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INVESTIGATION OF ANTIBACTERIAL PROPERTIES OF HYDROGEL CONTAINING SYNTHESIZED TiO_2 NANOPARTICLES

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ABSTRACT

In the present study, we prepared hydrogel containing synthesized TiO_2 nanoparticles to investigate the antibacterial properties of these hydrogels. TiO_2 nanoparticles were synthesized by a sonochemical method. The TiO_2 containing hydrogel was characterized using thermogravimetry (TG) and Fourier transform infrared spectroscopy (FTIR) techniques. The antibacterial activities of the hydrogels against *Escherichia coli* were measured by the airborne testing and modified Kirby Bauer disk diffusion method. Finally, we demonstrated the presence of antibacterial activity in the hydrogel containing synthesized TiO_2 nanoparticles.
Keywords: TiO_2 nanoparticles (NPs), Antimicrobial hydrogel, *Escherichia coli*.

SENTEZLENMİŞ TiO_2 NANOPARTİKÜLLERİNİ İÇEREN HİDROJELİN ANTİBAKTERİYAL ÖZELLİKLERİNİN ARAŞTIRILMASI

ÖZET

Bu çalışmada, antibakteriyal aktivitelerini araştırmak için sentezlenmiş TiO_2 nanopartiküllerini içeren hidrojeller hazırlanmıştır. TiO_2 nanopartikülleri sonokimyasal metod ile sentezlenmiştir. TiO_2 içeren hidrojel termogravimetri (TG) ve Fourier dönüşümlü infrared spektroskopisi (FTIR) teknikleri ile karakterize edilmiştir. Bu hidrojellerin, *Escherichia coli*' ye karşı antibakteriyal aktiviteleri airborne testi ve modifiye edilmiş Kirby Bauer disk difüzyon metotları ile ölçülmüştür. Sonuç olarak, sentezlenmiş TiO_2 nanopartiküllerini içeren hidrojelin antibakteriyal aktivitesinin varlığı gösterilmiştir.
Anahtar Sözcükler: TiO_2 nanopartikülleri (NPs), Antimikrobiyal hidrojel, *Escherichia coli*.

1. INTRODUCTION

Having studied from the microbiological point of view, today of the synthesizing of nano-sized drug particles those have physical and chemical properties, creates an interesting topic in the development of new pharmaceutical products. Nanoparticles' unique electrical, chemical, mechanical and optical properties thanks to their high surface area / volume ratio, and small dimensions it has been observed to represent effective antimicrobial property [1]. All these

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different features of nanoparticles allow them to become a center of interest in the diagnosis and treatment of diseases [2].

Especially on drug delivery, cancer therapy and antimicrobial applications, very promising results have been obtained in studies of nanoparticles. The metal oxide nanoparticles have good antibacterial activity and antimicrobial formulations comprising nanoparticles could be used as an effective bactericidal agent [3-8].

Titaniumdioxide (TiO_2) nanoparticles important products for nanotechnology due to their high-stability, corrosion and photocatalyst features. TiO_2 nanoparticles are materials used in everyday life all the time [9].

Recently, increasing studies on gels containing nanoparticles and these hydrogels having potential usage areas in selective drug transport have directed our efforts to this issue. In this study, we investigated antimicrobial activity of hydrogels containing synthesized TiO_2 nanoparticles on *Esherichia coli*. Our study is considered to be helpful for generating a new perspective on infectional diseases/wounds healing in the future and provide a possibility to realize the impact of hydrogel containing TiO_2 nanoparticles on *Escherichia coli* infection.

To the best of the authors' knowledge, investigation of antibacterial properties of hydrogels containing synthesized TiO_2 nanoparticles has not previously been reported. The scope of the present paper is to develop a new antimicrobial hydrogel with synthesized TiO_2 nanoparticles mechanism having potent long-lasting antibacterial activity toward Gram-negative bacteria.

2. EXPERIMENTAL SECTION

2.1. Materials

All reagents used in the synthesis of TiO_2 and hydrogel, antibacterial studies were analytical grade and employed without any further treatments. Distilled water was used for all synthesis and antimicrobial activity processes. Acrylamide (AAm,C_3H_5NO), N,N'-Methylenebisacrylamide (BAAm, $C_{12}H_{16}O_4$), tetramethylethylenediamine (TMEDA, $(CH_3)NCH_2CH_2N(CH_3)_2)$)), 2-Hydroxy-4-2(2-Hydroxyethoxy)-2-Methylpropiopherone (Irgacure 2959, $HOCH_2CH_2OC_6H_4COC(CH_3)_2OH$)) were purchased from Sigma Aldrich. *Esherichia coli* (*E.coli*, ATCC 25922) was obtained from American Type Culture Collection. All the media and chemicals were purchased from Sigma.

2.2. Instruments and Methods

The synthesized TiO_2 NPs containing hydrogel was characterized using Perkin Elmer Spectrum One FT-IR Spectrometer with Universal ATR in a wavenumber range of 4000-650 cm^{-1}. TG analysis was also performed to speculate the TiO_2 concentration in the hydrogel by using a TA Instruments TGA Q500 at the heating rate of 10°C/min under a 60 mL/min nitrogen flow.

The synthesized TiO_2 nanoparticles containing hydrogel and control hydrogel were prepared using Rayonet merry-go round photoreactor in which the samples were surrounded by a circle of 6 lamps emmitting light nominally at 300 nm.

2.3. Synthesis of TiO_2 Nanoparticles

In the present work, TiO_2 NPs were prepared by a sonochemical method according to our previous study [10]. The crystallite size of synthesized TiO_2 NPs were calculated using Scherrer's formula found to be in the range of 33.56 nm [11].

2.4. Synthesis of Hydrogels Containing Nanoparticles

Hydrogels were formed by photopolymerization pathway crosslinking-copolymerization of AAm with BAAm in aqueous solution at room temperature. A simultaneous free radical crosslinking-copolymerization was initiated with water soluble type 1 photoinitiator Irgacure 2959. In detail, hydrogels were prepared by mixing synthesized TiO_2 nanoparticles, AAm, BAAm, TMEDA and Irgacure 2959.

As an example of the formulation process, to formulate hydrogels (i.e containing synthesized TiO_2 nanoparticles 0.5 mg/mL) which is approximately 0.6 wt.% of solid content and amounts of AAm (8.113 wt.%), BAAm (0.203 wt.%) were dissolved in 4 ml water. Irgacure 2959 (3.15 mM) and TMEDA (1.25 mL/50 mL) were used as the redox initiator system. 1 mL stock solutions of Irgacure 2959 and 1mL TMEDA stock solution were added the reaction solution.

The reaction solution was stirred for 15 min over a magnetic stirrer plate at 100 rpm and after adding Irgacure 2959 immediately transferred into a glass tubes with a diameter of 10 mm.

The tubes were sealed off and irradiated for 1 hour at room temperature in a Rayonet merry-go round photoreactor in which the samples were surrounded by a circle of 6 lamps emmitting light nominally at 300 nm. UV curing was done by 1 ml of Irgacure 2959 solution. The reaction was allowed overnight to obtain a solid gel. After synthesis of hydrogels, the tubes were broken gently. The images of hydrogel containing synthesized TiO_2 nanoparticles and control hydrogel are given in Figure 1.

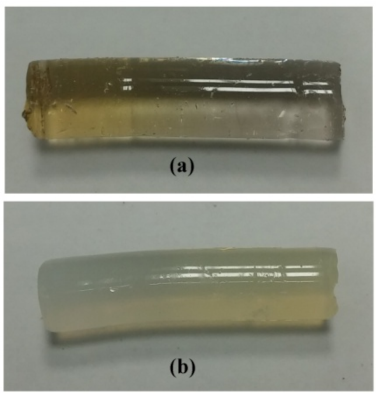

Figure 1. The images of hydrogels (a) control, (b) containing synthesized TiO_2 nanoparticles

2.5. Antibacterial Activity Testing

The antibacterial properties of the composites were tested against Gram-negative *E.coli* bacteria. All antibacterial activity tests were performed in triplicate. The airborne testing and the modified Kirby Bauer disk diffusion method were used to study the antibacterial activity of the solid hydrogel samples. We also tested the ability of synthesized TiO_2 nanoparticles containing hydrogels to serve as an antibacterial coating on surfaces.

Escherichia coli (ATCC25922) was grown overnight at 37°C and maintained on nutrient plates. The relation between absorbance at 590 nm (OD590) and colony forming units (CFU) per milliliter determined using the plate count method was used for standardization of bacterial solution concentrations [12].

A suspension (100 ml) of *E.coli* in 0.1 M aqueous PBS buffer (pH 7.0, 10^{11} cells/ml) were added to 50 ml of a nutrient broth in a sterile erlenmeyer flask. The cultured organisms were added to 10 ml of saline solution (0.9 % NaCl) to reach approximately the 10^6 colony forming units per milliliter ($CFU.mL^{-1}$). The airborne testing and the modified Kirby Bauer disk diffusion method were used to study the antibacterial activity of the solid gel samples.

For airborne testing, a saturated suspension of *E. coli* was centrifuged at 4000 rpm (room temperature) for 5 min. The cells were resuspended in ultrapure water to yield a concentration of 10^6 CFU/mL. The bacterial suspension (10^6 cells/mL) was sprayed on hydrogel. Sprayed surfaces were dried in air for about 5 min and then placed into empty sterile polystyrene Petri dishes (VWR, 100 mm × 15 mm). Nutrient agar (1% agar in a nutrient broth, autoclaved, and cooled to 37°C) was poured on the bacteria-sprayed samples and allowed to solidify. The samples were incubated at 37°C overnight and then colonies were quantified. A hydrogel sample without synthesized TiO_2 NPs was used as a control.

The results are expressed as;

$$\% \text{ kill} = \frac{\text{cell count of control - survivor count on test surfaces}}{\text{cell count of control}} \times 100 \quad \ldots\ldots \quad (1)$$

For disk diffusion method, a total of 100 μL of bacteria stock solution (10^6 CFU/mL) was plated on nutrient agar growth plates. The hydrogel samples were placed coating side down on top of the inoculated agar plates and incubated overnight at 37°C. The zone of inhibition were visualized the next day on the plates.

Statistical analysis. All data represent at least three independent experiments and were expressed as mean ± standard error of the mean (RSD). Statistical analyses were performed using Student 's t-test for comparison between two groups and analysis of variance (ANOVA) and Dunnett' s post hoc test for multiple comparisons among groups. A probability value of $P<0.05$ was considered significant.

3. RESULT AND DISCUSSIONS

3.1. Characterization of Hydrogels

The TG curves of hydrogels are given in Figure 2. The weight loss in control hydrogel between room temperature and 500°C is about 71.86 wt% which is attributed to release of absorbed water and decomposition of the hydrogel. The weight loss in hydrogel containing synthesized TiO_2 NPs is about 70.20 wt%. The weight loss differences between control hydrogel and hydrogel containing synthesized TiO_2 NPs can be explained by the presence of TiO_2 NPs in hydrogel.

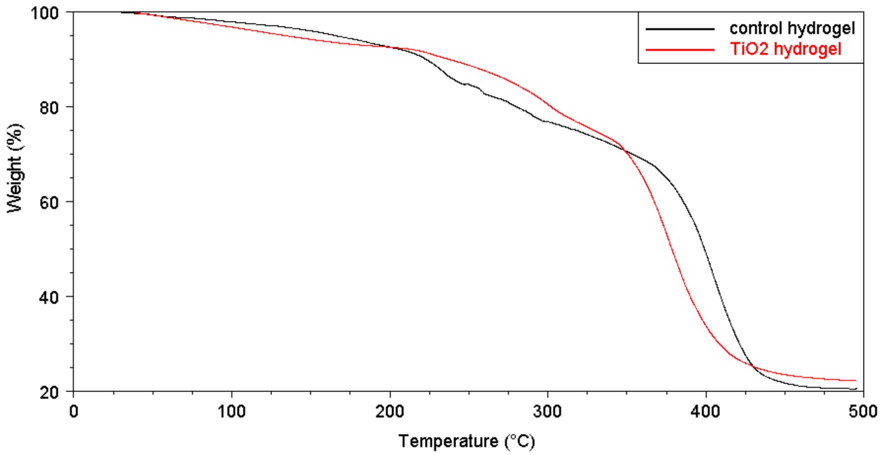

Figure 2. TG curves of hydrogels.

The FT-IR spectrum of the hydrogel containing synthesized TiO_2 NPs and control hydrogel are shown in Figure 3. The absorption band near at 2930 cm^{-1} are attributed to symmetric or asymmetric stretching vibration of the CH_2 groups of AAM or BAAm. The bands near at 3400 and 1645 cm^{-1} are associated to N–H stretching vibration in –NH-group of BAAm or–$CONH_2$ groups of AAm [13]. The absorption peak at 1408 cm^{-1} is assigned to stretching vibration of CN from AAm [14].

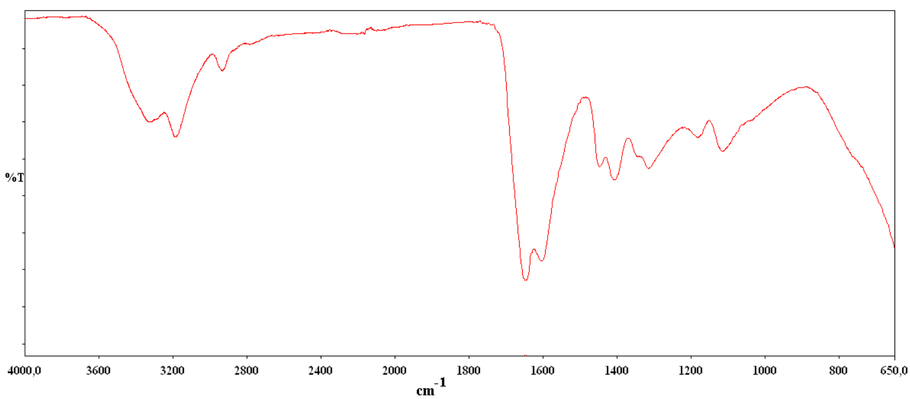

Figure 3. FTIR spectra of hydrogel containing synthesized TiO_2 NPs

3.2. Antibacterial Activity Results

According to the result of the modified Kirby Bauer disk diffusion method, zones of inhibition around the hydrogel consisting of synthesized TiO_2 NPs samples and control were not observed for *E.coli*. In addition, the airborne testing results shown that the synthesized TiO_2 NPs containing hydrogel showed efficiency in the killing bacterial strains (about % 65) while the control hydrogel did not show the killing efficiency. The % kill of *E.coli* pathogens for synthesized TiO_2 NPs containing hydrogels are given in Table 1 and Figure 5.

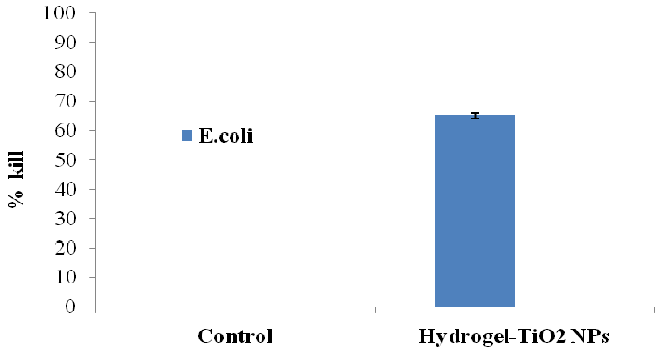

Figure 4. Antibacterial activity histograms of hydrogels (Error bars represent mean ± standart deviation of mean for n = 3)

Table 1. % Kill of pathogen on synthesized TiO_2 NPs hydrogel and control hydrogel

Sample	**% kill (*E.coli)***
TiO_2 NPs Hydrogel	65
Control Hydrogel	-

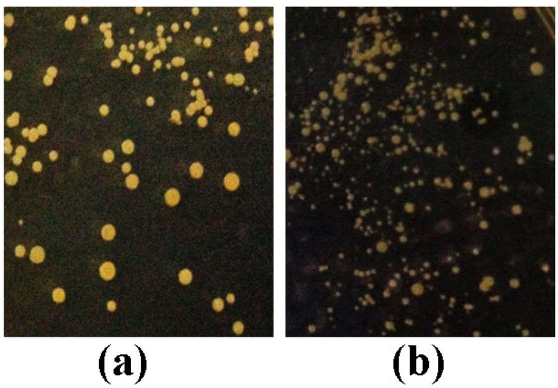

Figure 5. Imges of synthesized TiO_2 NPs hydrogel (a) and control hydrogel (b) onto which aqueous suspensions (10^6 cells per ml of distilled water) of *E.coli* cells were sprayed, air dried for 5 min, and incubated under 1% agar in a bacterial growth medium at 37°C overnight.

4. CONCLUSION

Hydrogel containing synthesized TiO_2 NPs was fabricated using photopolymerization pathway crosslinking-copolymerization of AAm with BAAm in aqueous solution at room temperature. A simultaneous free radical crosslinking-copolymerization was initiated with Irgacure 2959. Formation of hydrogel-TiO_2 nanocomposite was confirmed by TG and FTIR analysis. The antibacterial activity of the solid hydrogel samples were investigated by the airborne testing and the modified Kirby Bauer disk diffusion method. The analysis results were demonstrated that the hydrogel containing synthesized TiO_2 NPs possess excellent antibacterial properties.

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