Journal of Applied Polymer Science

Electrospun polyurethane membrane with Ag/ZnO microparticles as an antibacterial surface on polyurethane sheets

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ABSTRACT: A method of antibacterial modification of the polyurethane (PU) surface is presented in this article. An electrospun PU membrane with an incorporated antibacterial agent was applied as a coating of the PU sheets. As an antibacterial agent, a hybrid bimetallic filler was used; it combined the antibacterial effects of silver and zinc oxide. With an electrospun submicrometer-fiber membrane, the filler was uniformly and thinly applied on the PU surface by compression molding. The antibacterial activities of three filler concentrations were tested, and they demonstrated an effective antibacterial action against *Staphylococcus aureus* and *Escherichia coli*. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43020.

KEYWORDS: composites; polyurethanes; surfaces and interfaces

Received 26 March 2015; accepted 7 October 2015
DOI: 10.1002/app.43020

INTRODUCTION

Electrospun membranes have received a great deal of attention because of their potential to be processed for many different applications. The major advantage of electrospun membranes is their thin fibers. Their diameters may range from nanometers to a few micrometers. Depending on the material composition and component properties, they can be prepared for various separators, sensors, smart textiles, and topical applications in electronic, chemical, aerospace, or medical applications.[1–5] In medicine, they have been recently used in modern wound treatment and other applications where the controlled release of the drug is required.[5–9] Moreover, they have been applied in other medical applications where antibacterial properties and biocompatibility[10–13] are needed.

Considerable effort has been recently devoted to the development of efficient antibacterial surfaces. They can be developed either by a chemical method, including functionalization, polymerization, or derivatization, or a physical approach with consideration of the modification of the surface architecture.[14–18] In addition to polymers possessing an intrinsic antibacterial activity (*R*), the antibacterial properties can be achieved through (1) the coating or adsorption of an antibacterial agent onto the polymer surface, (2) the immobilization of an antibacterial agent in the polymer via ionic or covalent bonding, or (3) the direct incorporation of an antibacterial agent into the polymer during its synthesis or processing.[19,20] A number of conventional and nonconventional antibacterial agents have been discussed.[21,22] Recently, antibacterial agents with particle structures in their nanometric or submicrometer forms have been investigated. They usually consist of silver, copper, zinc oxide (ZnO), coated silica particles, and lately, carbon nanotubes[23–25] Silver (Ag) has long been known as antibacterial agent; it possesses a wide range of activities against bacteria, molds, and yeasts.[9–11,19–26] Also, ZnOs have proven to strongly inhibit the action of pathogenic microbes.[27] Zinc oxides ensure a durable antibacterial effect, even in small concentrations.[28,29] It is also known that Ag and ZnO can be used together in a combined bimetallic filler, and they synergistically improve the antibacterial effects of the original single-metal filler.[30] These advanced systems of metal–semiconductor materials have attracted great attention because of their large specific surface area, their high fraction of surface atoms, and also their unique electronic band structure, which results in a specific chemical activity.[31] The synergic effects of Ag and ZnO have shown antibacterial action in coatings, composite blends, and solutions.[32–34] With respect to electrospun membranes, the antibacterial effect of silver has been also used in the electrospinning of nanofiber membrane from solution.[9,11] Lately, Ag/ZnO was used for the electrospinning of a multifunctional nanocomposite membrane from sol–gel.[35]

In this study, electrospun membranes with an incorporated antibacterial agent were used for the creation of antibacterial

surface on polymers. Generally, fillers are used in polymer volumes to ensure the function for which they were selected. In this concept, an electrospun polyurethane (PU) membrane with incorporated Ag/ZnO filler was used to create a thin antibacterial surface detached from the bulk PU body. It was inseparably attached to the surfaces of PU sheets by compression molding. The use of this thin membrane ensured that the filler was distributed in a thin layer only on the PU surface. We considered this to be important from the perspective of this principle application when more expensive active metal particles were saved compared with their use as a bulk filler. The surface prepared by the proposed method proved to have *R* against *Staphylococcus aureus* and *Escherichia coli*. It follows that the procedure could be used as an original method for antibacterial surface preparation. This method does not require chemical or physical surface pretreatments, which would require advance planning in technological processes. This also allows additional antibacterial treatment of polymer surfaces or thermoplastic products. We chose PU first because it belongs to a family of polymers that has found a wide range of biomedical applications because of a number of useful properties, such as biocompatibility, good hydrolytic and oxidative biostability, and processability.[35,37] Furthermore, we dealt with it because there have been a few studies focusing on the electrospinning of PU.[38] Several authors have combined PU with virgin olive oil, dextran, or carbon nanotubes in nanofibers for wound-dressing or electronic applications.[3,9,39,40] They managed to achieve electrospun membranes with fibers with almost circular cross sections, smooth surfaces, and diameters ranging from nanometers to micrometers.

EXPERIMENTAL

Ag/ZnO Filler

Ag/ZnO particles were prepared by microwave-assisted synthesis with a domestic oven with a reflux cooling system.[30] The microwave oven (CWR-TECH, 1150W/230V-50 Hz) was modified for open-vessel solvothermal synthesis with an external condenser. Silver nitrate ($AgNO_3$; ≥99.5% purity) and zinc acetate dehydrate [$Zn(CH_3COO)_2{\cdot}2H_2O$; >99% purity] were obtained from Penta, and hexamethylenetetramine [HMTA ($C_6H_{12}N_4$); >99% purity] was supplied by Sigma-Aldrich.

Amounts of 0.05 mol of $Zn(CH_3COO)_2{\cdot}2H_2O$ and 0.005 mol of $AgNO_3$ were separately dissolved in 70 and 30 mL, respectively, of demineralized water. Obtained solutions were mixed together in a flask and placed into a microwave oven. Then, the flask was connected to an external condenser with a dropping funnel. After 2 min of microwave irradiation at the maximum power output, a precipitation agent (0.05 mol of HMTA dissolved in 50 mL of demineralized water) was added through the dropping funnel, and microwave irradiation was continued for another 3 min. HMTA was used as a precipitation agent and growth modifier. Finally, the product was washed and collected by filtration. The obtained powder was dried in a laboratory oven for 1 day.

PU Membrane

The PU submicrometer-fiber membrane was prepared by electrospinning from a PU (Desmopan DP 2590A, Bayer)–dimethyl formamide/methyl isobutyl ketone (1:3) solution.[3] The total PU concentration was 16 wt %. The electrospinning process was performed with a NanoSpider machine (Elmarco s.r.o. Liberec, Czech Republic, http://www.elmarco.com/) with one rotational electrode with needles and a power supply (Matsusada DC) under following conditions: a temperature of 20–25°C, a relative humidity of 25–35%, an electric voltage of 75 kV, an electrical conductivity adjusted to 20 μS/cm with sodium chloride.

Antibacterial Surface

An aqueous solution of sodium dodecyl sulfate with a concentration of 0.1 wt % was prepared with a total volume of solution of 200 cm^3. Then, 0.01 wt % of Ag/ZnO particles were added, and the dispersion was sonicated by a UZ Sonopuls HD 2070 kit for 5 min at 50% power and 50% pulse mode at room temperature. The prepared aqueous dispersion was then filtered through the PU membrane, which always had the same diameter and was dried in ambient air. Furthermore, the PU sheets were prepared from a thermoplastic PU elastomer (Desmopan DP 2590A, Bayer) by compression molding. An adequate molding temperature of 175°C was set according to DSC measurements (PerkinElmer Pyris 1) performed at a heating rate of 10°C/min. Finally, membranes with Ag/ZnO fillers were placed onto the PU sheets and hot-pressed at a temperature of 175°C. Three concentrations of Ag/ZnO were prepared (0.04, 0.08, and 0.42 g/L).

Measurements and Methods

The crystalline structure of the obtained powder was characterized by X-ray diffraction with a multipurpose X-ray diffractometer PaNalytical X'Pert PRO MPD with a Cu Kα X-ray source ($\lambda = 1.5418$ Å) operating at 40 kV and 30 mA. The phase composition was evaluated by the use of PaNalytical X'Pert HighScore software. It used the ratio between the integrated normalized intensities of the peak of interest and that of a known standard.

A scanning electron microscopy (SEM) analysis was performed with a Vega II/LMU (Tescan) operated at a voltage source of 10 keV. A backscattered electron detector was used to distinguish between the particles. The samples were fractured under cryogenic conditions with liquid nitrogen and coated with gold/palladium by a SC 7640 sputter coater (Quorum Technologies) to reduce any charge buildup if needed. The obtained SEM picture of the PU membrane was consequently used for the fiber diameter/pore size distribution determination with the recently proposed digital image analysis technique.[37]

R of the composite surface was assessed *in vitro* against *E. coli* (ATCC 8739) and *S. aureus* ATCC 6538P as representatives of Gram-negative and Gram-positive bacteria, respectively, according to ISO 22196:2007 (E). Nutrient broth with 1% peptone (M244) and nutrient agar no. 2 (M1269) were used in the test (HiMedia Laboratories). The results are expressed according to the aforementioned standard as the number of viable bacteria per square centimeter of the test specimen (*N*) and *R*:

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t \quad (1)$$

where U_0 is the average of the logarithm of the number of viable bacteria (cells/cm^2) recovered from the untreated test specimens immediately after inoculation, U_t is the average of the

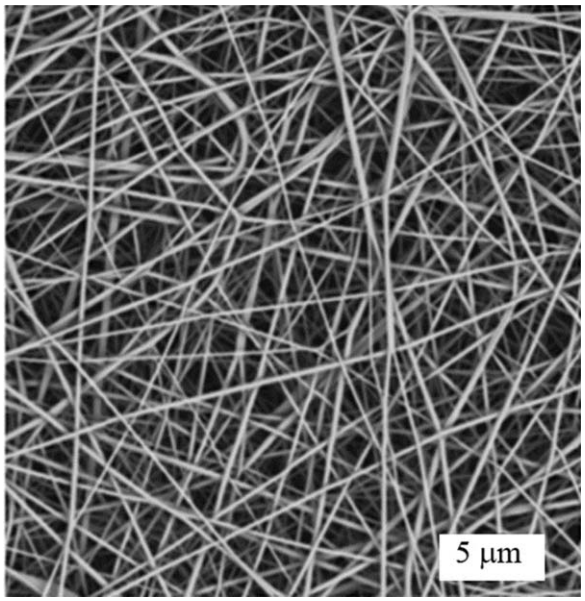

Figure 1. SEM image of the PU nanofiber membrane.

logarithm of the number of viable bacteria (cells/cm^2) recovered from the untreated test specimens, and A_t is the average of the logarithm of the number of viable bacteria (cells/cm^2) recovered from the treated test specimens.

RESULTS AND DISCUSSION

Electrospun PU Membrane and Hybrid Ag/ZnO Filler

The SEM image of the PU membrane prepared here is shown at Figure 1. The membrane was formed from the straight fibers with circular cross sections. The mean fiber diameter was determined to be 182 nm from the measured fiber diameter distribution with a Gaussian function; this represented the measured data very well, as shown in Figure 2. The mean pore size diameter of the membrane according to the distribution of pore sizes (Figure 3) was determined to 227 nm. Under given conditions of electrospinning, the submicrometer membrane thus prepared was ideal for capturing the antibacterial agent particles.

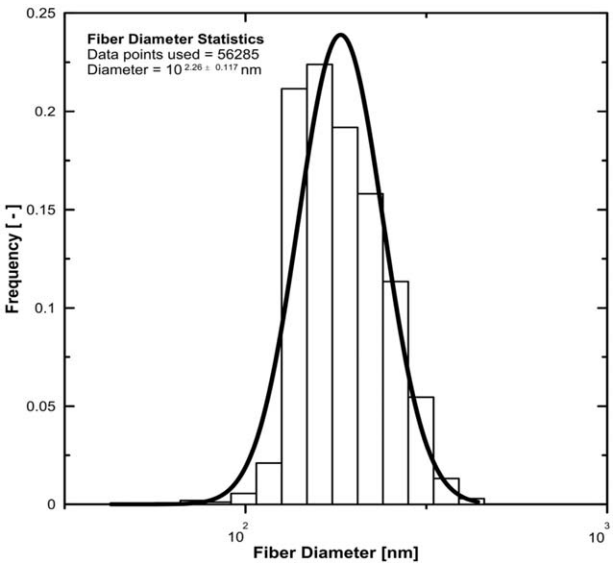

Figure 2. Distribution of the fiber diameters for the PU nanofiber membrane. The columns represent the measured data, and the line represents the Gaussian function fit.

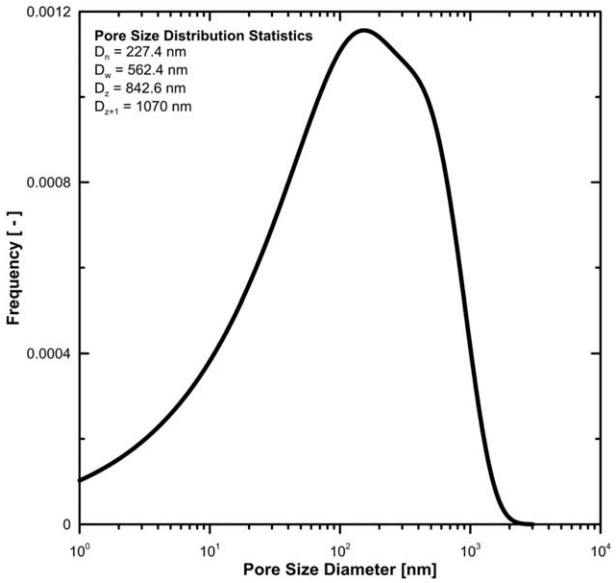

Figure 3. Distribution of the pore sizes for the PU nanofiber membrane. Where D_n, D_w, D_z, D_{z+1}, represents basic pore size averages further defined by appropriate equations in previous paper [42].

As an antibacterial agent, the hybrid Ag/ZnO filler was chosen and prepared. The pattern from the X-ray diffractometer (Figure 4) confirmed the presence of both zinc oxide and silver in the filler. The powder exhibited patterns typical for known positions of diffraction lines of two materials: the hexagonal structure of ZnO (JCPDS-ICDD PDF-2 entry 01-079- 0207) and face-centered-cubic Ag metal (JCPDS-ICDD PDF-2 entry 01-087-0720). All diffraction peaks were assigned by the appropriate structure and reflection plane indices, and no other

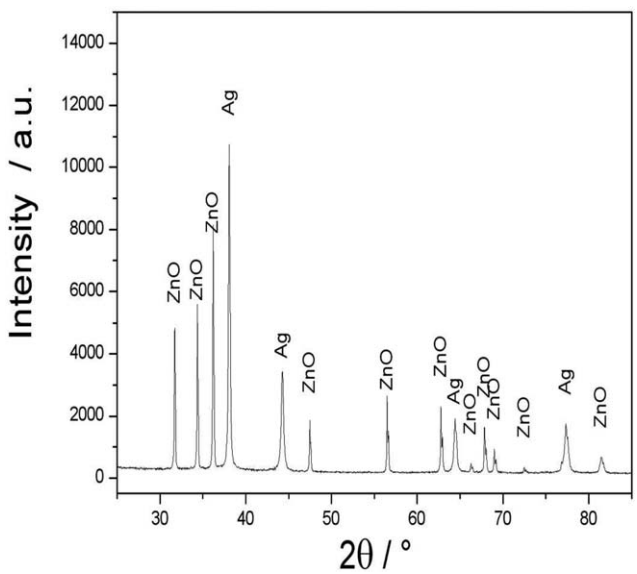

Figure 4. X-ray diffraction of the hybrid Ag/ZnO filler.

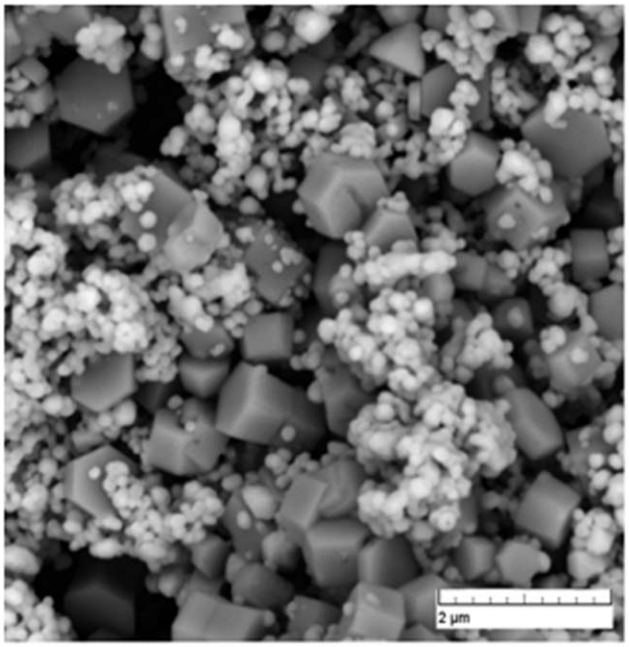

Figure 5. SEM image of the hybrid Ag/ZnO filler.

crystalline phases were found. The crystalline phase composition was estimated to be 71% ZnO and 29% Ag. These findings were consistent with earlier research,[30] although the percentage of each metal was slightly different because of the slightly modified Ag/ZnO filler preparation procedure.

Figure 5 shows the SEM image of the prepared hybrid Ag/ZnO filler. The backscattered electron detector enabled us to distinguish between the material components by color contrast. Materials containing elements with higher atomic numbers were shown to be brighter. Therefore, particles of ZnO and silver were clearly distinguishable. ZnO particles (dark gray in Figure 2) were present and were defined as flat hexagonal prisms. The dimension of the edge base of the hexagonal prism was around 400 nm. Ag nanoparticles (light gray in Figure 5) occurred as clusters of spherically shaped particles with a diameter in the range 150–200 nm. Note that even when the Ag nanoparticle size was lower than the particular pore size of the PU membrane at a given location, they were still trapped at top of the membrane and not passed out because of the fact that the adhesive force (i.e., van der Waals and/or electrostatic interactions) and the friction force between Ag particle and PU nanofibers were higher than the drag and lift forces occurring during the filtration process.[41,42]

Characterization of the Antibacterial Surfaces of the PU Sheets

As mentioned in the introduction, bimetalization can improve the antibacterial properties of an original single-metal filler. Recently, attention has been focused on bimetallic Ag/ZnO nanoparticles embedded into polymers for antibacterial purposes.[30,35,43,44] Antibacterial properties of ZnO nanoparticles have previously been studied,[45,46] but the mechanism of action is not yet fully understood. Unlike the *R* of silver, it is well known. Silver is known as a widely used broad-spectrum biocidal agent; it is effective against bacteria, fungi, and viruses.[47–49]

The silver-release behavior of silver-based antibacterial materials is the most important for the growth of bacterial inhibition. It was reported that concentration levels as low as 0.1 parts per billion could render effective *R*.[50] With respect to the mechanism of action, some researchers[51,52] share the opinion that Ag^+ hinders DNA replication and inhibits the expression of ribosomal proteins and enzymes for ATP (adenosine triphosphate) hydrolysis. It is believed that Ag nanoparticles display the same mechanism as Ag^+ and create a redox imbalance; this causes extensive bacterial death. In this investigation, a bimetallic Ag/ZnO filler was prepared to create an effective antibacterial surface, which acted by a synergistic effect of both metals.

We prepared the antibacterial surface in the first step by filtering the dispersion of Ag/ZnO filler over the PU membrane. Three final concentrations of filler on the membranes were achieved (0.04, 0.08, and 0.42 g/L), and one reference sample without any treatment was also retained. The appearance of the membrane after the filler incorporation is presented at Figure 6(a). The presence of the filler particles was clearly apparent. The concentration of the filler at the displayed surface was 0.42 mg/cm^2.

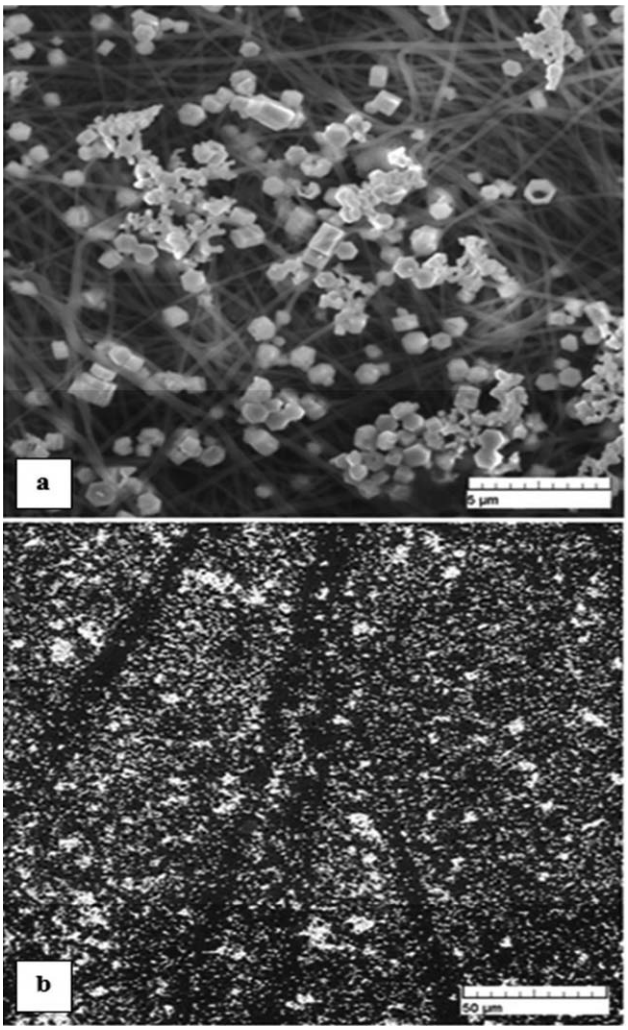

Figure 6. SEM images of the (a) PU nanofiber membrane with the Ag/ZnO filler filtered on it and (b) designed surface with *R*.

Table I. R Values of Ag/ZnO on the PU Surfaces According to ISO 22196:2007

Concentration of Ag/ZnO at surface (mg/cm^2)	N (cfu/cm^2) for *S. aureus*	R	N (cfu/cm^2) for *E. coli*	R
0[a]	5.8×10^4	$U_t = 4.8$	4.8×10^6	$U_t = 6.7$
0.04	9.3×10^1	2.8	7×10^2	3.9
0.08	<1	>4.8	<1	>6.7
0.42	<1	>4.8	<1	>6.7

[a] This was the reference sample.

In the second step, membranes with filler were placed on the PU sheets and hot-pressed. This way, the membranes became an integral part of the PU sheet surfaces. The SEM micrograph [Figure 6(b)] shows the surface of the PU sheet with 0.42 mg/cm^2 Ag/ZnO filler. The random distribution of filler on the whole surface was evident even after hot pressing.

The surfaces prepared as described previously with three different concentrations of Ag/ZnO filler and one reference surface without treatment were subjected to antibacterial testing (see Table I).

In general, silver has a stronger antibacterial performance against Gram-negative bacteria, whereas ZnO provides a better R against Gram-positive types.[30] All of the prepared surfaces with Ag/ZnO filler as an antibacterial agent proved to be more efficient against *E. coli* than against *S. aureus*.

The difference in activity against these two types of bacteria were attributed to structural and chemical differences in the cell-wall composition. *E. coli* is a Gram-negative type of bacteria; it has a more complex cell wall structure, with a layer of peptidoglycan between the outer membrane and the cytoplasmic membrane. *S. aureus* belongs to the group of Gram-positive bacteria, which are characterized by the high level of peptidoglycan in the cell wall and the absence of lipopolysaccharide and outer membrane layer. The difference in the antibacterial action toward *E. coli* and *S. aureus* was assumed to be caused by different sensitivities toward H_2O_2 generated by Ag/ZnO, although the exact mechanisms responsible for the R of silver and ZnO nanostructures are still not fully clear, and the exact cause of the membrane damage requires further study.[30]

With respect to the overall evaluation of R of all of the prepared surfaces, the surface with the lowest concentration of Ag/ZnO (0.04 mg/cm^2) reached an R of about 2.8 against *S. aureus*, whereas against *E. coli,* it was about 3.9. As the critical value of R should not be less than 2.0 for materials that can be categorized as having an effective antibacterial surface,[53] the amount of 2.8 mg of Ag/ZnO filler was sufficient for creating an antibacterial surface. However, it is important that surfaces treated with 0.08 and 0.42 mg/cm^2 of Ag/ZnO filler achieved even higher *Rs*; for example, that against *S. aureus* exceeded 4.8, and that against *E. coli* was higher than 6.7. Thus, all three concentrations of Ag/ZnO were sufficient for creating an effective antibacterial surface on PU.

CONCLUSIONS

The application of surface coatings is generally considered to be a chemical approach to antibacterial surface modification. In this study, a method of surface modification was proposed, in which the antibacterial agent became a part of the surface without chemical modification. Because the filler was incorporated between the fibers of the membrane, it was firmly bound in a thin layer to the surface of PU sheets. The method also enabled an additional treatment of polymer surfaces or products. As an antibacterial agent, the bimetallic Ag/ZnO filler was used. Even at a filler concentration of 0.04 mg/cm^2, the surfaces had an R sufficient to be considered as an effective antibacterial surface, whereas at higher filler concentrations, even higher R values were achieved. For all of the concentrations of filler used, R was higher against *E. coli* than against *S. aureus*.

ACKNOWLEDGMENTS

This article was written with the support of Operational Program Research and Development for Innovations, which was cofunded by the European Regional Development Fund and the national budget of the Czech Republic within the framework of the Centre of Polymer Systems project (registration number CZ.1.05/2.1.00/03.0111).

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