Fabrication of Cross-linked Gelatin Electrospun Nanofibers Containing Rosemary Oil for Antibacterial Application

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Abstract

In this study, fabrication of nanofibers with antibacterial property was aimed to obtain biopolymer-based nano-fiber product that is used for wound healing. For this aim, natural rosemary oil was used to give antibacterial activity to the nanofibers and gelatin polymer was used to produce nanofibers. According to literature survey, rosemary oil has antibacterial activity and it is also used in aromatherapy, topically to sooth muscles, and medicinally thanks to its antibacterial and antifungal properties. Gelatin is a natural biopolymer and extensively used in medical products such as wound dressings, drug delivery systems etc. Therefore, the combination of inherently beneficial effects of gelatin material with enhanced properties of nanofibers mats and rosemary oil was aimed at.

In the study, gelatin nanofibers containing rosemary oil were fabricated by an electrospinning method. Gelatin was dissolved in distilled water/acetic acid at concentration of 10 % at first step. Then, rosemary oil and surfactant (Span 20) was added to solution and stirred for 6 hours. To get a cross-linked nano-fibrous mat, two different cross-linking methods and different cross-linkers were applied. In the first method, glutaraldehyde or tannic acid as a cross-linker was added to polymer solution before electrospinning. In the second method, nanofibers were spun from rosemary oil/gelatin solution and then cross-linked by GA and tannic acid, separately. Morphology and fiber diameter were investigated using SEM. FT-IR spectroscopy was used to identify cross-linked fiber structure and the presence of rosemary oil in electro spun mat. The solubility of cross-linked fiber mat was also investigated.

Introduction

Electrospinning is an inexpensive, effective and simple method to produce nanoscale fibers, which have intrinsically high surface to volume ratios, increased flexibility in surface functionalities, improved mechanical performances, and smaller pores than fibers produced using traditional methods [1]. Electrospun fibers have been used for advanced applications especially in biomedical field such as a smart wound dressing material and artificial scaffold for tissue engineering.

According to literature, numerous synthetic biocompatible and biodegradable polymers such as poly(lactide), poly(glycolic acid), poly(caprolactone) and poly(ethylene glycol) and naturally derived biopolymers such as collagen, silk fibroin, alginate, gelatin, chitin and a blend of various biocompatible polymer pairs have been electro-spun into nanofibers for a multitude of biomedical applications such as scaffolds for use in tissue engineering, wound dressing, drug delivery and vascular grafts [2, 3].

Gelatin is a protein-based biopolymer derived from partial hydrolysis of native collagens, which are structural proteins found in parts of animal bodies, such as skin, tendon, cartilage and bone. Gelatin is extensively used in medical products such as wound dressings, drug delivery systems, sealants for vascular prostheses thanks to its high biocompatibility, biodegradability and bioactivity. In this study, gelatin nanofibers containing rosemary oil was fabricated for antibacterial applications. According to literature survey, rosemary oil has antibacterial activity against Gram-positive and Gram-negative bacteria [4, 5]. Therefore it was added into nanofibers' structure during electrospinning.

Despite their potential, the electrospun fibers of gelatin are water soluble and mechanically weak. Thus, further treatments such as cross-linking to improve these properties are required. As seen from previous studies, electrospun gelatin fiber mats are successfully cross-linked by GA vapor by adding small amounts of GA to the gelatin solution before electrospinning process (one step cross-linking process) [6]. Another cross-linker used in study, tannic acid (TA), is a hydrolysable tannin as a natural phenolic cross-linker to modify gelatin and improve its mechanical performance [7]. This study focused on cross-linking of gelatin nanofibers containing rosemary oil. In the study, glutaraldehyde and tannic acid as cross-linkers were used. Cross-linking was carried out before and after the electrospinning process.

1 Materials and Methods

1.1 Materials

Gelatin (from porcine skin, type A) in powder form, acetic acid (99.8- 100, 5 %), tannic acid ($C_{76}H_{52}O_{46}$) in powder form, glutaraldehyde (GA) solution (25 %) were purchased from Sigma-Aldrich. Hydrochloric acid (37 %) and toluene were obtained from the Ricdel-de Haör. Surfactant (Span 20) was a product of Merck. Sodium hydroxide (50 %) purchased from J.T. Baker. Natural rosemary oil was obtained from Botalife (Turkey).

1.2 Electrospinning Process

Gelatin of 10 wt % solution was prepared by dissolving gelatin in the mixture of distilled water and acetic acid. Then, 5 grams of rosemary oil and 1 gram of surfactant (Span 20) were added to the solution and stirred at room temperature for 6 hours. The distance between the grounded collector and needle tip was set to 12 cm. Electrospinning carried out at 17 kV, solution feed rate of 2 ml/h.

1.3 Cross-linking of Gelatin Nanofibers Containing Rosemary Oil

The cross-linking process of gelatin/rosemary oil electrospun mat was carried out by two different methods and cross-linkers. In the first method, 0.5 grams of glutaraldehyde or 0.09 grams of tannic acid were added to the polymer solution before electrospinning. Then, polymer solutions were electrospun.

In the second method, nanofibers were spun from gelatin solutions containing rosemary oil and then cross-linked by GA or tannic acid. In the glutaraldehyde cross-linking process, a 0.5 grams of glutaraldehyde and 0.05 M, 37% hydrochloric acid that were used to adjust pH were mixed in 50 ml of toluene. Electrospun nanofiber mat was dipped in the prepared mixture for 24 hours at room temperature. Cross-linked nanofiber mat was washed to remove residual GA solutions. In the cross-linking process with tannic acid (TA), 0.09 grams of TA were dissolved in 100 ml distilled water and the pH of the solution was adjusted to 8 by adding NaOH solution. Electrospun nanofiber mat was dipped in this solution for 24 hours at room temperature. Cross-linked nanofiber mat was treated by series of washing process to remove residual TA. The information about nanofibers cross-linked is given in Table 1.

Tab. 1: *Cross-linked nanofiber properties*

Nanofiber samples	Cross-linker	Cross-linking step
Sample 0	-	-
Sample 1	GA	Before electrospinning
Sample 2	TA	Before electrospinning
Sample 3	GA	After electrospinning
Sample 4	TA	After electrospinning

Source: Own

1.4 Characterizations of Nanofibers

To investigate dissolvability of nanofibers, fabricated nanofiber mats containing rosemary oil were cut into pieces having the area of 3x3 cm^2 and immersed in water at room temperature. In this test, to examine whether the sample was dissolved or not, the time required for dissolution was measured in case dissolution was performed. Photos of insoluble samples were taken at certain time intervals in 24 h. The morphology of the nanofibers was characterized by SEM images. FT-IR spectroscopy was used to identify cross-linked gelatin/rosemary oil nanofiber structure and the presence of rosemary oil in electrospun fiber mat.

2 Results and Discussion

2.1 Results of Dissolvability Test

The dissolvability test was applied to investigate whether nanofibers were cross-linked or not. Gelatin polymer is soluble in water at room temperature. Once cross-linking of gelatin was achieved, its solubility could be prevented. Therefore, nanofibers treated with cross-linkers by different methods were tested for dissolvability. Figure 1 shows dissolvability test results of uncross-linked nanofibers. Uncross-linked electrospun gelatin fiber mat containing rosemary oil began to form gel and shrink as it contacted with water and then almost dissolved.

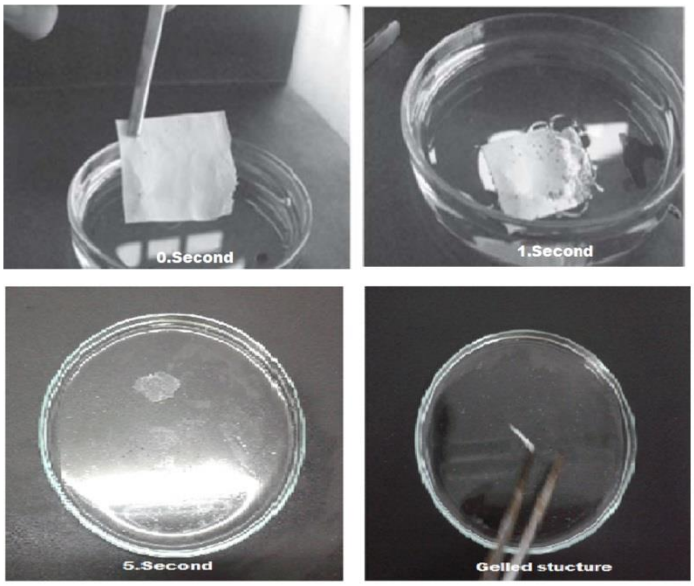

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Fig. 1: *Dissolvability test of uncross-linked gelatin nanofiber (Sample 0)*

Dissolubility test results of Samples 1 that were cross-linked with GA before electrospinning, were given in Figure 2. According to the photos, nanofibers did not dissolve during 24 h in the water.

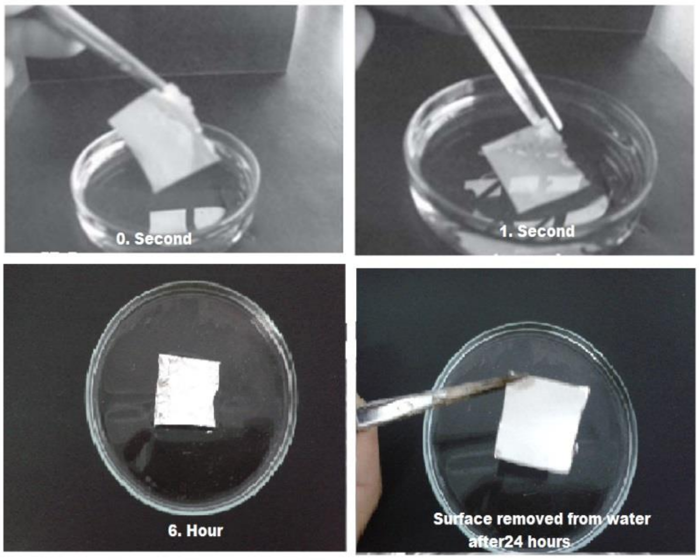

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Fig. 2: *Dissolvability test result of Sample 1*

According to literature, TA cross-links the gelatin polymer at pH 8. Cross linkage between reactive groups of gelatin and tannic acid occurs at this pH [7]. In the study, electrospinning of this solution could not be carried out due to salt crystals formation between the acid and basic (NaOH used for pH adjusting) groups in solution when the pH of the polymer solution was adjusted to pH 8. For this reason, nanofibers were spun from the gelatin polymer solution containing TA at pH 5. Figure 3 shows dissolubility test results of this sample (Sample 2). As seen from Figure 3, nanofiber sample was entirely dissolved in water.

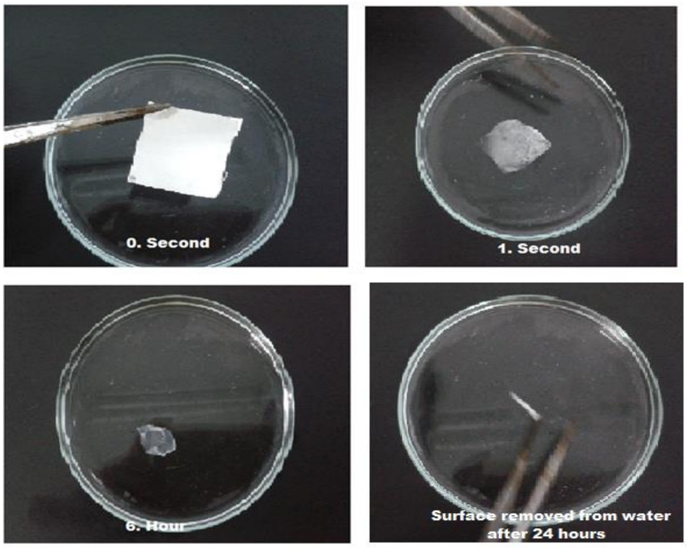

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Fig. 3: *Dissolvability test result of Sample 2*

Cross-linked gelatin nanofiber mat containing rosemary oil by glutaraldehyde after electrospinninig process showed no gelation, shrinkage and deformation as cross-linked gelatin nanofiber immersed in water. There was no gelation, shrinkage and deformation even after six and twenty four hours following the moment surface of electrospun gelatin fiber mat immersed in water (Figure 4). The water resistant ability of the surface was a proof of cross-linking gelatin nanofiber mat containing rosemary oil by glutaraldehyde.

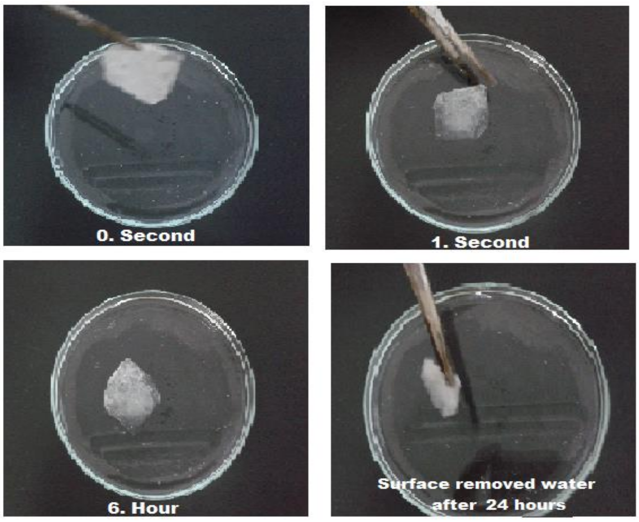

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Fig. 4: *Dissolvability test of Sample 3*

Figure 5 shows the dissolubility test photos of Sample 4. There was no gelation, shrinkage and deformation of electrospun gelatin fiber mat immersed in water even after six and twenty four hours. The water resistant ability of surface was a proof of cross-linking gelatin nanofiber mat containing rosemary oil by tannic acid.

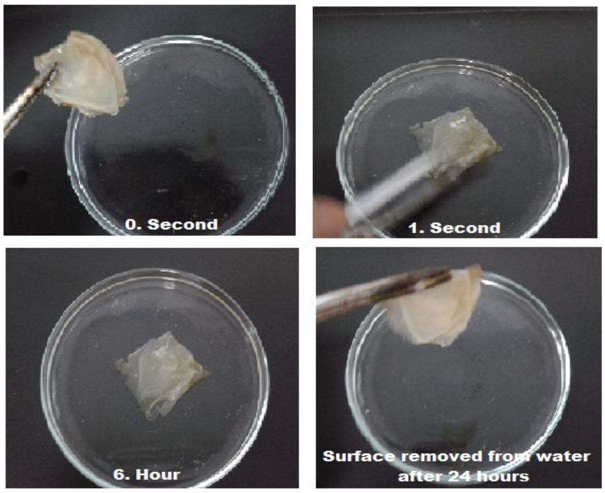

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Fig. 5: *Dissolvability test result of Sample 4*

2.2 Results of SEM Analysis

The SEM image of gelatin/rosemary oil nanofibers were given in Figure 6. It clearly shows the fibers with nano-size have almost uniform diameters. There are no beads and continuous fiber structure was achieved.

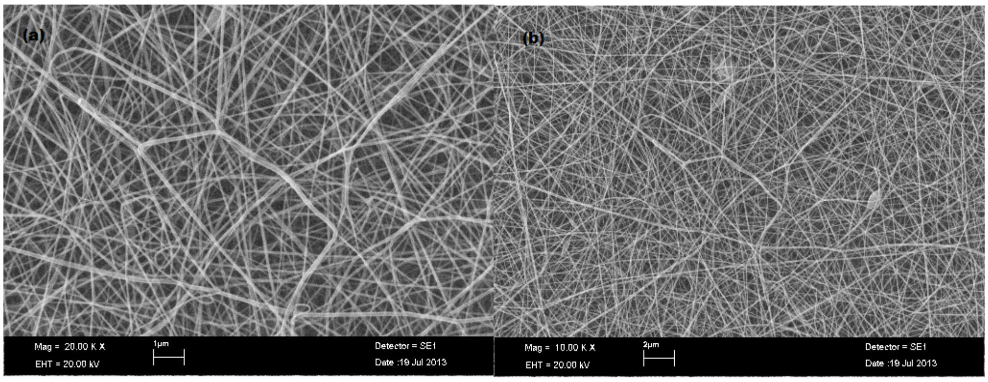

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Fig. 6: *SEM images of Sample 0 (a) X20000 (b) X10000*

To investigate the effect of the cross-linking process on the morphology of nanofibers, SEM images of gelatin/rosemary oil nanofibers cross-linked with GA before electrospinnig process were given in Figure 7. According to the SEM images, nanofibers which belong to Sample 1 have nano-sized and uniform diameter distribution. There is no negative effect of GA addition on the morphology of electro spun nanofibers.

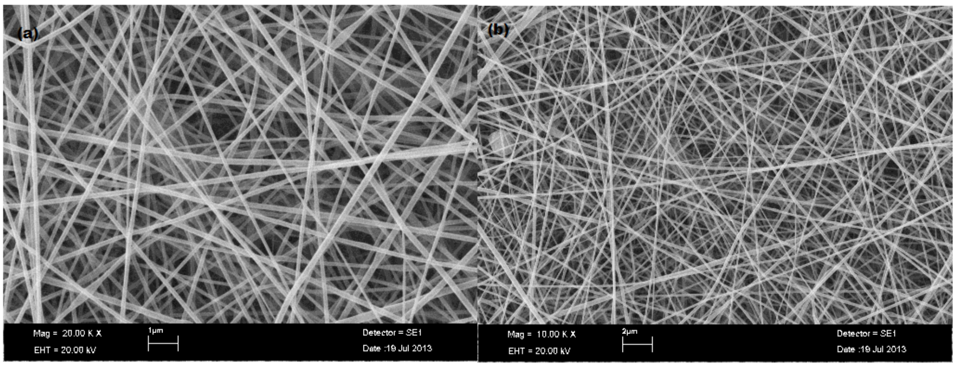

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Fig. 7: *SEM images of Sample 1 (a) X20000 (b) X10000*

SEM images of nanofibers cross-linked by GA after electrospinning given in Figure 8 clearly show the change in morphology. Net-like structure formed due to cross-linkage occurrence between fibers by GA cross-linking. It was concluded that net-like structure caused the morphological change.

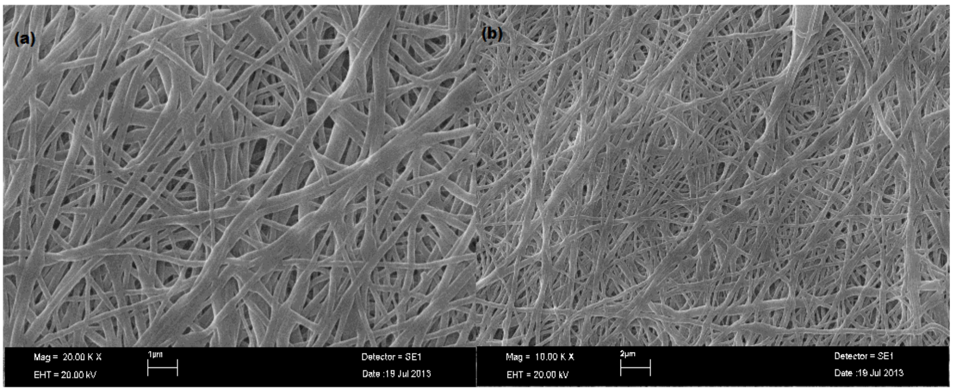

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Fig. 8: *SEM images of Sample 3*

SEM images of Sample 4 given in Figure 9 show deformation of the structure and loses fibrous structure. Furthermore, formation of crystal particles on the surface nanofibers was observed. These nanofibers were crosslinked by TA in the water at pH 8. It was thought that crystal particles formed on the surface nanofibers due to chemical interaction between TA that shows weak acidity and sodium hyroxide used for adjusting pH.

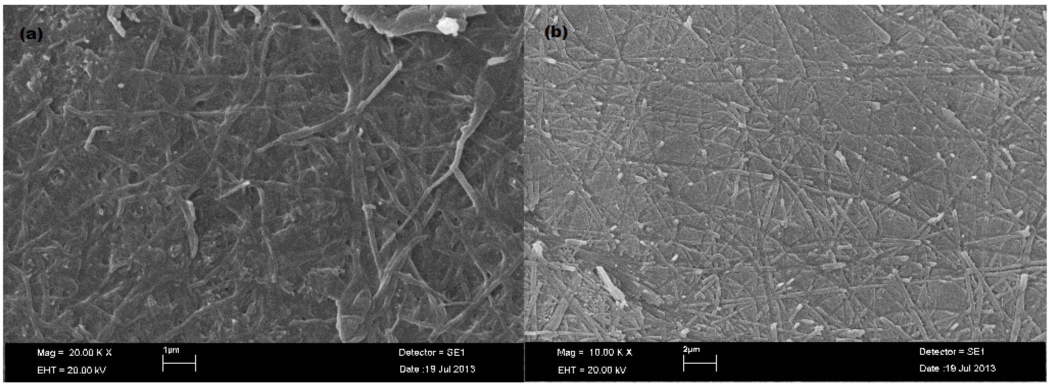

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Fig. 9: *SEM images of Sample 4*

2.3 Results of FT-IR

According to the results of the dissolubility test and SEM analysis, gelatin/rosemary oil nanofibers encoded as Sample 1 can be chosen as ideal cross-linked nanofibers. Therefore FT-IR analysis was performed on these nanofibers to investigate the presence of rosemary oil in structure and identify cross-linked structure. Figure 10 shows the FT-IR spectrums of nanofibers, gelatin, rosemary oil and glutaraldehyde. According to FT-IR spectra of gelatin, there is a wide peak at 3462 cm^{-1} and this peak is N-H stretching peak in gelatin structure. These peaks arise at 3300 cm^{-1} and 3426 cm^{-1} in IR spectrum of cross-linked gelatin nanofibers (Figure 10 c).

According to FT-IR spectra of rosemary oil (Fig.10), there are sharp peaks at 2923 cm^{-1} and 2854 cm^{-1} that are C-H stretching peaks of rosemary oil [8]. The peaks at 2928 cm^{-1} wave length in FT-IR spectrum of nanofibers are characteristic peaks of rosemary oil and prove presence of rosemary oil in the structure of nanofibers.

During the cross-linking process by glutaraldehyde, aldimine linkage (CH=N) occurs due to chemical reaction between amino groups of gelatin and aldehyde groups of glutaraldehyde. The characteristic absorption of the aldimine groups arises at 1450 cm^{-1} [2]. This peak was observed in FT-IR spectrum of cross-linked gelatin nanofibers containing rosemary oil (Figure 10 c). This finding is a proof of cross-linking of gelatin with GA.

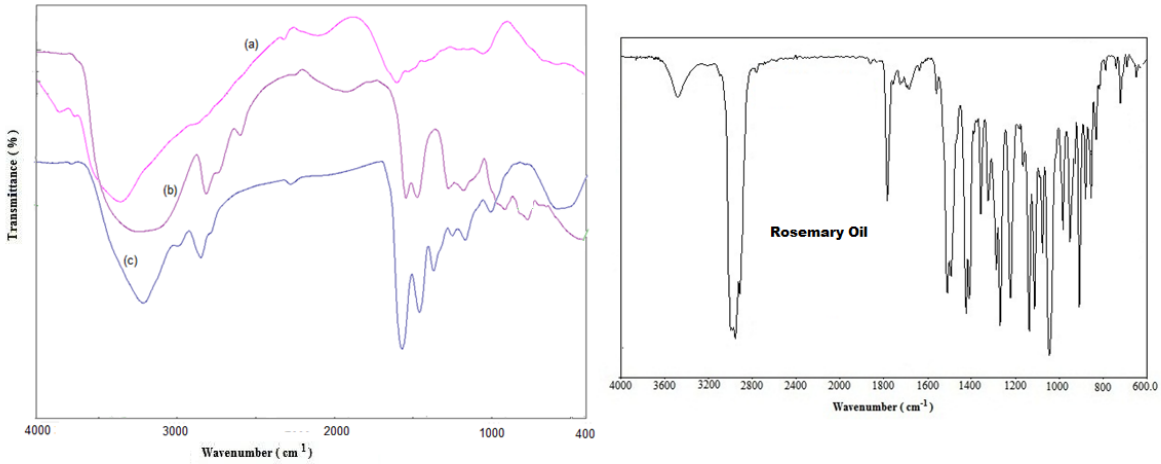

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Fig. 10: *FT-IR spectra of gelatin (a), glutaraldehyde (b), cross-linked gelatin nanofibers containing rosemary oil by adding glutaraldehyde to the gelatin/rosemary solution before electrospinning process (c)*

Conclusion

In this study, fabrication of cross-linked gelatin nanofiber containing rosemary oil was carried out. Rosemary oil was dispersed in gelatin solution and the solution was electrospun to produce gelatin nanofibers containing rosemary oil. In the study GA and TA were used as cross-linkers. Cross-linking was carried out by two different methods. One of the methods focused on adding cross-linkers to polymer solution before electrospinning. In another method, cross-linking was carried out after electrospinning process. Nanofibers were tested and characterized by a dissolubility test, SEM and FT-IR spectroscopy analysis. According to the test and the analysis results, cross-linking with TA couldn’t be achieved before electrospinning as the morphology was affected negatively by TA cross-linking after electrospinning. GA cross-linking before electrospinning gave the most suitable results related to dissolubility and morphology. The rosemary oil content and cross-linkage with GA of these nanofibers were also proved by FT-IR analysis.

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VÝROBA ŽELATINOVÝCH NANOVLÁKEN OBSAHUJÍCÍCH ROZMARÝNOVÝ OLEJ S ANTIBAKTERIÁLNÍMI ÚČINKY

Tato studie se zabývá výrobou nanovláken s antibakteriálními vlastnostmi zaměřenou na získání nanovlákenného produktu na bázi biopolymeru, který se používá pro hojení ran. Za tímto účelem byl použit přírodní rozmarýnový olej, který posiluje antibakteriální aktivitu nanovláken. K výrobě nanovláken byl použit želatinový polymer. Jak potvrzuje studium literatury, rozmarýnový olej má antibakteriální účinky, a je také používán v aromaterapii, lokálně k uklidnění svalů, a díky svým antibakteriálním a antimykotickým vlastnostem má i léčivé účinky. Želatina je přírodní biopolymer a její využití v medicíně je široké – například se používá při ošetřování ran nebo je součástí různých léčiv atd. Proto jsme se zaměřili na kombinaci nepopiratelně blahodárných účinků želatiny s vysoce užitnými vlastnostmi nanovlákenných látek a rozmarýnu.

HERSTELLUNG VERNETZTER ELEKTRONISCH GESPONNENER NANOFASERN AUS GELATINE, DIE ROSMARINÖL FÜR ANTIBAKTERIELLE ANWENDUNG ENTHALTEN

In dieser Studie ging es darum, Nanofasern mit antibakteriellen Eigenschaften zu produzieren. Das Ziel bestand in der Gewinnung von Nanofaserprodukte auf Biopolymerbasis, das zur Wundbehandlung Verwendung findet. Zu diesem Zweck wurde natürliches Rosmarinöl benutzt, um die antibakterielle Aktivität der Nanofasern zu verstärken. Zur Produktion von Nanofasern wurde Gelatinpolymer verwendet. Laut Literatur wirkt Rosmarinöl antibakteriell und wird ebenfalls in der Aromatherapie angewendet, namentlich um die Muskeln zu beruhigen. Außerdem die antibakteriellen und antifungiellen Eigenschaften des Textils heilsame Wirkung. Gelatine ist ein natürliches Polymer und wird hauptsächlich bei der Wundbehandlung usw. verwendet. Daher richtete sich unser Interesse auf die Kombination der inhärent heilsamen Effekte von Gelatinematerial mit den verbesserten Eigenschaften von Nanofasermatten und Rosmarinöl.

PRODUKCJA ŻELATYNOWYCH NANOWŁÓKIEN ZAWIERAJĄCYCH OLEJ ROZMARYNOWY O DZIAŁANIU ANTYBAKTERYJNYM

Niniejsze opracowanie poświęcone jest produkcji nanowłókien o działaniu antybakteryjnym, dotyczącej pozyskiwania produktu z nanowłókien na bazie biopolimeru, który stosowany jest do gojenia ran. W tym celu zastosowano naturalny olej rozmarynowy, który wzmacnia antybakteryjne działanie nanowłókien. Do produkcji nanowłókien użyto polimeru żelatynowego. Jak wynika z literatury, olej rozmarynowy ma działanie antybakteryjne i stosowany jest również w aromaterapii, miejscowo do uspokojenia mięśni i dzięki swoim cechom antybakteryjnym i antygrzybiczym ma także działanie lecznicze. Żelatyna to naturalny biopolimer, który jest szeroko stosowany w medycynie – przykładowo używany jest do opatrunku ran lub jako element różnych substancji leczniczych itd. Dlatego skupiliśmy się na połączeniu niepodważalnego korzystnego działania żelatyny i wysoce skutecznych właściwości substancji nanowłóknowych i rozmarynu.