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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

Antibacterial Characteristics of Nanofiber Structures Obtained by Benzalkonium Chloride Additive Poly (Vinyl Alcohol)/Gelatin

Benzalkonyum Klorür Katkili Pva/Gelatin Nanolif Yapilarinin Antibakteriyel Özellikleri

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Abstract

Polyvinyl alcohol (PVA) is a hydrophilous and semi-crystallized. It has attracted much relevancy due to its pretty chemical endurance, fine thermal determination, decent physical specialities, wonderful bio-compatibility and cheapness. Gelatin (G) is a natural polymer and that are interesting materials for biomedical applications. Electrospinning is a simple method that provides very porous nanofiber production with high surface area. It is possible to produce biomedical, filtration, energy storage and protective materials by using electrospinning method. Benzalkonium chloride (BAC) is a kind of anti-microbial cationic surface-active agent, which has been pretty used in merchant wound dressings and has a powerful status toward Gr+ bacteria.

In this study, nanofibers were produced from electrospinning of BAC, PVA and G containing solutions at various concentrations. The fibers of obtained nanofiber structure were uniform, continuous and intensive. The optimum parameters in terms of good mechanical and antibacterial properties were determined. S2 sample, electrospun from 11.63% PVA + 1.0% G +1.0% BAC containing solution, had the best morphological and mechanical properties due to having the thinnest fiber diameter (51±13nm) and the highest vertical strength (4.299MPa) and horizontal strength (4.058MPa). It also had antibacterial activity against all the bacteria tested (*E. coli, P. aeruginosa, B. subtilis and S. aureus*). Due to owning good mechanical and antibacterial properties, S2 sample can have many uses in medical sector.

Keywords: Electrospinning, poly(vinyl alcohol), gelatin, benzalkonium chloride, antibacterial, nanofiber

Öz

Poli (vinil alkol) (PVA), iyi kimyasal dayanımı, iyi termal kararlılığı, iyi fiziksel özellikleri, mükemmel biyouyumluluğu ve ucuzluğu nedeniyle çok dikkat çeken hidrofilik, yarı kristalli bir polimerdir. Jelatin (G) biyomedikal uygulamalar için ilginç ve doğal bir polimerdir. Elektroeğirme, yüksek yüzey alanına sahip çok gözenekli nano elyaf üretimi sağlayan basit bir yöntemdir. Elektrospinning yöntemi kullanılarak biyomedikal, filtrasyon, enerji depolama ve koruyucu malzemeler üretmek mümkündür. Benzalkonyum klorür (BAC), ticari yara sargısında yaygın olarak kullanılan ve Gram-pozitif bakterilere karşı güçlü bir role sahip olan bir tür antimikrobiyal katyonik yüzey aktif malzemedir. Bu çalışmada, çeşitli konsantrasyonlarda BAC, PVA ve G içeren çözeltilerin nano lifler üretilmiştir. Elde edilen nano lif yapısının lifleri homojen, sürekli ve yoğundur. İyi mekanik ve antibakteriyel özellikler açısından optimum parametreler belirlenmiştir. 11.63% PVA + 1.0% G +1.0% BAC içeren S2 örneği en iyi morfolojik ve mekanik özellikler göstermesinin yanında en ince elyaf çapına (51 ± 13nm) ve en yüksek dikey dayanıma (4.299MPa) ve yatay dayanıma (4.058MPa) sahiptir. Ayrıca test edilen tüm bakterilere (E. coli, P. aeruginosa, B. subtilis ve S. aureus) karşı antibakteriyel aktiviteye sahiptir. İyi mekanik ve antibakteriyel özelliklere sahip olması nedeniyle, S2 örneğinin tıbbi sektörde birçok kullanımı olabileceği sonucuna ulaşılmıştır.

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Anahtar Kelimeler: Elektroeğirme, PolivinilAlkol, PVA, Benzalkonyum Klorür, BAC, Antibakteriyel, Nanolif

I. INTRODUCTION

Electrospinning is a basic technique that dissolved materials is processed into nano-scale and micro-scale continuous fibers [1]. A typical electrospinning comprise of mainly three components: a capillary tube with pipette or needle of small diameter, a high voltage supplier and a metal collecting screen. There are four different regions within electrospinning process: the base region, the jet region, the splay region, the collector region [2]. The various synthetic polymers, natural polymers and a blend of both including proteins are used in the electrospinning process [3].

Polyvinyl alcohol (PVA) is a hydrophilous and semi-crystallized. It has attracted much relevancy due to its pretty chemical endurance, fine thermal determination, good physical specialities, wonderful bio-compatibility and cheapness. When diameter sizes of polymeric fibers reduce from micro-meter up to nano-meter variety. It has appeared several amazing properties. For example high surface area per bulk or per gob ratio, size probability for area operationalization and enhanced mechanical productivity due to a development in the constructional organization. These amazing specialities make superfine electrospinning polymeric fibers great applicants for many significant implementation, such as filtration, consolidating materials, wound dress, tissue scaffold, releasing of drug, etc [4].

Gelatin (G) is a connatural polymer. It can be popularly found in muscle, skin and bone of animal. Therefore, it has bio-degradability and bio-compatibility qualities. Gelatin has been jointly used in biomedical implementations. For example, wound healing and tissue scaffolds [5]. These apparent advantages of gelatin make it a goal constituent to enhance protein based constructs with similar mechanical strength to extracellular matrix (ECM) [6]. Also, gelatin is a promising choice for generation nano fiber due to it is inexpensive and present [5].

Benzalkonium chloride is a kind of anti-microbial cationic surface-active agent belonging to quaternacy ammonium compounds (QACs) with long alkyl chains of C_8 to C_{18}. It has antimicrobial activity against many microorganisms especially Gram-positive bacteria [7]. Due to its high antimicrobial activity, it is used extensively as biocides at hospitals and food procedure industries, and personal care products. Also, it is used at wound dressings in medical textile industries. Moreover, it is also environmentally friendly. During the waste-water treatment procedures, most BACs are generally removed by bio-degradation in assembly with adsorption on sewerage trash and the rest is drained in the effluence [8].

There are lots of studies about antibacterial activity of BAC containing membranes produced via electrospinning. For example; You et al. (2006) attached BAC to a solution of poly Lactic-co-Glycolic Acid (PLGA) for electrospun and obtained several diameters of BAC-PLGA nano fibers [9]. Wang et al. (2011) dissolved BAC in a polymer solution of poly Hydroxybutyrate – co-Hydroxyvalerate to enhance the conductivity at the electrospinning procedure [10]. Kim et al. (2007) prepared anti-microbial polycarbonate (PC) nano fibers using BAC as an anti-microbial spy and they stated that the BAC-PC nano fibers had fine anti-microbial activity toward Gr+ (S. aureus) and Gr – (E. coli and K. pneumonia) bacteria [11]. Electrospinning BAC – PVA nano fibers have been beforehand equiped by Arumugam et al. (2009), who put to use BAC as a conductive ingredient to a solution of PVA in electrospinning [12].

Addition of gelatin in PVA/BAC containing elecrospun membrane may add versatile properties of gelatin especially in terms of biocompability. There is no studies on production and anti-microbial activity of electrospinning BAC-PVA-G nanofibers. This study aims production of electrospun BAC-PVA-G nanofibers and optimization of the process in terms of mechanical and antibacterial properties.

II. MATERIALS AND METHODS

2.1 Materials

Polyvinyl alcohol (PVA) with molecular weight of 70,000 $gmol^{-1}$ and degree of hydrolysis of 85%, was supplied from Merck. Gelatin powder (bovine gelatin, 250 Bloom) was supplied from Alfasol. Alkyldimethylbenzylammonium chloride (benzalkonium chloride) (50%) was purchased from Kimetsan. The chemicals were used as received.

Main cultures of Gr – bacteria (*Es. coli* ATCC 35218 and *Ps. aeruginosa* ATCC 27853) and Gr+ bacteria (*Ba. subtilis* ATCC 6633 and *St. aureus* ATCC 25293) were supplied from Microbiologics.

2.2. Methods

2.2.1. Preparation of PVA/G/BAC electrospinning solution

Aqueous 12% (w/w) PVA solution was prepared by gently stirring for 2 hours at 70ºC. After formation of homogenous solution, gelatin powder was added, and the resulting mixture was stirred at 70ºC for 2 hours to make homogenous solution. Finally, various amount of BAC was added to produce

the spinning solutions. The final concentrations of the electrospinning solutions were listed at Table 1.

Table 1: The final concentrations of the sample solutions for electrospinning. The concentrations were adjusted to 100% with distilled water.

Sample code	PVA (%)	Benzalkonium chloride (%)	Gelatin (%)
S1	11.75	0.5	1.0
S2	11.63	1.0	1.0
S3	11.27	2.5	1.0
S4	11.03	3.5	1.0
S5	10.79	4.5	1.0

2.2.3. Electrospinning

A 10 ml plastic syringe was filled with PVA/G/BAC solution for electrospinning. Inovenso NE300 Nanospinner model electrospinning device was used to produce nanofibers. A high voltage power was applied to generate the high electric field between the nozzle and the cylindrical collector. Cylindrical collector surface was covered with grease-proof paper. The set parameters of electrospinning process were listed at Table 2.

Table 2: Electrospinning parameter

Parameter	S1	S2	S3	S4	S5
Applied Voltage (kV)*	36	36	39	30	27
Feeding Speed (ml/h)	1	1	1	0.7	0.7
Velocity of cylindrical rotating collector (rpm)	100	100	100	100	100

* The applied voltage was adjusted according to the solution conductivity.

2.2.4. Viscosity and conductivity of solutions

Viscosities of the polymer solutions were determined by using viscometer (Brookfield DV-E Viscometer, USA) with S21 spindle at 50rpm and 60rpm. Conductivities of the polymer solutions were gauged by conductivity meter (WTW Cond 3110, Germany). All experiments were fulfiled out at room temperature.

2.2.5. Morphology of nanofiber structure

The morphologies of the electrospinning nanofibers were analyzed with SEM images (JEOL JSM-5910 LV, Japan). The average fiber diameters of the PVA/G/BAC nanofibers were measured by Image J software from the SEM images.

2.2.6. Mechanical test

All nanofiber membranes were cut to 50x10mm (length x width) for the mechanical test. Instron 4411 universal test device was used to examine the mechanical properties of nanofiber membranes. The piston speed was set 30mm/min.

The thicknesses of nanofiber membranes were measured with a Mitutoyo digital thickness gauge.

2.2.7. Antibacterial activity

ISO 20645:2004 method was simulated to specify the anti-bacterial activity of electrospun mats [13]. The bacterial strains preserved at 80ºC were pre-cultured in 10ml liquid medium in waging incubator at 37±1ºC for 16h. The liquid media were Nutrient Broth for *E. coli* and *B. subtilis* bacteria, and Trypticase Soy Broth for *S. aureus* and *P. aeruginosa* bacteria. 10µl of the pre-cultures were vaccinated into the requested capacity of fresh liquid media. Proximate CFU numbers were guessed from McFarland densitometer measurement and the bacterial culture was generated by incubating at 37±1ºC till the bacteria concentration arrived 10^8 CFU/ml [14].

To test anti-bacterial activities of the mats, soft agar plaques were made by adding 7.5g/L Agar into the broth media described above. After cooling to 40-45 ºC, the bacterial culture was added such a way that bacterial concentration reaches to 10^6 CFU/ml, which was adjusted via McFarland measurement. After gelation of the agar mediums, each mat to be tested was cut in 20mm x 20mm size and was placed on the soft agar medium. The petri plates were incubated at 37±1ºC for 24 hours. For accuracy, the tests were executed and rehearsed three times. Then, the inhibition zones of S1-S5 samples were compared with each other [15].

III. RESULTS

3.1. Viscosity And Conductivity Of Solutions

Table 3 shows viscosity and conductivity of all solutions.

Table 3: Viscosity and conductivity of the solutions

Number	Viscosity values of solutions (cP)	Conductivity values of solutions (mS/cm)
S1	322	1645
S2	348	1935
S3	414*	3000
S4	344	3690
S5	616	4570

*Measured at 60rpm, while the other samples were measured at 50rpm.

As shown at Table 3, solution viscosities generally increased in parallel to increase in BAC concentration and the highest viscosity was observed at the most BAC including sample. Increase in BAC concentration increased the conductivities of the solution and the highest conductivity was observed at the most BAC including sample. Increase in the conductivity made it necessary to reduce the applied voltage at S4 and S5.

3.2. Morphology of Nanofiber Membrane

The morphologies of the electrospinning nanofibers were analyzed with SEM images.

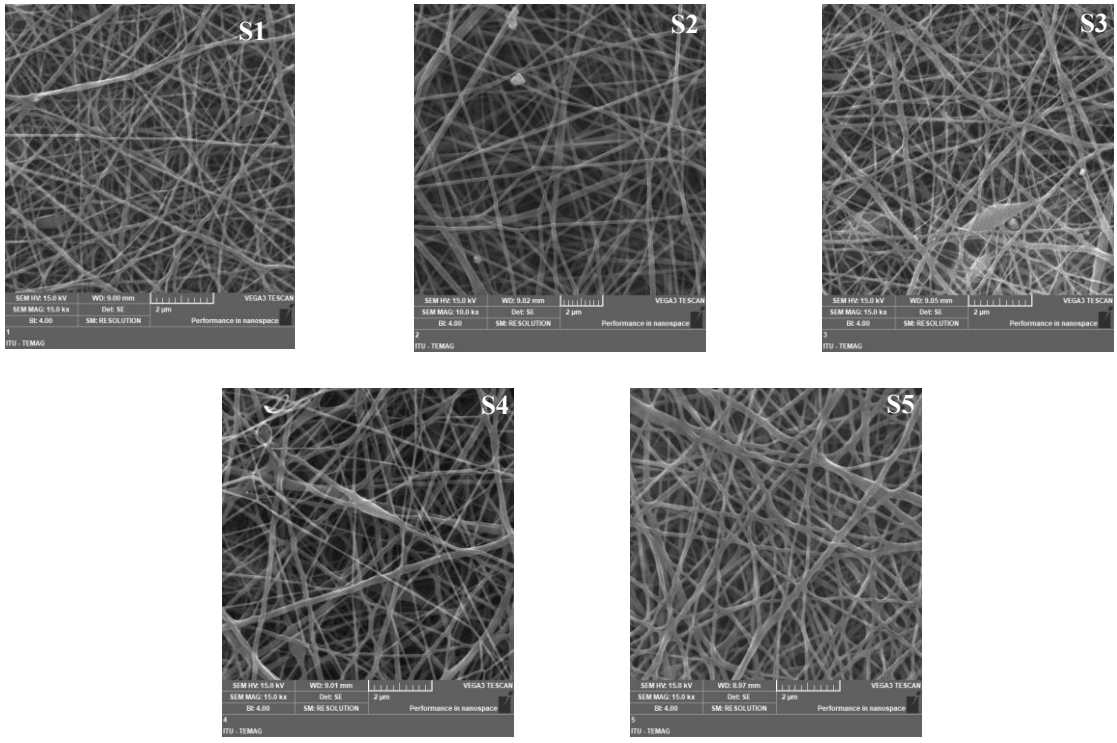

Figure 1: The SEM images of S1-S5 samples.

The fiber diameters of samples S1-S5 were 51±13nm, 135±19nm, 67±18nm, 82±18nm and 93±13nm respectively. When BAC concentration increased, the nanofiber diameters of PVA/G/BAC membrane increased. But, the average nanofiber diameter increased at 1.0% BAC concentration (S2). The thinnest fiber diameter (51±13nm) was observed at the least BAC containing sample (S1).

3.3. Mechanical Test

Vertical and horizontal strength values of PVA/G/BAC nanofiber membranes were measured (Chart 1 and Chart 2).

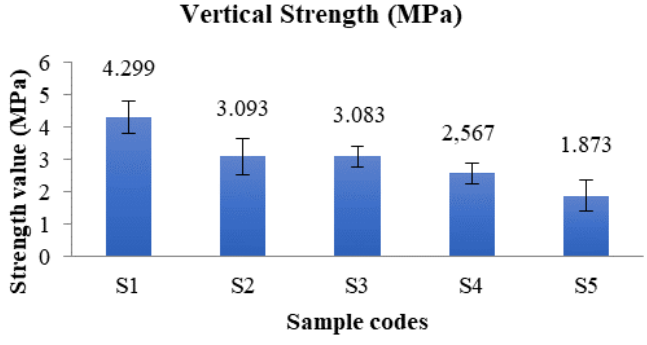

Chart 1: The measured vertical strength values of nanofiber membranes

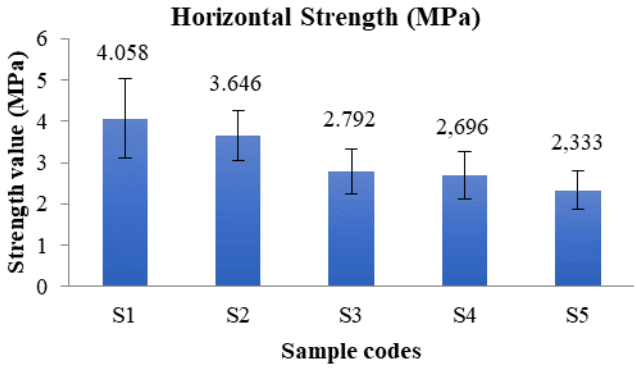

Chart 2: The measured horizontal strength values of nanofiber membranes

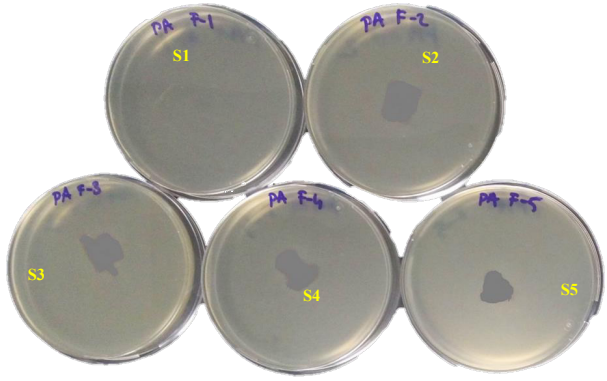

Figure 2: Antibacterial activity of S1-S5 samples against *P. aeruginosa*.

The highest vertical strength and horizontal strengths were observed at the least two BAC concentrations (0.5% at S1 and 1.0% at S2). When BAC concentration increased, vertical and horizontal strengths decreased gradually.

The thicknesses of nanofiber membranes were also measured (Chart 3).

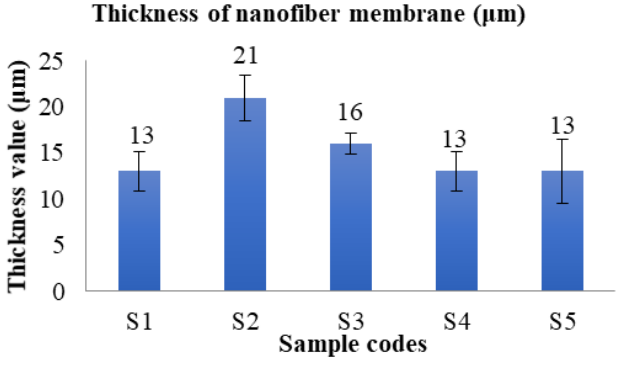

Chart 3: The thickness values of nanofiber membranes

The thickest membrane (21mm) was observed when BAC concentration was 1% (S2). The thicknesses (13mm) of the membranes were close the each other at S1, S4 and S5 samples.

3.4. Antibacterial Activity

Due to the water solubility of PVA, the membranes dissolved in the agar medium and effective samples created inhibition zones. The most resistant bacterium was *P. aeruginosa,* which was not inhibited by S1, produced from 0.5% BAC containing solution (Figure 2).

As shown at Figure 2, S1 sample did not create an inhibition zone against *P. aeruginosa*. The minimum BAC concentration that produced an inhibition zone against *P. aeruginosa* was 1% (S2). S2 sample produced inhibition zone against all the bacteria tested (Figure 3). Therefore, among the tested samples (S1-S5), S2 sample with minimum amount of chemicals seems optimum for inhibition of all the bacteria tested.

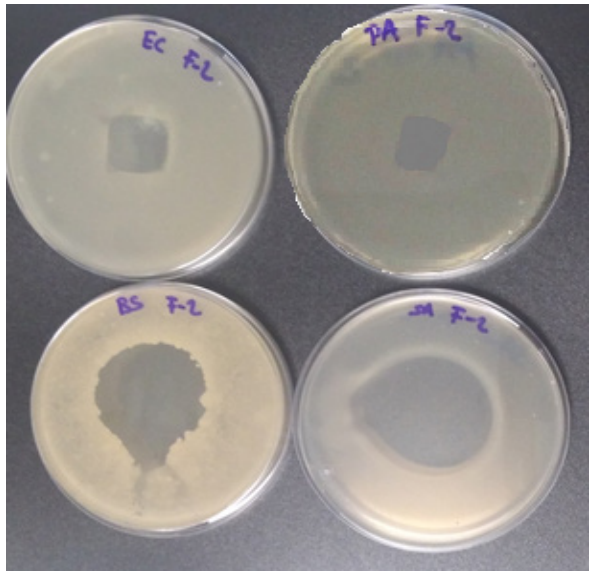

Figure 3: Antibacterial activity of S2 against *E. coli* (EC), *P. aeruginosa* (PA), *B. subtilis* (BS) and *S. aureus* (SA) bacteria.

IV. CONCLUSION

In this study, PVA/G/BAC aqueous solutions were electrospun to produce nanofiber structure with high mechanical strength and with antibacterial activity against Gr – (*E. coli*

and *P. aeruginosa*) and Gr+ (*B. subtilis* and *S. aureus*) bacteria. Viscosities of the solutions generally increased as increase in BAC concentration. Also, conductivities of the solutions increased in parallel to increase in BAC concentration.

The membranes produced from electrospinning of PVA/G/BAC aqueous solutions had uniform and intense nanofiber distribution with fiber diameter of 51±13nm-135±19nm. The highest vertical and horizontal strengths nanofibers belonged to S1 and the order of the strengths were S1>S2>S3>S4>S5. Antibacterial activities of the samples showed that all the samples were effective against *E. coli, B. subtilis* and *S. aureus* but S1 sample (including 0.5% BAC) was ineffective against *P. aeruginosa*. S2-S5 samples were effective toward all the bacteria tested. 1.0% BAC (S2) was the minimum concentration for inhibition of all the bacteria tested. Therefore, due to its second best mechanical properties and its low BAC concentration (1.0%), which decreases environmental concerns and production costs, S2 sample (11.63% PVA + 1.0% G +1.0% BAC) was thought ideal for electrospinning of PVA/G/BAC solution system.

The membranes electrospun from S2 solution can be good candidates for medical applications like roll bandage for its good mechanical properties and antibacterial activities against common bacteria (*E. coli* and *B. subtilis*) and pathogenic bacteria (*S. aureus* and *P. aeruginosa*) causing nosocomial infections and wound infections.

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