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Anti-Inflammatory/Bacteria Potential of Gelatin/ZnO Nano Fiber Scaffold

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Abstract

Based on healthy properties of gelatin, strengthening it for using as multifunctional material is essential. In this paper nano scaffold of gelatin was doped with nano ZnO (with different percent) and electrospun. The morphology of obtained nano scaffold was study by FESEM and elemental mapping proved the present and distribution of nano ZnO in scaffold. Also, XRF test was done to confirm the amount of used nano material. The anti-bacterial property of the obtained scaffold was investigated against both gram-negative and positive bacteria (Shigella and Staphylococcus Aureus respectively) and the results show that the nano scaffold has more than 96% anti-bacterial property. On the other hands, the anti-inflammatory effect of nano scaffold was investigated on rats and the results illustrated that nano scaffold is effective for edema decrease.

Keywords: Gelatin, Zinc Oxide, Nano-Scaffold, anti-bacterial, anti-inflammatory.

1. Introduction

Inflammation is one of the allergic signs that cause to swelling. Anti-inflammatory is the typicality of material which decreases the swelling and inflammation. In recent years, many researches are done towards producing anti-inflammatory materials which has low toxicity. Hekmatimoghaddam et al. reported that using gelatin hydrogel including nanoparticles of cerium oxide has an anti- inflammatory effect [1]. Also, Hadisi et al. came to conclusion that nano fibers of gelatin-starch has anti-bacterial and anti-inflammatory properties [2]. So, as it seems, gelatin is a good candidate for researching and using in textile production. Gelatin (or

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gelatus) is a semi-translucent biomaterial which can be reached from collagen [3-5]. The scientist can obtain it by hydrolysis of collagen, so a protein will be gaining which is soluble in water and by drying it, a breakable layer will appear [6, 7]. Most of the polar solvents can solve gelatin and one of the most applications of gelatin is in nourishment industry. Also, this material is used in medicine and hygienic products [8-12]. In the field of textile industry, some researches are done about producing gelatin/silver fiber by wet spinning and investigate the anti-microbial property of produced fiber [4] while some researchers reported that using nano silver has cellular toxicity[13]. But the lack of researches in this field is felt.

In medicine, the scaffold has many applications [14, 15]. The polymeric scaffolds are create by various techniques [16, 17] but electrospinning method is one of the best techniques. Khodadadi et al. reported the electrospinning of nano fiber scaffold in order to drug delivery [18]. In this method, a polymer solution will charge and by ejection this solution, a fine layer of polymer scaffold is formed. In this method ultra fine fiber web will produce [19-24]. Choosing this method for producing nano scaffolds has many advantages such as excellent oxygen exchange rate in nano fiber, great porosity and high ratio of surface area to volume [25, 26]. Erencia et al. and Salles et al. reported that using some acids can improve the shape and cause to have finer nano fibers in electrospinning [27, 28] while some researches indicate that using acetic acid caused to better electrical conductivity of electrospinning [29]. On the other hands, electrospinning of scaffolds has many other applications such as wound dressing, dental applications and filtration [30-34]. Also, the produced nano scaffolds have some disadvantages such as low mechanical property, but by doping the scaffolds with some nano materials, we can overcome this problem. Recently, nano particle of semiconductors gain much attention based on their newfound specifications and properties which can gives to the final produced fibers. Using these nano particles in fiber/fabric (such as nano titania, nano zinc oxide, nano cerium, nano iron and nano cadmium sulfide) was study by some researchers [35-40]. One of interesting these semiconductors is zinc oxide (in the scale of nano) which has 3.3eV of energy ban gap and has some properties like ultra violet blocking, low toxicity, activating by the photo and antiseptic. Many methods are studied for grafting nano ZnO on surface of fiber/fabric such as padding, spinning, ultrasonic irradiation and etc.[41-44].

In this paper, nano scaffold of gelatin/ZnO(NSGZ) has been prepared by electrospinning and the structure and its properties investigated. The goal of producing NSGZ was preparing a suitable anti-inflammatory and anti-bacteria web by using gelatin scaffold.

2. **Experimental**

2.1. Material

Nano ZnO and the powder of gelatin were purchased from Sigma-Aldrich and needed acetic acid was prepared from Merck. First of all, distilled water and acetic acid were mixed with the ratio of 70/30 respectively and then 1g of gelatin powder was added to above solution. Then the solution was sonicated (a Euronda ultrasonic bath model Eurosonic 4D, 350 W, 50/60 Hz, Italy was used) with ultrasonic device at 40oC for half an hour. Beside preparing this solution, in another beaker three percentage of nano zinc oxide solution prepared and these two above solution was added to each other. So we have prepared three specimens: 0.5ZnO/gelatin, 1%ZnO/gelatin and 1.5%ZnO/gelatin. These three samples then sonicated again for an hour in order to gain homogenous solution. Blunt needle syringe was used for electrospinning of these three samples and so nano fibers of ZnO/gelatin produced. The conditions of electrospinning are present in table1. All samples were prepared on aluminum sheet.

Table1. Conditions of electrospinning

Voltage (kV)	Pumping rate (ml/h)	Drum speed (rpm)	Distance between needle and collector(cm)
25	0.4	350	15

The morphology of the electrospun nano fibers was investigated by Field Emission Scanning Electron Microscope (FESEM) (MIRA3 TESCAN). AATCC 100-2004 was used in order to study the antibacterial property of produced samples against Staphylococcus Aureus (gram-positive bacteria) and Shigella (gram-negative bacteria).

3. Results and discussion

3.1 Morphology study

As it was mentioned in introduction, the morphology of produced nano fibers was investigated by FESEM. The voltage of the device was 15kV and the magnification was 100kx. Figure1 show the FESEM of prepared nano fiber. As it was shown, NSGZ formed by electrospinning and based on Figure1(a), the diameter of nano scaffolds are about 32nm in average. On the other hands, for proving the existence of nano ZnO, elemental mapping analysis of FESEM image was done and as it was shown in Figure1(b), the nano particles ha, the nano particles have excellent distribution. In other words, the prepared nano scaffold doesn't have any nano ZnO agglomeration. So we come to conclusion that the used method and prepare condition of NSGZ was suitable. Beside, in order to investigate the quantity of used nano particle in final scaffold, XRF analysis was done. In this test, we can investigate the amount of nano particles in inner layers of the scaffold. So, 2.0 g of each sample were prepared, and XRF analysis was done. The result of XRF is presented in table2. As it was demonstrating, the ratio of nano ZnO to weigh of used fabric is the same with connivance. So, the XRF analyses prove the existence of nano particle in the scaffold. It must be mention that the present of Fe_2O_3 in results is due to device holder.

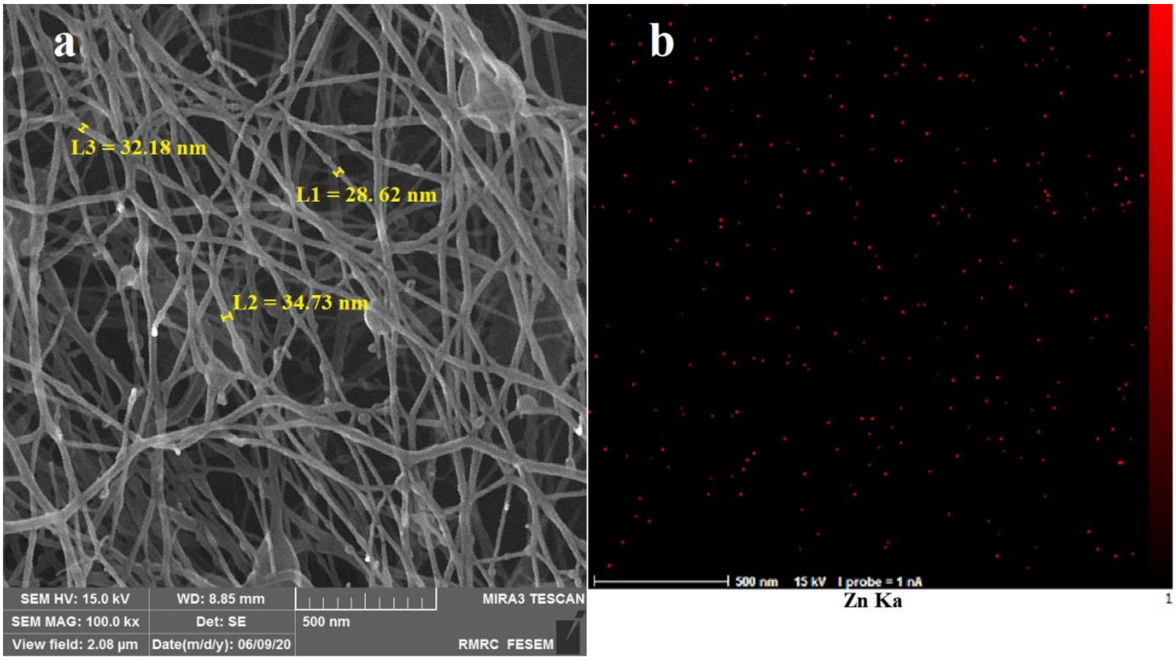


Figure1. FESEM images of (a) NSGZ, (b) elemental mapping of NSGZ

Table2. Specification and XRF quantity result of samples

Sample Code	Specimen	Existing Oxide	wt%	Std.Err.
A	NSG + 0.5% ZnO	ZnO	0.009	0.08
		Fe_2O_3	0.004	0.02
B	NSG + 1.0% ZnO	ZnO	0.018	0.07
		Fe_2O_3	0.005	0.02
C	NSG + 1.5% ZnO	ZnO	0.027	0.08
		Fe_2O_3	0.005	0.01

3.2 Antibacterial property analysis

The two common gram-negative/positive bacteria are Shigella and Staphylococcus Aureus respectively. Wang et al. reported that nano zinc oxide has ability to destroy the gram-negative and gram-positive bacteria[45]. Therefore, in order to study the antibacterial property of NSGZ, Shigella and Staphylococcus Aureus were selected. Figure2 show the results of antibacterial property of NSGZ. As it can see, the anti-bacterial property of nano scaffold which contains 1.5% nano ZnO is 100% for both gram-negative and gram-positive bacteria. Also the results for 1% and 0.5% nano ZnO are more than 96% while the sample contains 0% nano ZnO doesn't have any anti-bacterial property.

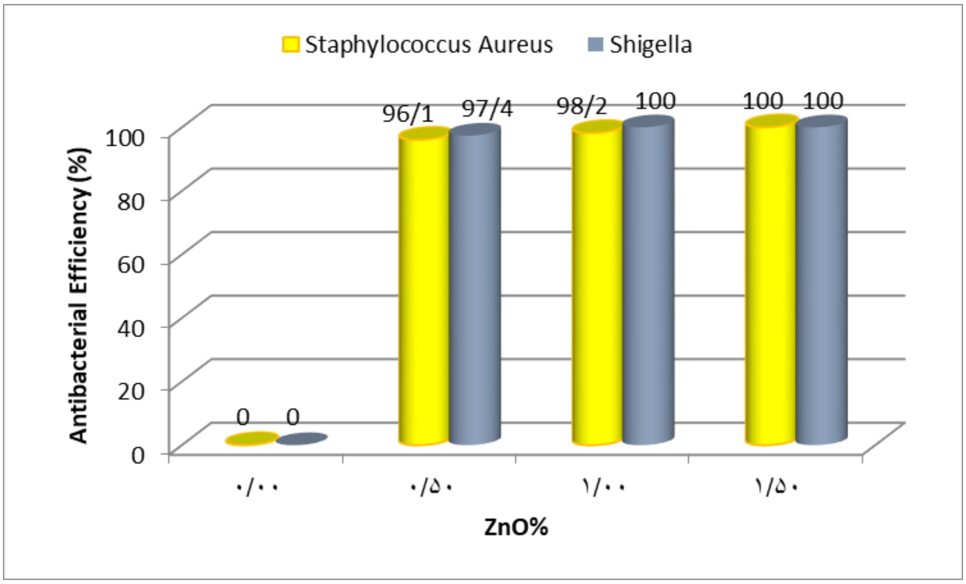
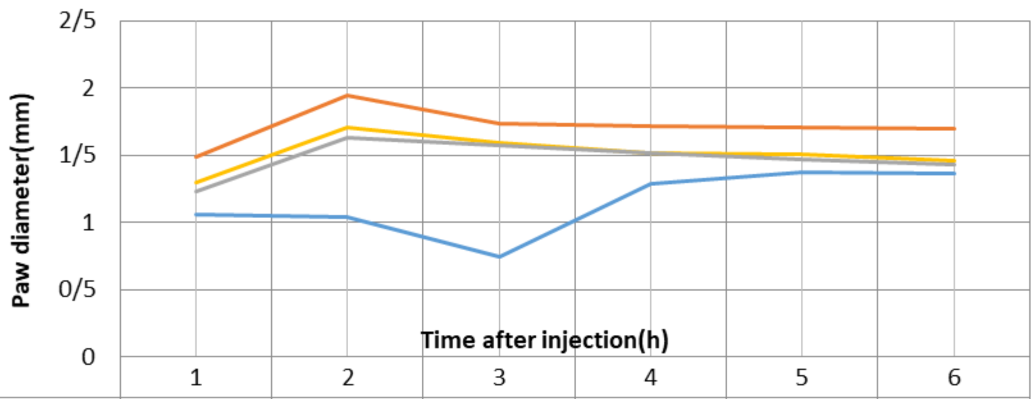


Figure2. Antibacterial property of NSGZ

3.3 Anti-inflammatory analysis

For anti-inflammatory analysis of NSGZ, Wistar rates were used. For each NSGZ sample, three rats were selected and all of the twelve rats were kept in same condition for a day. It must be mention that for this analysis, animal rights laws were performed. Then, the shaved rates were coated with NSGZ (except control sample) and tied firmly. For control sample, the back of one rat was coated with Indomethacin cream (as reference sample). Winter et al. were introduced a novel method for study the inflammatory property in 1962 by injection of carrageenan to the rats[46]. First of all, a digital caliper was used in order to measuring the hind paw diameter and the data was saved. Then, the injection of carrageenan was done and diameter of the hind paw was recorded every hour. After that, the edema diagram of samples prepared and as it was shown in figure3, Indomethacin has rapid result on reduction of edema. For NSGZ samples, after the second hour, the anti-inflammatory effect of NSGZ is demonstrated and in the fifth hour, the anti-inflammatory effect of NSGZ was closed to control sample.



	1	2	3	4	5	6
Control sample	1/061	1/037	0/741	1/288	1/374	1/362
Sample A	1/483	1/941	1/735	1/712	1/709	1/701
Sample B	1/301	1/704	1/589	1/511	1/506	1/457
Sample C	1/234	1/626	1/576	1/514	1/466	1/431

Figure3. Inflammatory diagram of specimens

4.Conclusion

This study reports the fabrication of nano scaffold of gelatin/ZnO (NSGZ) with electrospinning method. Different concentration of nano ZnO was used in NSGZ and the FEEM image and elemental mapping proved the formation of the scaffold in nano scale and the present of nano particles and good distribution of ZnO without agglomeration. Also, XRF analysis confirmed the used amount of nano particle. On the other hands, anti-bacterial property of scaffolds demonstrates that NSGZ has excellent antibacterial property against gram-negative/positive bacteria (Shigella and Staphylococcus Aureus respectively) (more than 96%). The anti-inflammatory property of NSGZ show that Indomethacin has rapid result on reduction of edema but for NSGZ samples, the anti-inflammatory effect appears after the second hour, and after five hours it was closed to control sample. So the nano scaffold of gelatin/ZnO has anti-inflammatory property.

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