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Research Article

Antiacne Effects of PVA/ZnO Composite Nanofibers Crosslinked by Citric Acid for Facial Sheet Masks

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The water-insoluble polyvinyl alcohol (PVA) nanofibers containing ZnO nanoparticles were prepared by an electrospinning technique for antiacne facial masks. Zinc oxide (ZnO) nanoparticles prepared by a sol-gel method were dispersed in aqueous PVA and citric acid solution for electrospinning. The electrospun nanofibers were treated at 160°C to induce the thermal crosslinking between PVA and citric acid. The crosslinked PVA with citric acid become water-insoluble, which can be used for facial masks. PVA/ZnO composite nanofibers were characterized by structural and morphological analysis techniques including Fourier transform infrared spectroscopy (FT-IR), field-emission scanning electron microscopy (FE-SEM), and transmission electron microscopy (TEM). Also, the swelling ratios according to the ZnO content (0, 1, 4, and 7 wt.% with respect to PVA) were measured. Antibacterial activity tests were also studied. The clear zone of *Cutibacterium acnes* of PVA/ZnO 7% nanofibers was 2.25 mm, indicating antiacne activities of nanofibers.

1. Introduction

Nanofibers form the broadest class of nanomaterials due to their unique properties such as high surface area to volume ratios, high porosities, high strength values, light weight, and small diameters [1, 2]. These remarkable properties make them suitable candidates for various applications including tissue-engineered scaffolds, biomedical devices, drug delivery system [3], filtration [4], and energy generation [5]. Cosmetics are also one of these various application areas. The unique characters of nanofibers provide excellent interaction with the skin, high liquid absorption capacity, and high oxygen and water vapor permeability. They can be used as facial masks and skin care and regenerative products in the cosmetic area [2].

An electrospinning method is one of the promising methods for fabricating organic and inorganic nanofibers. This method provides a simple and cost-effective process to produce continuous and homogeneous nanofiber of submicron diameter [6]. In the process, an electric field of high voltage is applied to the solution in a syringe to form a jet from a nozzle. The jet becomes nanofibers with a uniform diameter during drying and deposition on the collector [7]. The morphology and diameter of synthesized nanofibers depend on the rheological property of polymeric solutions, distance between the nozzle and collector, and strength of the applied electric field [8]. Various polymers have been employed in the electrospinning method to fabricate ultrafine fibers in recent years for cosmetic applications. Polyvinyl alcohol (PVA) is an environmentally friendly polymer which is soluble in water and biodegradable. Also, it is a nontoxic and good biocompatible polymer which can be applied for biomedical materials and cosmetics [9–11]. But PVA itself is water-soluble. The PVA nanofibers can be collapsed by absorbing or contacting with water. Thus, the conversion of water-soluble PVA nanofibers into water-insoluble nanofibers is inevitable for the application as facial masks. This can be done through the crosslinking of PVA polymeric chains with crosslinking agents such as glutaraldehyde, maleic acid, and citric acid [12].

Acne vulgaris is a chronic skin disease that affects the individuals of all ages. The current standard of treatment includes topical (washes, lotions, and creams) and systemic (oral isotretinoin, oral antibiotics) treatments. Nevertheless,

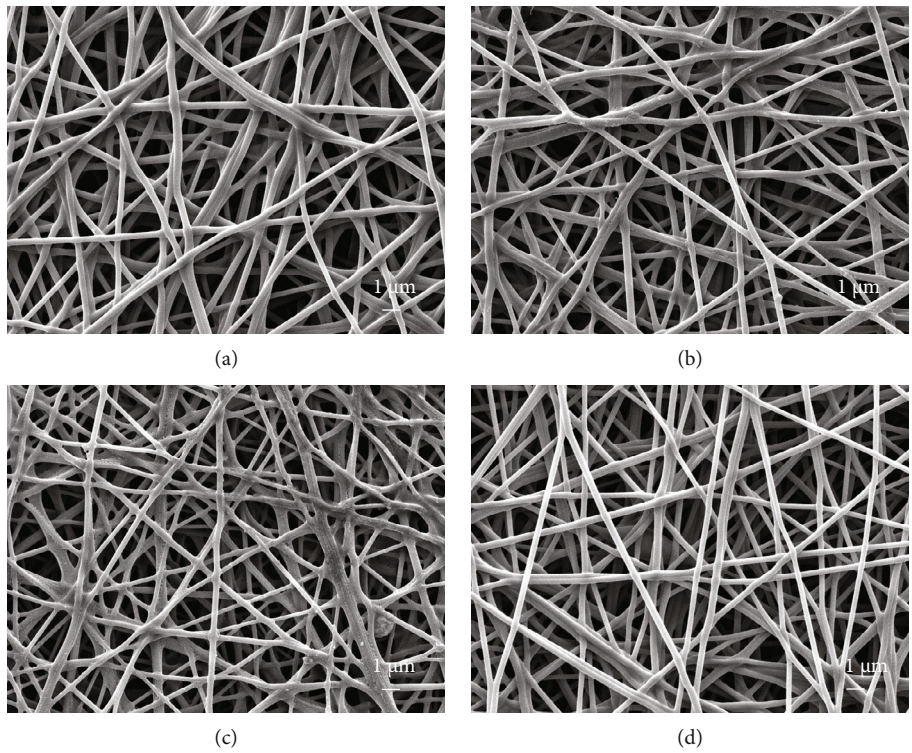

Figure 1: FE-SEM images of PVA/ZnO nanofibers with different relative concentrations of ZnO NPs: (a) 0%, (b) 1%, (c) 4%, and (d) 7%.

these cares have shown to cause a variety of side effects such as dryness, peeling, and erythema. Zinc is a promising alternative to other acne treatments due to its low cost, efficacy, and lack of systemic side effects [13].

Zinc oxide (ZnO) has been widely studied as an important semiconductor photocatalyst due to its large band gap, easy process ability, nontoxicity, and inert properties [14, 15]. It is a major inorganic ingredient of sunscreen along with TiO_2. ZnO and TiO_2 were also shown to have antibacterial activity against microorganisms such as *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), and *Bacillus subtilis* (*B. subtilis*), and more antibacterial activity of ZnO is shown. In addition, ZnO has the function of inhibiting *Cutibacterium acnes* (*C. acnes*) which is a gram-positive bacterium that induces acne. It has been shown that these inorganic nanoparticles can more effectively inhibit *P. acnes* when mixed with citric acid [16]. Citric acid is a polycarboxylic acid that can be easily obtained in nature, and there are three carboxyl groups capable of esterification with the OH group of PVA, which can serve as a crosslinking agent [17].

When ZnO nanoparticles are directly applied into the skin in cosmetics, the ZnO particles are adsorbed into the skin. This adsorption of ZnO particles might induce toxic effect on the skin [18]. In this study, the ZnO nanoparticles were embedded into PVA nanofibers which have a large surface area. Thus, the adsorption of ZnO nanoparticles into the skin can be prevented by embedding into polymeric nanofibers on the skin. And the large contacting area between the skin and nanofibers containing ZnO nanoparticles resulted in high antibacterial effect against acne. The ZnO nanoparticles were embedded into PVA nanofibers by electrospinning techniques. Since the PVA is water-soluble, the PVA/ZnO nanofibers were crosslinked with citric acid through thermal treatment. The crosslinked PVA/ZnO composite nanofibers with citric acid are water-insoluble. In addition, these nanofibers are made of nontoxic materials such as ZnO, PVA, and citric acid and have the advantage of being biocompatible and biodegradable, so they can be used for facial masks in cosmetics. The prepared nanofibers were characterized by FE-SEM, HR-TEM, and FT-IR, and antibacterial effects of nanofibers were investigated.

2. Experiment

2.1. Materials. Zinc acetate dehydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$), polyvinyl alcohol (PVA; 87-89% hydrolyzed, Mw: 85,000-124,000 g/mol), citric acid (99%), and polyoxyethylene sorbitan monolaurate (Tween 20) were purchased from Sigma-Aldrich. Potassium hydroxide (KOH; pellet 85%) was obtained from JUNSEI in Japan. Absolute ethanol (99.9%) was purchased from Daejung, Korea. The water was double-distilled and deionized using a Milli-Q Plus system, Millipore, France, with 18.2 MΩ·cm electrical resistivity at 25°C. All chemical reagents used in experiments were of analytical grade and were used without further purification.

2.2. Synthesis of ZnO Nanoparticles. The ZnO nanoparticles were prepared by the sol-gel method from our previous study [19]. In detail, the zinc acetate dehydrate (4.38 g) was added to 200 mL of ethanol and stirred at 60°C for 30 min until dissolved to obtain transparent zinc acetate solution.

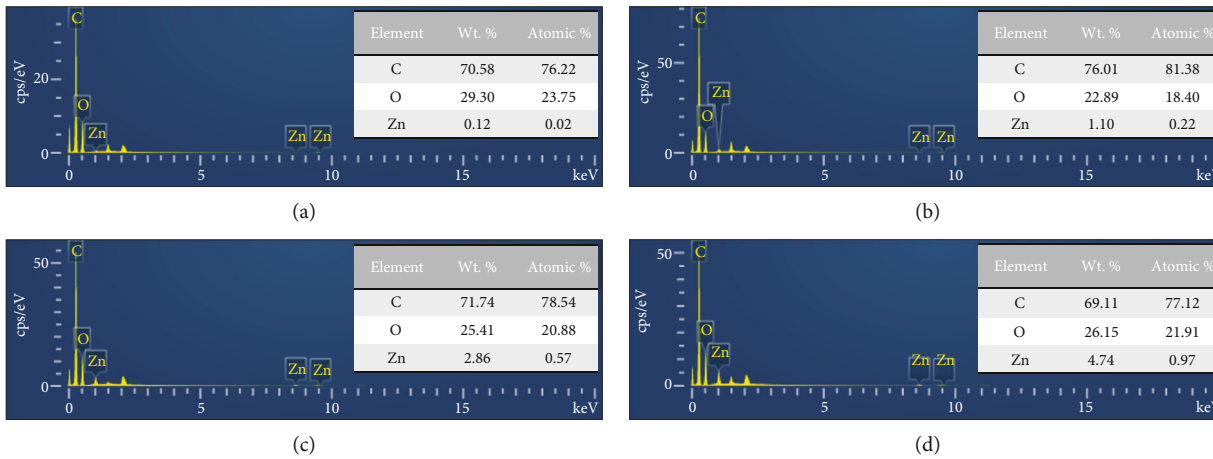

Figure 2: EDS spectrum of PVA/ZnO nanofibers with different relative concentrations of ZnO NPs: (a) 0%, (b) 1%, (c) 4%, and (d) 7%.

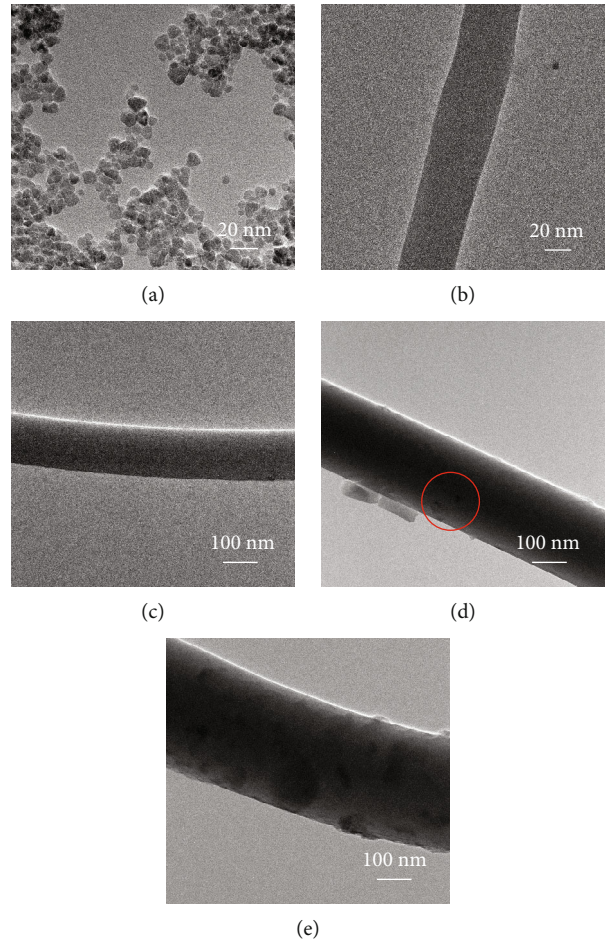

Figure 3: HR-TEM images of (a) ZnO nanoparticles and PVA/ZnO nanofibers with different relative concentrations of ZnO NPs: (b) 0%, (c) 1%, (d) 4%, and (e) 7%.

Then, KOH (2.24 g) completely dissolved in ethanol (100 mL) at 60°C for 30 min was added into the zinc acetate solution. The mixture was stirred at 60°C for 3 h and washed with ethanol for several times with centrifugation at 3,000 rpm. The precipitates were redispersed in ethanol.

2.3. Preparation of PVA/ZnO Composite Nanofibers. PVA solution was prepared with distilled water at 80°C while stirring for 3 h until completely dissolved. The relative weight percentage of ZnO to polymer was varied from 0 to 7 wt.% by adding 10 wt.% ZnO solution. Citric acid and Tween 20

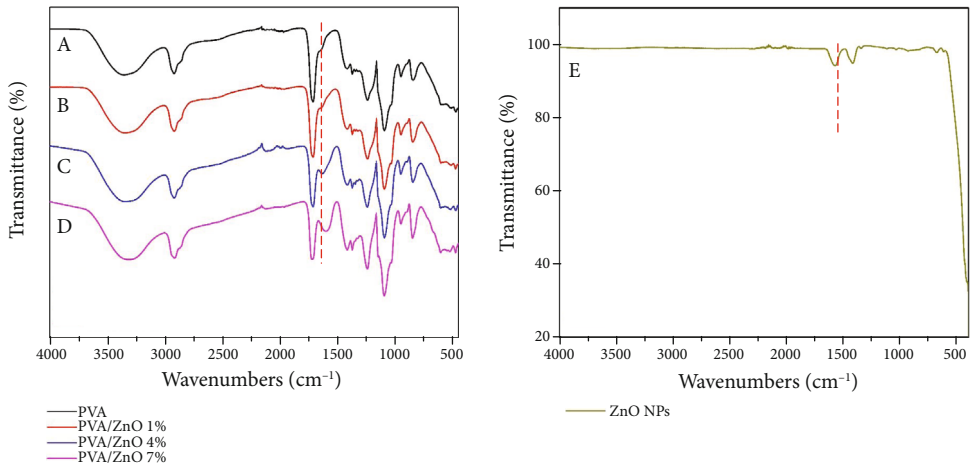

Figure 4: FT-IR spectra of (A) PVA nanofiber, (B–D) PVA/ZnO nanofibers, and (E) ZnO nanoparticles.

were added each 0.3 g in solution and stirred overnight. The polymer and ZnO composite solution were loaded in the plastic syringe and connected for injection to 21-gauge (0.5 mm of internal diameter) tip needle. The solution was pumped with a syringe pump (KDS 100 Scientific) at a feed rate of 0.8~1.0 mL/h. The distance from the tip to the collector was 15 cm, and the supply of voltage was 17~20 kV. The fibers were collected on a grounded drum (DC90L, Nano NC) rotating at 250 rpm. The electrospinning chamber was maintained at 20~25°C and 40% humidity. The nanofibers were thermally treated at 160°C under vacuum conditions for 1 h and dried for 1 h more remaining heat.

2.4. Characterizations. The morphology of nanofibers was observed with a field-emission scanning electron microscope (FE-SEM, JEOL JSM-6700F). Prior to FE-SEM analysis, the samples were coated with platinum at 20 mA for 1 min under vacuum condition using a sputter coating machine. Chemical composition of the prepared nanofiber membranes was analyzed using energy-dispersive X-ray spectroscopy (EDS) equipped with FE-SEM. The transmission morphology of the synthesized ZnO and PVA/ZnO nanofibers was investigated with high-resolution electron microscopy (HR-TEM, JEOL JEM-3010) accelerated with 300 kV. Fourier transform infrared spectroscopy (FT-IR) was also carried out to examine functional groups in nanofibers by using a FT-IR spectrometer (Thermo Scientific, Nicolet iS550).

2.5. Swelling Properties. The swelling ratio (equation (1)) of nanofiber membranes was obtained by placing the samples with unit size in water at 37°C and removing after 24 h. The excess moisture on the surface of swollen membranes was removed with filter paper and weighed (W_S). The weight of the sample before being soaked was measured (W_d) after drying in an oven at 40°C for 1 day.

$$\text{Swelling ratio} = \frac{W_s - W_d}{W_d} \times 100(\%). \quad (1)$$

2.6. Antibacterial Activities. The antibacterial activity of PVA/ZnO nanofibers was performed against *Escherichia coli*

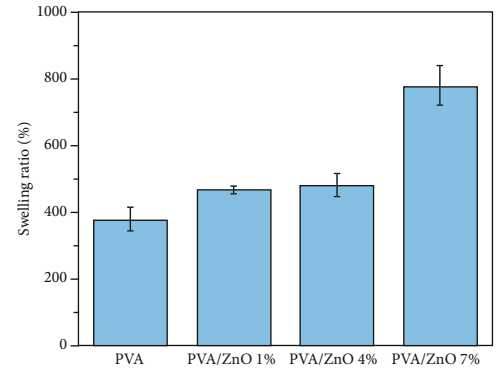

Figure 5: Swelling ratio of PVA nanofiber and PVA/ZnO nanofibers.

(*E. coli*: ATCC 10536, gram-negative bacteria), *Staphylococcus aureus* (*S. aureus*: ATCC 6538, gram-positive bacteria), and *Cutibacterium acnes* (*C. acnes*: ATCC 6919, gram-positive bacteria) according to the KS K 0890 Parallel Streak Standard Method (qualitative test for antibacterial activity of fabric and textile materials, similar to AATCC 147 in USA) by the ALLPASSBIO Institute, Korea. The test specimen was used in a size of 25 × 50 mm. The *E. coli* and *S. aureus* were incubated at 37°C for 24 h in Tryptic Soy Agar (TSA) under aerobic condition, and *C. acnes* was incubated in a 5% blood TSA at 37°C under anaerobic condition for 7 days. The clear zone was measured after incubating each bacterium.

3. Results and Discussion

3.1. Morphology of ZnO and PVA/ZnO Composite Nanofibers. The diameters of electrospun nanofibers decreased with the addition of ZnO nanoparticles, but as the ZnO content increased from 0 wt.% to 7 wt.%, the diameter of the samples did not show a large variation to 360 ± 51, 301 ± 60, 300 ± 53, and 325 ± 48 nm, respectively (Figure 1). In general, the diameter of PVA/ZnO composite nanofibers prepared by the electrospinning technique decreases

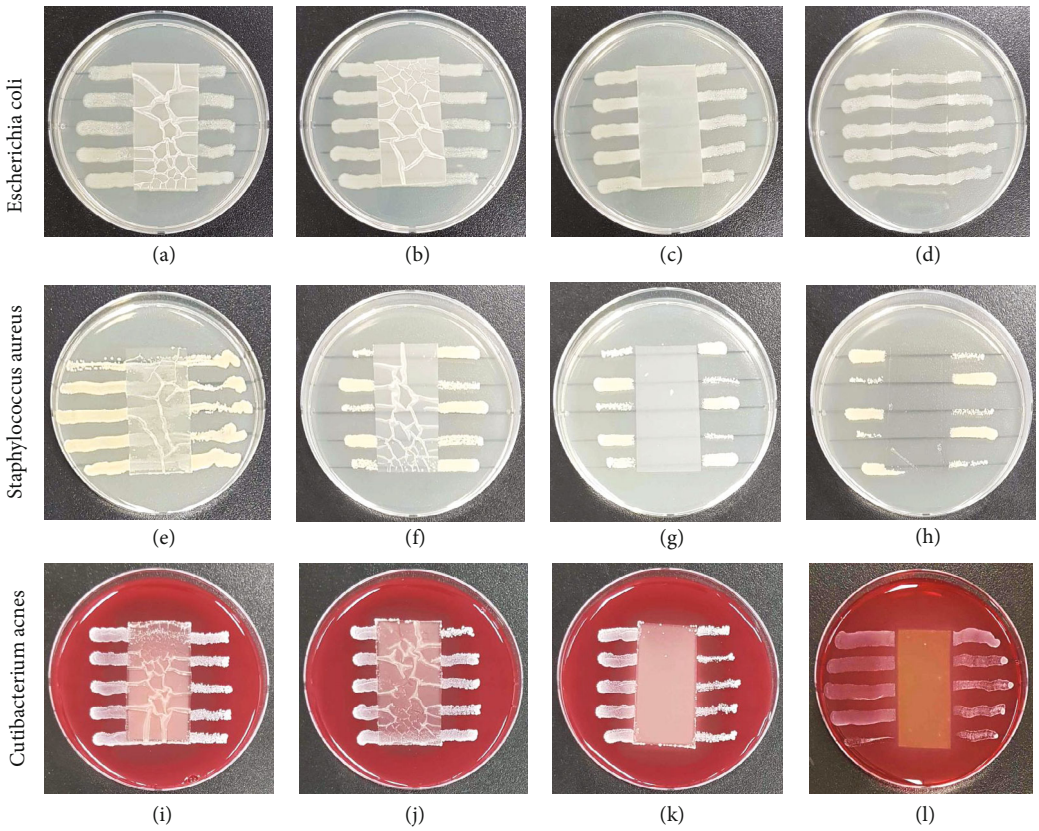

Figure 6: Antibacterial result of PVA nanofibers with different relative concentrations of ZnO nanoparticles: (a, e, i) 0%, (b, c, j) 1%, (c, g, k) 4%, and (d, h, l) 7% each against (a–d) *E. coli*, (e–h) *S. aureus*, and (i–l) *C. acnes*.

as the amount of ZnO increases [20]. But in this study, the diameters of nanofibers were not changed significantly. This might be due to the small amount of ZnO compared with other studies.

EDS analysis was performed to confirm the ZnO particle content of the nanocomposite fibers (Figure 2). The Zn element content showed 0.12 wt.% and 0.02 atomic % in neat PVA nanofibers, which are negligible (Figure 2(a)). As the content of ZnO in nanofibers increased to 1, 4, and 7 wt.%, the weight percentage of Zn elements gradually increased to 1.10, 2.86, and 4.74 wt.% and the atomic percentage also increased to 0.22, 0.57, and 0.97 atomic % (Figures 2(b)–2(d)). A small amount of Zn element content was determined because 7% or less of ZnO was added to the polymer solution.

The ZnO nanoparticles prepared by the sol-gel method are spherical and monodispersed (Figure 3(a)). The average diameter of nanoparticles was 6.11 ± 1.48 nm. There was no significant difference in appearance in the SEM, and TEM analysis was performed to confirm the inside of the nanofibers. As shown in Figures 3(b)–3(e), it was difficult to find ZnO nanoparticles at ZnO 1% (Figure 3(c)). Because 0.01% of ZnO was added to the polymer solution, it appears that the inorganic nanoparticles were not evenly dispersed in the fibers. In PVA/ZnO 4% (Figure 3(d)), a very small amount of ZnO nanoparticles was found inside the nanofiber, and in 7% (Figure 3(e)), it was confirmed that it increased noticeably. However, it can be confirmed that the nanoparticles are agglomerated rather than well dispersed and are complexed inside the nanofiber.

Table 1: Size of the clear zone in mm about each bacterium.

Samples	*E. coli*	*S. aureus*	*C. acnes*
PVA	—	—	—
PVA/ZnO 1%	—	—	—
PVA/ZnO 4%	—	1.5 mm	—
PVA/ZnO 7%	—	1.5 mm	2.25 mm

3.2. FT-IR Analysis. The FT-IR analyses of PVA/ZnO nanofibers and ZnO nanoparticles are compared in Figure 4. As the ZnO content increased, the absorption peak at the 3600-3100 cm^{-1} broad region (O–H stretching) increased and the intensity of peak at 1730 cm^{-1} for the C=O stretching decreased. It can be confirmed that the esterification reaction between the hydroxyl group of PVA and the carboxyl group of citric acid occurred less, resulting in less crosslinking [9, 10, 12]. The nanofiber composites showed absorption peak at 1240 cm^{-1} (C–H wagging) and 1087 cm^{-1} (C–O stretching), indicating that it was banded by the PVA polymer [21]. As shown in (E) in Figure 4(b), the FT-IR spectra of ZnO nanoparticles synthesized by the sol-gel method showed distinct peaks at 1570, 1417, and 450 cm^{-1} relating

to vibration of C=O, C–O, and Zn–O. The intensity peak of C=O and C–O groups can be seen to be caused by zinc acetate precursors [22]. The peak increased at 1570 cm^{-1} as the ZnO content increases in PVA/ZnO nanofibers, which can be thought to increase due to ZnO.

3.3. Swelling Property. The swelling ratio was measured to confirm the possibility of a facial mask. As the amount of ZnO in nanofibers was increased, swelling ratios were increased to 378 ± 35.35, 466 ± 11.26, 480 ± 34.90, and 780 ± 58.77% (Figure 5). As seen in the FT-IR section, it can be confirmed that crosslinking occurs less as the ZnO content increases. The more ZnO nanoparticles were added, the greater the swelling rate was by interfering with the esterification reaction between PVA and citric acid.

3.4. Antibacterial Activities. The antibacterial test was conducted using *E. coli*, *S. aureus*, and *C. acnes*. PVA and PVA/ZnO 1% showed no antibacterial activity for all bacteria (Figures 6(a), 6(b), 6(e), 6(f), 6(i), and 6(j)). In PVA/ZnO 4%, the clear zone was observed only for *S. aureus*, and the thickness was 1.5 mm. PVA/ZnO 7% also showed no antibacterial activity in *E. coli*, but in *S. aureus* and *C. acnes*, they formed the inhibition zone of bacteria of 1.5 and 2.25 mm, respectively, as shown in Table 1, indicating antibacterial activity. PVA/ZnO nanofibers did not inhibit *E. coli* and *S. aureus*; PVA nanofibers containing more than 4% ZnO NPs showed an inhibitory effect. The antiacne effect was shown at the ZnO content of 7% against *C. acnes*, an acne-inducing bacterium. And it seems that it is possible to suppress the induction of acne bacteria when the content of ZnO is at least 7%.

The antibacterial mechanisms of ZnO nanoparticles have been proposed, such as formation of reactive oxygen species (ROS) [23, 24], release of Zn^{2+} [25, 26], internalization of ZnO NPs into bacteria [27], and electrostatic interactions [28]. ZnO nanoparticles in aqueous solution can produce various ROS such as hydroxyl radicals ($OH^{\bullet}$), superoxide ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2) [29] and release the zinc divalent ions [30]. Antibacterial activity occurs because ROS and Zn^{2+} damage bacterial cellular constituents. The ZnO nanoparticles contained in the PVA/ZnO nanofiber also have antibacterial activity by the same mechanisms.

4. Conclusions

It was confirmed through TEM that the ZnO nanoparticles synthesized via the sol-gel method had uniform size and spherical shape. Inorganic nanoparticles were dispersed in PVA polymeric solution to prepare antiacne nanofibers through electrospinning. From SEM and TEM analyses, it was confirmed that nanofibers were prepared with a uniform diameter without beads, and the ZnO nanoparticles were aggregated and combined into nanofibers. The swelling ratios of each nanofiber increased as the amount of ZnO added to the polymer increased, and the PVA/ZnO 7% nanofiber showed a swelling ratio of about 2.06 times more than that of the pure PVA nanofiber. Through the antibacterial test, the antimicrobial activity of nanofibers containing ZnO particles did not occur against *E. coli*. The activity against *S. aureus* produced a 1.5 mm of inhibition zone as 4% of ZnO nanoparticles entered, but the zone size did not grow even if the amount of ZnO increased. PVA/ZnO nanofibers have an effect against acne-inducing bacteria when at least 7% of ZnO particles are added. The PVA/ZnO nanofiber, which has a high swelling ratio and acne-preventing effect, seems to be applicable as a facial mask without penetration of ZnO nanoparticles into the skin.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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