(REFEREED RESEARCH)

IN VIVO EVALUATION OF ELECTROSPUN POLY (VINYL ALCOHOL)/SODIUM ALGINATE NANOFIBROUS MAT AS WOUND DRESSING

ELEKTRO ÇEKİM YÖNTEMİ İLE ÜRETİLMİŞ POLİ(VİNİL ALKOL)/SODYUM ALGİNAT NANOLİFLİ YÜZEYİN YARA ÖRTÜSÜ OLARAK IN VIVO DEĞERLENDİRMESİ

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ABSTRACT

In this study, potential of the alginate based electrospun nanofibrous mats as wound dressings was investigated macroscopically in vivo experiments. 2/1 (v/v) %9 PVA / %1 NaAlg nanofibrous mats were produced by electrospinning method. In order to improve water resistance of the electrospun mats, the cross-linking was performed. SEM analyses, thickness and air permeability tests were applied for the nanofibrous mats produced as wound dressing and Suprasorb-A commercial alginate wound dressing. In vivo experiments, the healing performances of the wounds dressed by using cotton gauze, Bactigras, Suprasorb-A and the electrospun PVA/NaAlg nanofibrous mat were macroscopically evaluated and compared. The wound healing potential of the electrospun mat was better than that of cotton gauze including a drug and looked like antibacterial Bactigras. The results indicated that the electrospun PVA/NaAlg nanofibrous mat as wound dressing provided a good means for wound healing.

Key Words: Electrospinning, Nanofiber, Alginate, Wound dressing, In vivo.

ÖZET

Bu çalışmada, elektro çekim yöntemi ile elde edilen alginat nanolifli yüzeylerin yara örtüsü olarak kullanım potansiyeli, gerçekleştirilen in vivo çalışmalarla makroskobik olarak değerlendirilmiştir. 2/1 hacimsel karışım oranında %9 PVA / %1 NaAlg çözeltisinden elektro çekim yöntemi ile nanolifli yüzeyler üretilmiştir. Bu yüzeylerin suya dayanımını geliştirmek için, çapraz bağlama işlemi uygulanmıştır. Yara örtüsü olarak üretilen nanolifli yüzeylere ve ticari alginatlı yara örtüsü olan Suprasorb-A'ya SEM analizleri, kalınlık ve hava geçirgenliği testleri uygulanmıştır. In vivo çalışmalarda, gazlı bez, Bactigras, Suprasorb-A ve elektro çekim ile üretilen PVA/NaAlg nanolifli yüzey ile kapatılmış yaraların yara iyileştirme performansları makroskobik olarak değerlendirilmiş ve kıyaslanmıştır. Elektro çekim yöntemi ile üretilmiş yüzeyin yara iyileştirme potansiyeli; ilaç emdirilmiş gazlı bezden daha iyi, antibakteriyel bir örtü olan Bactigras ile benzer olmuştur. Sonuçlar, PVA/NaAlg nanolifli yüzeylerin yara örtüsü olarak kullanılabilir olduğu öngörüsünü doğrulamıştır.

Anahtar Kelimeler: Elektro çekim, Nanolif, Alginat, Yara örtüsü, In vivo.

Received: 22.06.2010 Accepted: 04.09.2010

1. INTRODUCTION

Electrospinning is a unique fiber spinning technique of producing nanofibers from a large variety of bulk starting materials in a moderately easy, repeatable and simple fashion (1). It is a process that uses an electric field to control the formation and deposition of nanofibers from a liquid polymer. An old technology rediscovered, refined and expanded into nanotextile applications in recent years, electrospinning has attracted great attention as the simplest and least expensive means in producing polymer nanofibers with diameters ranging from microns down to a few nanometers (2-6). Electrospun nanofibrous mats with high specific surface area, high aspect ratio and high porosity as a result of random deposition of fibers, could have a great potential in biomedical applications such as tissue engineering scaffolds, drug delivery carriers, wound dressings etc. (7-8).

Alginate is a natural biopolymer extracted from brown seaweed. Since it was discovered by Stanford in 1881, alginate has been used in a wide range of industries, such as food, textile printing, paper, pharmaceuticals, and for many other novel end-uses. Because of its some characteristics such as biological origin, biodegradability, biocompatibility and gel forming ability, alginate has been extensively reevaluated and used recently as an attractive natural resource in biomedical applications especially in wound management industry. Today, there are more than 10 types of alginate wound dressings, mainly available for the treatment of wounds with a large amount of exudates, on the British Drug Tariff alone (9-12).

When the unique properties of alginate are combined with the exciting nano-effects that nanofibrous mats have to offer, enhanced products can be manufactured. However, electrospinning of alginate is still a challenge because of its polyelectrolyte characteristics and insufficient chain entanglement. Therefore, pure alginate can not be electrospun into nanofibers. The electrospinability of alginate may be improved by blending its aqueous solution with appropriate nontoxic, biocompatible synthetic polymers such as poly(ethylene oxide) (PEO) or poly(vinyl alcohol) (PVA) [10, 13]. There are only a few studies about electrospinning of alginate in literature.

Safi et al. (4) investigated the electrospinning of sodium alginate/PVA and sodium alginate/PEO solutions with different concentrations and ratios and performed scanning electron microscope and fourier transform infrared analyses on the mats. Lu et al. (7) measured the solution properties of sodium alginate/PEO blends with different ratios and investigated the morphology and mechanical properties of the electrospun mats of these solutions. Nie et al. (10) produced pure sodium alginate electrospun mats by using glycerol as a co-solvent. They studied the electrospinnability and the physical properties of the sodium alginate/water/glycerol system. Lee et al. (14) prepared nanowebs from different blend solutions of sodium alginate and PVA by electrospinning and investigated their structure and morphology through a series of instrumental analyses. Bhattarai and Zhang (15) explored the influence of physical and chemical properties of sodium alginate/PEO solutions with different ratios on the structures of electrospun products and compared several cross-linking methods for the alginate-based nanofibers. Moon et al. (16) studied the properties of sodium alginate/PEO blend solutions by changing concentrations and volume ratios. Then, they observed the morphology, thermal and mechanical properties of the electrospun nanofibers.

The main functions of wound dressings are to facilitate wound healing and minimize scarring. They provide a physical barrier to protect the wound from further physical damages and any contaminations of exogenous organisms. They should also be permeable to moisture and air, and allow the extraction of extra body fluid from the wound area to maintain a partially immobilized moist environment. The electrospun mats usually have pores which are small enough to prevent bacterial penetration. Also, their high surface area is of importance for fluid absorption and dermal drug delivery (17, 18). Advantageous of electrospun nanofibrous mats as wound dressing are indicated at studies focused on some natural and synthetic polymers such as collagen (19), chitosan (20, 21), poly(caprolactone)/gelatin (22), PVA/silver (23), silk fibroin (24), polyurethane (25). However, any study about electrospun alginate nanofibers as wound dressing and their experiments *in vivo* has not been coincided during literature research.

In this study, aqueous poly(vinyl alcohol) (PVA) and sodium alginate (NaAlg) solutions were blended in proper volume ratio and were electrospun under constant conditions. Electrospun PVA/NaAlg nanofibrous mats were used as wound dressing *in vivo* experiments and their wound healing performances were macroscopically compared with some commercial wound dressings.

2. MATERIALS AND METHODS

2.1. Materials

The NaAlg polymer $(NaC_6H_7O_6)_n$ used for production of nanofibrous mats had viscosity of 700-900 cp and was a commercial product, Cecalgum® S1300, purchased from Cargill (Turkey). In order to overcome poor electro-spinnability of sodium alginate, the PVA polymer $(CH_2CHOH)_n$ with Mw 31.000-50.000 g/mol was obtained from Sigma-Aldrich (USA). Distilled water was used as the solvent.

The electrospun mats were cross-linked by immersing in 37% technical hydrochloride acid (HCl) (Merck) and 50% biological grade glutaraldehyde ($OHC-C_3H_6-CHO$) (EMS) to improve their water resistance. Acetone (CH_3COCH_3) (Merck) with Mw 58.08 g/mol was used as solvent in cross-linking solution. The cross-linked mats were then washed at two stages thoroughly with 99.5% ethyl alcohol (C_2H_5OH) (Merck) and phosphate tampon (Euroimmun), respectively.

In the experiments in vivo conditions, 5 male New Zealand rabbits those were 3 months in age, 2-2.5 kg. in weight, white in color obtained from Uludag University Medical Faculty Experimental Animals Breeding and Research Center have been used. Turkish national regulations for the care and use of laboratory animals have been observed. The necessary permission for animal use was taken from the Center (dated 20.05.2008, no. 8/2).

In vivo experiments, three commercially available wound dressings, woven cotton gauze (Eczacibasi) impregnated with furacin pomade, woven cotton antibacterial Bactigras (Smith & Nephew) containing paraffin and chlorhexidin, and nonwoven Suprasorb-A (Lohmann) made from calcium alginate fibers, were used to compare with PVA/NaAlg nanofibrous wound dressing produced.

2.2. Preparation of the electrospinning solutions

The blend ratios in volume and the concentrations of polymer solutions used in this study were chosen by considering the results of the previous study (26). According to this, NaAlg powder was dissolved in distilled water by mixing on magnetic stirrer at 50°C temperature for 6 h. and the NaAlg solution with concentration of 1% (w/v) was prepared. PVA powder was dissolved in distilled water by mixing on magnetic stirrer at 80°C temperature for 6 h. and the PVA solution with concentration of 9% (w/v) was prepared. The solutions were matured at room temperature for 12 h.

Table 1. The properties of the solution

Density (g/cm^3)	Viscosity (cp)	Electrical conductivity (μs/cm)	pH	Surface tension (mN/m)
1.0155	476.4	1200	6.82	53.42

PVA solution with concentration of 9% and NaAlg solution with concentration of 1% were blended in the volume ratio of 2/1 PVA/NaAlg. This blend was stirred for 3 h. to obtain a homogeneous solution and was waited at room temperature for 24 h. prior to electrospinning. The properties of this solution are given in Table 1.

2.3. Electrospinning

The productions of electrospun mats in this study were carried out on the electrospinning apparatus at Uludag University Textile Engineering Lab. The electrospinning apparatus was consisted of a high voltage power supply, an infusion pump with syringe and a collector, enclosed in a chamber. The high voltage power supply (Matsusada AU-50*1.2 DC, Japan) was used to generate the electric field of 10-50 kV. The infusion pump (TOP 5300, Japan) with syringe was used to control constant mass flow of the polymer solution during the electrospinning. The polymer solution was fed into the syringe fitted with a brass spinneret. A circular aluminum collector (20 cm dia.) covered with aluminum foil was preferred for formation of dried polymer jet deposited as a fibrous mat. As shown in Figure 1, the collector was located over against to the spinneret to prevent the solution dripping on the mat during electrospinning. All the spinning experiments were performed at ambient conditions.

The process parameters for the electrospinning of 2/1 9% PVA / 1% NaAlg solution, which were chosen after preliminary experiments, are demonstrated in Table 2.

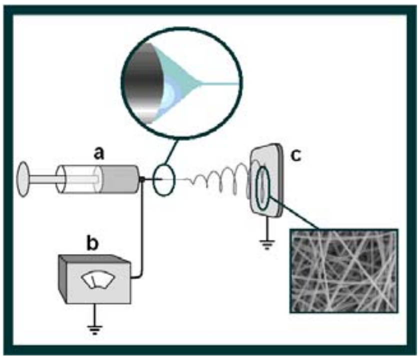

Figure 1. Schematic diagram of the electro-spinning process (27)
[(a) Polymer solution, (b) High voltage power supply, (c) Collector]

Table 2. The process parameters of the electrospun mats

Voltage	20 kV
Flow rate of the solution	1.2 mL/h
Tip-to-collector distance	17 cm
Spinneret inner diameter	830 µm
Amount of the solution	60 mL

2.4. Cross-linking and Sterilization

Water has a solvent effect on both polymers because of hydroxyl groups in their structure. So, the water resistance of the PVA/ NaAlg nanofibrous mats produced from these polymers was fairly low. Therefore, in order to improve the water resistance of the mates, cross-linking process was required for application of electrospun mats on experiment animals.

To stabilize the PVA/NaAlg nanofibers, electrospun mats were cross-linked by immersing in acetone solution with 0.15 M glutaraldehyde (50%) and 0.05 M hydrochloric acid (37%) at room temperature for 24 h. The cross-linked nanofibrous mats were neutralized in ethyl alcohol (99.5%) for 5 min. The cross-linked and neutralized mats were washed with phosphate tampon solution (1%) three times and then dried in an oven at 50^{o}C temperature for 1 h. To investigate the anti-water property of the cross-linked electrospun nanofibrous mats, they were dipped in distilled water at room temperature for 24 h.

Cross-linked electrospun PVA/NaAlg mats were sterilized with ethylene oxide gas for 4 h. and aerated for 8 h. at Uludag University Medical Faculty prior to in vivo experiments.

2.5. Characterization

JEOL 840 Scanning Electron Microscope (SEM) was used for analyses of electrospun PVA/NaAlg nanofibrous mats, measurement of fiber diameters and taking photographs. The mean and standard deviation values were calculated by measuring ten nanofiber diameters based on SEM images. Also, cross-linked nanofibrous mats immersed in water for 24 h. were investigated and photographed on SEM to ascertain whether fibrous structure was deformed.

Thickness and air permeability properties of the cross-linked electrospun mats purposed as wound dressing and Suprasorb-A commercial wound dressing were determined and compared. The thickness analyses of electrospun mats and Suprasorb-A were carried out on L&WBK Thickness Gauge for film and plastics and on DM100 Thickness Gauge for textiles, in respectively, according to TS 7128 EN ISO 5084 (28). For both of sample, the test was repeated ten times. The air permeability tests of electrospun mats and Suprasorb-A were performed on Textest F3300 Air Permeability Tester according to TS 391 EN ISO 9237 (29). The pressure differential was set to 200 Pa. and a 20 cm^2 orifice size was used. The test was repeated three times. The means and standard deviations of data were determined.

2.6. *In vivo* experiments

In vivo experiments purposed assessment of the electrospun alginate-based nanofibrous mats as wound dressing and comparison with commercially available wound dressings produced with conventional methods were performed in Medical Faculty Experimental Animals Breeding and Research Center at Uludag University. Twenty five rabbits were used for macroscopically evaluation.

The rabbits were anesthetized with 30-50 mg/kg intraperitoneal Ketalar (Pfizer) and 10 mg/kg intramuscular Rompun (Bayer) injections. The hairs of the incision area were shaved and sterilized with Baticon. The back and lumbar regions were cut with lancet and four full-thickness experimental wounds with a surface area of 2x2 cm^2 were excised for each rabbit. The wounds were dressed with an equal size of gauze (G), Bactigras (B), Suprasorb-A (S) and electrospun PVA/NaAlg nanofibrous mat (N). The entire surgical site was covered with a sponge and cotton gauze as a second layer. The sponge was fixed on the skin with staplers (Figure 2). Then, the rabbits were housed individually in cages by numbering, and fed with water and laboratory feed during the observation.

Wound healing efficiencies of the wound dressings used in this study were evaluated macroscopically. For this purpose, the original wound areas were identified by measuring the wound dimensions with a ruler on the application day (day 0). On the 4th, 6th, 12th, 15th and 21st postoperative days, measurements of the wound dimensions were continued. For every assessment day, the measurement was repeated five times and the percent wound contractions were calculated according to Equation 1 and the results were considered for comparison of the wound dressings.

$$\% K = 100 - [(A_n / A_0) \times 100] \qquad (1)$$

Where;

K: Wound contraction

A_0: Original wound area (day 0)

A_n: Wound area on the assessment day

The means and standard deviations of wound contractions for every wound dressing and every assessment day were determined. Furthermore, the percent wound contraction results were evaluated according to one-way variance analysis (ANOVA) at significance level of 0.05 by using a statistical package program, separately for every assessment day.

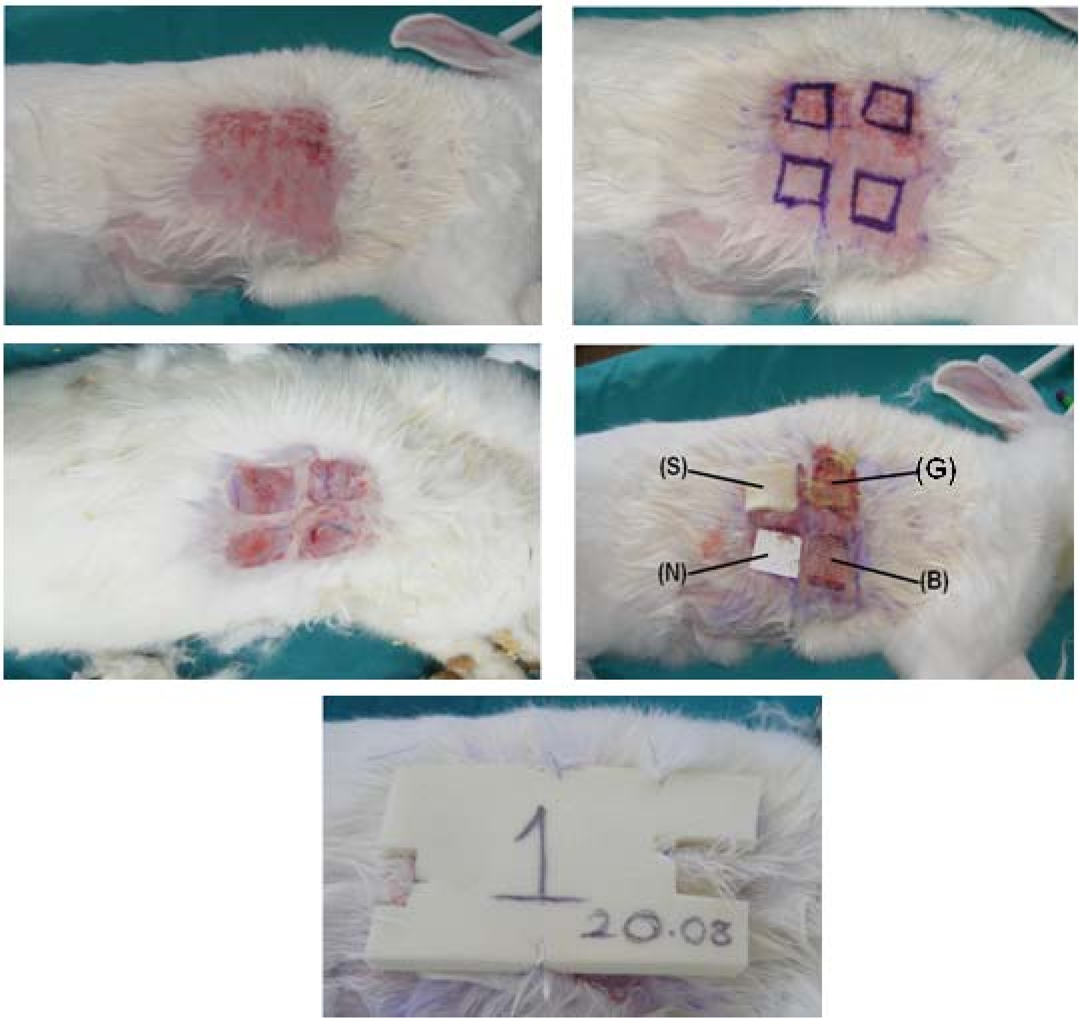

Figure 2. Formation of the wounds on rabbits and application of the wound dressings

3. RESULTS AND DISCUSSION

3.1. SEM analysis

Bead defects which are often encountered during electrospinning were not consisted on the electrospun mats from 2/1 9% PVA / 1% NaAlg solution. Uniform and continuous fiber formations were observed (Figure 3). Fiber diameter was 100.35 ± 12.79 nm.

After the nanofibrous mats consisted of PVA and NaAlg polymers which are soluble in water and any hydrated solution were cross-linked, a water resistance test was carried out (Figure 4). Results showed that the mats became completely resistant to water. As it was seen from SEM image (Figure 5) of nanofibrous mat immersed in water for 24 h., there was not any deformation on nanofibrous structure.

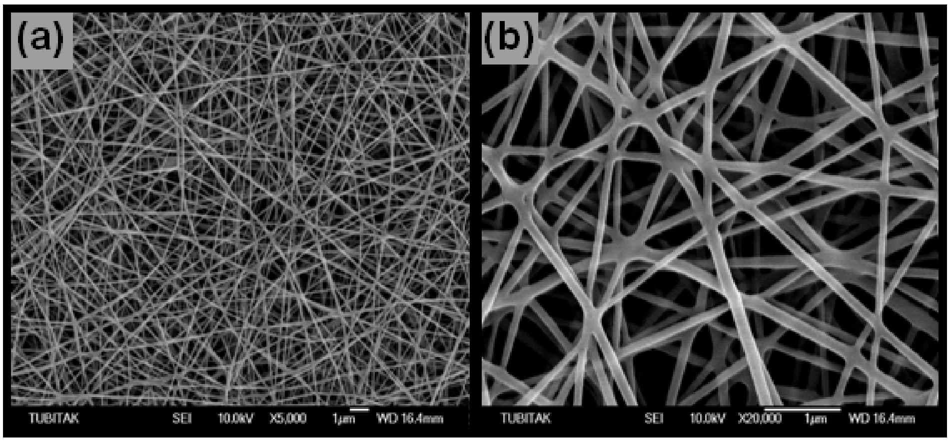

Figure 3. SEM photographs of the nanofibrous mat [(a) X5000 (b) X20.000]

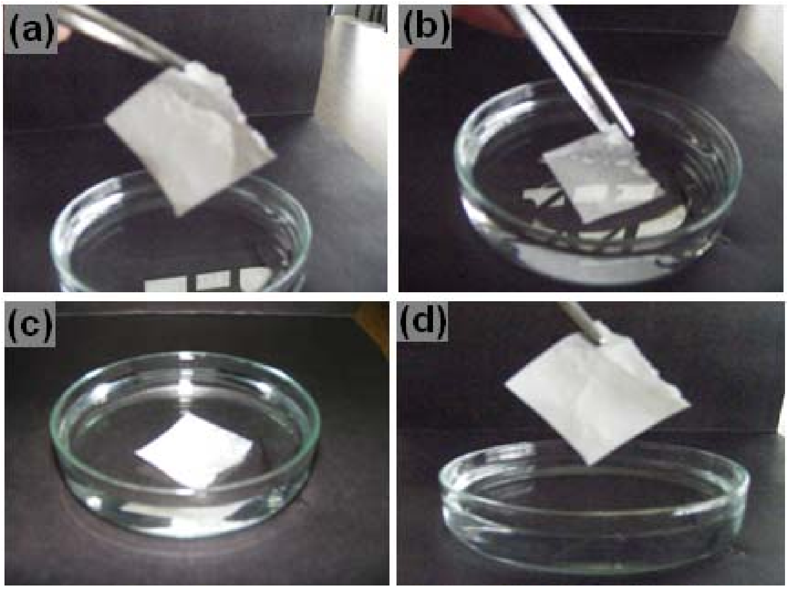

Figure 4. Water resistance test of the cross-linked nanofibrous mat [(a) Initial, (b) 1st s., (c) 3rd h. (d) After 24 h.]

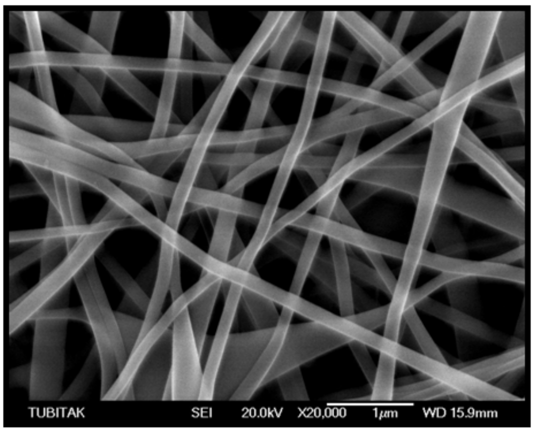

Figure 5. SEM image of the cross-linked nanofibrous mat immersed in water for 24 h. [X20.000]

3.2. Thickness analysis

The results of thickness measurement for the electrospun mat and Suprasorb-A are given in Table 3.

Table 3. Results of the thickness test (mean ± sd)

Material	Thickness (mm)
Cross-linked nanofibrous mat	0.31 ± 0.08
Suprasorb-A	1.63 ± 0.09

According to this, Suprasorb-A formed with nonwoven technique was approximately five times more thick than cross-linked nanofibrous mat produced in this study. The thickness difference of these two mats is clearly seen from photograph in Figure 6. The fineness which is one of the advantageous of nanofibrous mats is an important choice criteria for its usage as wound dressing.

Figure 6. PVA/NaAlg nanofibrous mat and Suprasorb-A

3.3. Air permeability test

The results of the air permeability test are given in Table 4.

Table 4. Results of the air permeability test (mean ± sd)

Material	Air permeability ($m^3/m^2/min$)
Cross-linked nanofibrous mat	0.14 ± 0.02
Suprasorb-A	144.00 ± 12.00

It was seen from the results that cross-linked nanofibrous mat had approximately 1000 times lower air permeability value than Suprasorb-A. When it was considered that the pore dimensions of microfibrous Suprasorb-A were highly greater than those of nanofibrous mat (Figure 7), the difference in values of permeability to air was an expected result.

The membrane porosity and permeability to air and moisture were important aspects affecting the healing process. The finer the fibers, the smaller the pores. A high porosity led to high membrane permeability to air/moisture. When the membrane thickness increases, air passage from the pores will get difficult due to rise of friction and pressure loss (17, 30, 31). Therefore, air permeability value of the electrospun mat produced in this study was lower than expectation value because of the process parameters.

So, PVA/NaAlg nanofibrous mats due to its pores in nano size not only prevented the passing of bacteria into the wound but also were permeable to air, when they were used as wound dressing. This property could be the important criteria for a successful wound dressing.

3.4. Wound healing test

The mean percentages of wound contractions are listed in Table 5. Higher percentage indicates a higher healing efficiency. The healing performances of wound dressings are compared via graphics in Figure 8. Also, the photographs of wounds on the assessment days are presented in Figure 9.

As it was seen from Table 5, Figure 8 and 9, the wound healing performance for all of the wound dressings was above of 90% from 12th day forward and the wounds were entirely closed at the end of 21 days. Especially on the 4th day, the most rapid wound healing belonged to the wound area dressed with Suprasorb-A (S) following by Bactigras (B) and PVA/NaAlg nanofibrous mat (N), respectively. The most slow wound healing was also eventuated on the wound area dressed with gauze (G).

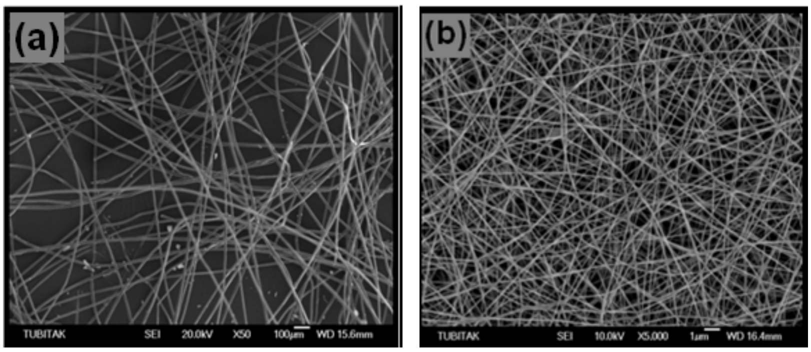

Figure 7. (a) Suprasorb-A wound dressing [X50], (b) PVA/NaAlg nanofibrous mat [X5000]

Table 5. The percent wound contractions (mean ± sd)

% K	4th day	6th day	12th day	15th day	21st day
$\%K_G$	33,50 ± 19,27	50,20 ± 8,84	93,70 ± 2,34	97,00 ± 2,43	100,00
$\%K_B$	51,20 ± 15,61	61,10 ± 2,42	94,70 ± 2,41	98,70 ± 1,80	100,00
$\%K_S$	60,90 ± 7,82	62,90 ± 9,58	95,20 ± 0,80	99,20 ± 1,20	100,00
$\%K_N$	55,50 ± 12,60	60,50 ± 17,33	93,80 ± 1,96	98,00 ± 1,75	100,00

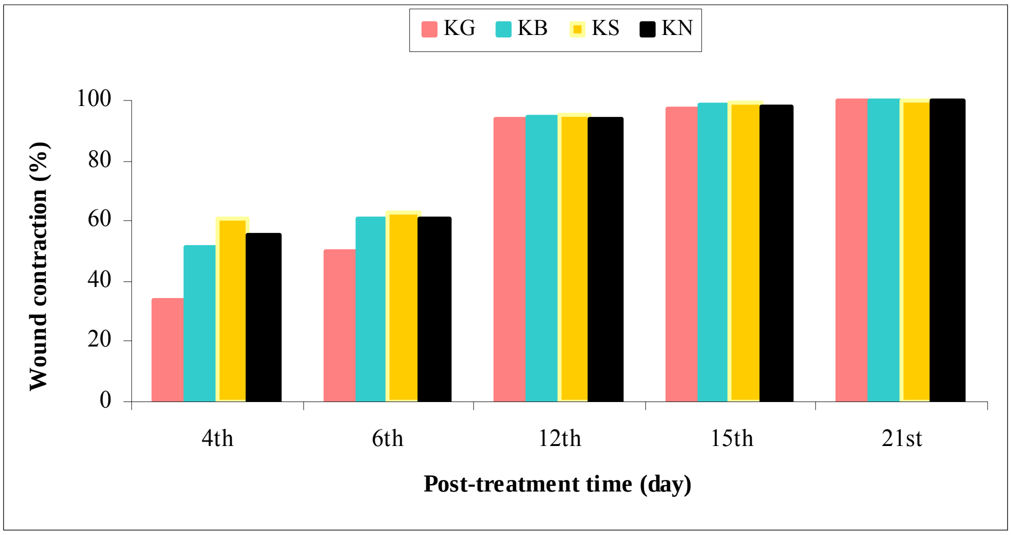

Figure 8. Comparison of the percent wound contraction for the wound dressings

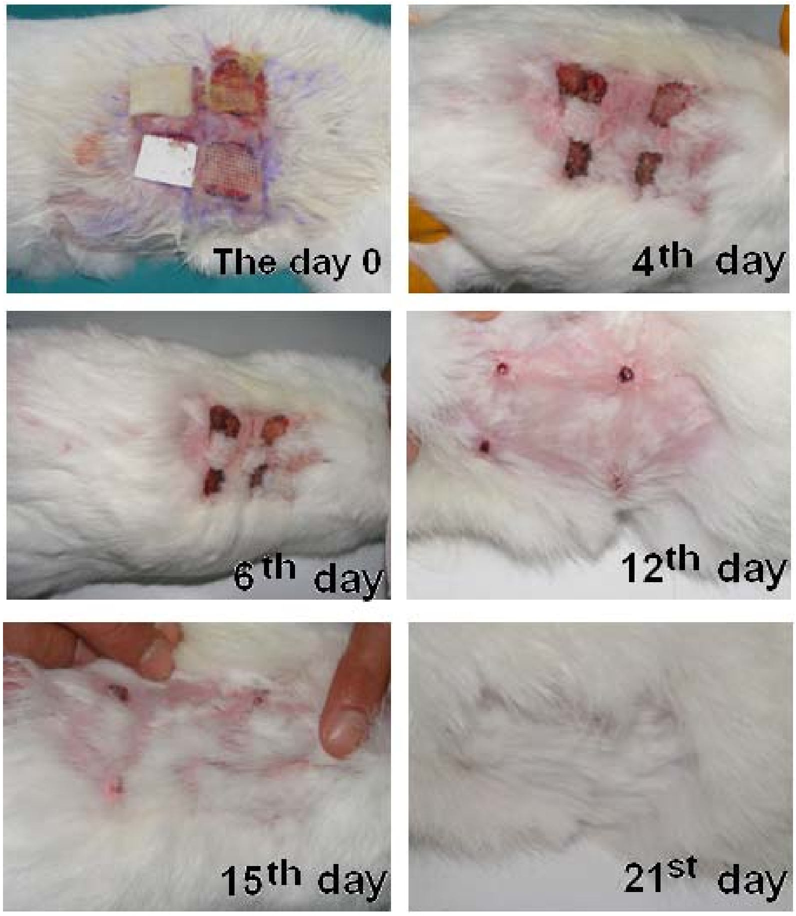

Figure 9. Views of the wounds on post-treatment days

Acceleration of the wound contraction for Suprasorb-A resulted from exchanging of calcium ions in calcium alginate with sodium ions in the body fluid and turning itself into a gel which helps keep a moist interface on the wound surface (11). The wound healing potential of electrospun PVA/NaAlg nanofibrous mat was better than that of gauze including a drug and looked like antibacterial Bactigras. The results indicated that the electrospun PVA/NaAlg mat as wound dressing provided a good means for wound healing.

When the statistical analyses results in Table 6 were also considered, the percent wound contractions for the wound dressings were not statistically different (P>0.05) from each other for every assessment day. This outcome was also evidenced that the electrospun PVA/NaAlg nanofibrous mat had a wound healing performance like the commercial wound dressings.

Table 6. Results of the statistical analysis

Post-treatment days	F value	P value (Significance)
4	2.0207837050	0.1896 (non-significant)
6	0.8325471698	0.5126 (non-significant)
12	0.3840625661	0.7675 (non-significant)
15	2.0203703704	0.1897 (non-significant)

4. CONCLUSIONS

In this study, macroscopically investigation of potential of the electrospun 2/1 %9 PVA / %1 NaAlg nanofibrous mats as wound dressing *in vivo* was purposed.

In order to improve water resistance of nanofibrous mat electrospun from PVA and NaAlg polymers which are soluble in water, the cross-linking was performed. The SEM analysis indicated that there were not any deformation and diameter change in nanofibrous structure after cross-linking process.

The thickness and air permeability tests were applied for electrospun PVA/NaAlg nanofibrous mat produced as wound dressing and Suprasorb-A commercial alginate wound dressing. Suprasorb-A was five times thicker than nanofibrous mat. The air permeability of nanofibrous mat was considerably lower than that of Suprasorb-A because of differences in their pore dimensions.

In vivo experiments, the healing performances of the wounds dressed by using gauze, Bactigras, Suprasorb-A and electrospun PVA/NaAlg nanofibrous mat were macroscopically evaluated and compared. It was concluded that the earliest and the latest wound contractions were obtained on the wounds dressed with Suprasorb-A and gauze, respectively. It is noteworthy that the performance of the electrospun PVA/NaAlg mat as wound dressing was higher than the performance of antibacterial Bactigras and gauze impregnated with a drug healing wound. For future studies, it is suggested that the electrospun PVA/NaAlg nanofibrous mats aided antibacterial agents, silver nano-particules or drugs may produce for improvement of their performance as wound dressing. A paper in which wound healing performance of the PVA/NaAlg nanofibrous mats will be histological evaluated in vivo will be published.

ACKNOWLEDGEMENT

The authors thank to TUBİTAK (The Scientific and Technical Research Council of Turkey) for its financial support in the research project (No. 108M396)

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Bu araştırma, Bilim Kurulumuz tarafından incelendikten sonra, oylama ile saptanan iki hakemin görüşüne sunulmuştur. Her iki hakem yaptıkları incelemeler sonucunda araştırmanın bilimselliği ve sunumu olarak ***"Hakem Onaylı Araştırma"*** *vasfıyla yayımlanabileceğine karar vermişlerdir.*