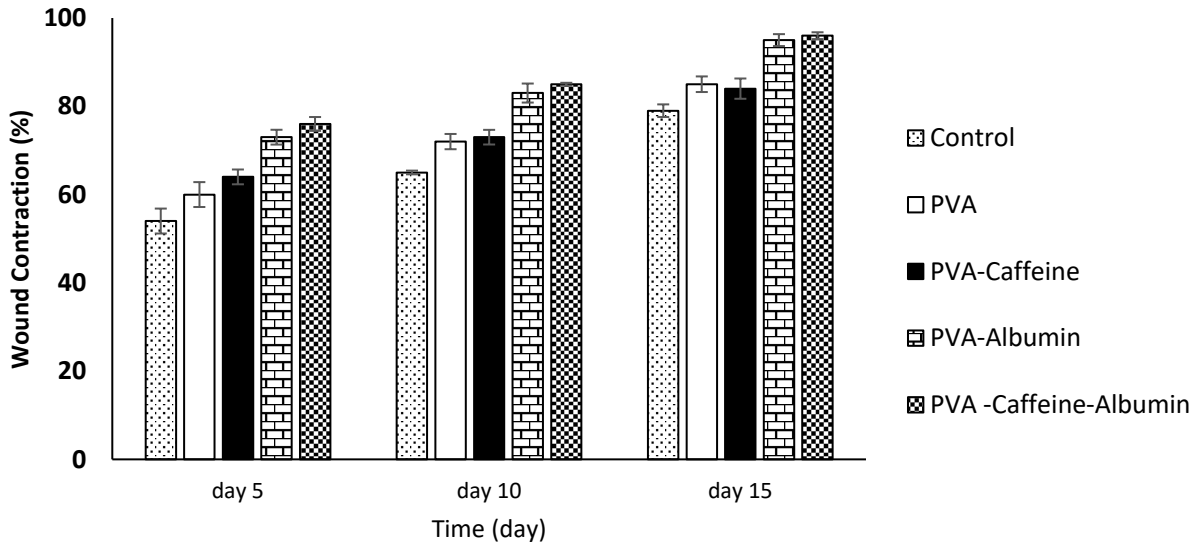



**Fig. 3.** Wound healing (%) in rat models after treatment with various nanofiber formulations

The effect of caffeine has been tested on cell proliferation and differentiation in an ex vivo wound healing model. It was found that topical application of caffeine may delay epithelialization and keratinocyte proliferation. This shows that caffeine, as an adenosine-receptor antagonist may show marked reduction in cell proliferation in a dose dependent manner. However this hypothesis needs to be further investigated [19].

The repair of tissue structure was studied through histological examination of the treated wounds shown in Fig. 4. It is seen that the epithelium of the control group show infiltration of inflammatory cells. Group treated with PVA show similar results. The group treated with PVA show white empty spaces in the dermis layer which indicates poor collagen deposition [20]. The wound treated with caffeine show sparse formation of blood capillary.

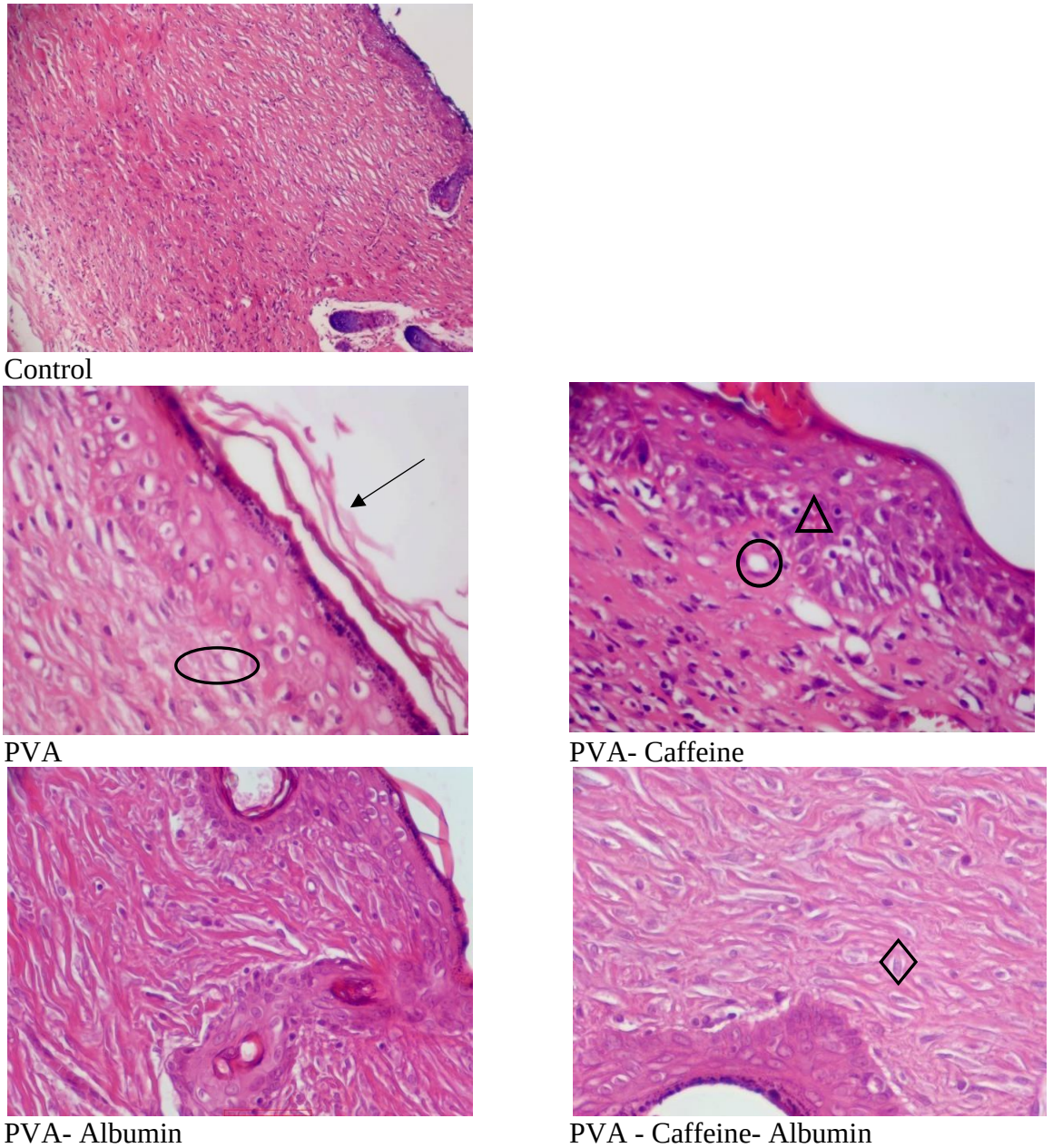



**Fig. 4.** Photomicrographs of haematoxylin and eosin stained wound tissue of rats 15 days after treatment. The symbols like ○ denotes blood vessles, ⬭ white empty space, △ RBC, □ eosinophils, ◇ refers to fibroblasts, and the ↓ indicate stratum corneum.

Wound treated with albumin show well-developed dermis and epidermis. Fibroblasts can replace damaged areas with ground substances. It is observed that fibroblasts are more abundant in the group treated with albumin and caffeine. This may indicate a better healing process than the control

group. Sufficient vascularization of the newly formed skin tissue is necessary for wound healing and skin regeneration. Although caffeine did not show any significant effect on wound closure but its application was proven to be effective in the formation of blood vessels.

Many studies have reported on the use of albumin as an accelerator for its beneficial effect on wound healing. It is reported that albumin may enhance the function of inflammatory cells. Blood flow to the skin and skin microcirculation is also related to serum albumin as a parameter of malnutrition and inflammatory [21]. The preliminary results obtained in this work may suggest the beneficial effect of topical albumin for wound healing.

## Conclusion

In this work, albumin and caffeine loaded nanofibers were successfully prepared by electrospinning method and examined for their effect on wound closure. Nanofiber formation was assessed via SEM and evaluated using FT-IR spectroscopy. Wound healing potential of the formulations was tested on excisional wound model in rats. Data show that daily albumin-loaded wound dressing displayed good healing properties and may serve as a promising tool in wound healing therapy.

## Conflict of interest

The authors have no other relevant affiliations or financial involvement with any organization and declare no conflict of interest.

## Consent for publications

All authors approved the final manuscript for publication.

## Availability of data and material

The data of the current study is available from the corresponding author on reasonable request.

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