Antibacterial Efficiency of Nanofiber Membranes with Biologically Active Nanoparticles

Daniela Lubasova, and Smykalova Barbora

Abstract—In this study, the antibacterial properties of nanofiber membranes prepared using an electrospinning technique from poly(vinyl alcohol) with incorporated zinc oxide, zirconium dioxide, tin dioxide and titanium dioxide nanoparticles were compared. Scanning electron microscopy and X-ray maps confirmed that all tested nanoparticles were dispersed homogenously whereas energy-dispersive X-ray spectra confirmed a semi-quantitative estimation of the nanoparticles amount presented in the nanofiber membranes. Antibacterial properties of prepared nanofiber membranes were evaluated by a modified AATCC test method 100-2004 using both gram-negative Escherichia coli and gram-positive Staphylococcus aureus bacteria. All results showed that the nanofiber membranes with zinc oxide and zirconium dioxide nanoparticles exhibit antibacterial properties against both tested microorganisms.

Keywords—Nanofibers, nanoparticles, antibacterial efficiency, electrospinning.

I. INTRODUCTION

The human society co-exists and constantly interacts with the world of microorganism. Unfortunately, the modern life is characterized by objective increase of infections. Many factors and indicators of infection activation are subject to revision because of changing relations between the agent and its host organism. Moreover, there are microorganisms resistant to the majority of antibiotics and antiseptics [1-3]. Therefore the necessity of new effective bactericidal products is obvious. Processing and use of biocompatible nanomaterials with antimicrobial properties are believed to be perspective from a long-term point of view. On that account, the application of advanced nanocompositions based on water soluble polymers and the use of biologically active metals (copper, zinc, zircon etc.) seems to be desirable. [4-7]

Several papers have reported that various types of nanoparticles or additives can be entrapped in the nanofiber membranes or encapsulated within the nanofibers. In addition, by varying of additives and their chemical base it is possible to obtain different characteristics of nanofiber membranes. The incorporation of antimicrobial nanoparticles into nanofibers prepared using the electrospinning process has attracted a great deal of attention because of almost unlimited variability of nanofiber membranes properties. [8-10] An electrospinning process is a well-known and simple but efficient technique to produce nanofibers with unique properties such as high surface area, high porosity, and diameters of fibers in the nanoscale. [11-13]

Herein, the incorporation of titanium dioxide (TiO$_2$), zinc oxide (ZnO), zirconium dioxide (ZrO$_2$) and tin dioxide (SnO$_2$) nanoparticles into nanofiber membranes made of polyvinyl-alcohol (PVA) was studied. Morphology of nanofiber membranes and distribution of incorporated nanoparticles were characterized by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). Antimicrobial activity of individual nanofiber membranes were tested as well. Different inhibition effects against gram-positive Staphylococcus aureus (S. Aureus) and gram-negative Escherichia Coli (E. Coli) bacteria were observed.

The nanofiber membranes with incorporated antibacterial nanoparticles can be potentially used in several fields such as bacterial air filtration, sanitary means and cosmetics, antibacterial textiles, and so forth.

II. MATERIALS AND METHODS

A. Materials

PVA (Slovio1 R16) was obtained from Chemical Company Novaky-SK and used as received. All nanoparticles (TiO$_2$, ZnO, ZrO$_2$ and SnO$_2$) with average diameter under 100 nm were purchased from Sigma Aldrich. For consistent distribution of nanoparticles in PVA solution was used surfactant Triton X-100 obtained from Sigma Aldrich. For the bacterial test, E. Coli (ATCC 8739) and S. Aureus (ATCC 6538) were purchased from Fisher Company.

B. Electrospinning of PVA nanofiber membranes with incorporated nanoparticles

10wt.% PVA solution was prepared by dissolving PVA in DI water under magnetic stirring at room temperature. For consistent distribution of nanoparticles, 1wt.% of surfactant Triton X-100 was added into PVA solution. Nanoparticles (TiO$_2$, ZnO, ZrO$_2$ and SnO$_2$) were mixed individually in PVA solution in different concentrations: 1, 5 and 10wt.%.

The polymer solutions were placed to a plastic syringe and then charged with a high voltage of 20 kV through a metal syringe needle (21 gauges). Fibers were electrospun at the needle tip and collected on a spun-bond mounted onto collector. The collector was electrically ground and placed 10 cm away from the tip of the needle. The flow rate of the polymer solution was controlled at 0.8 ml/hr by a syringe pump.

Ing. Daniela Lubasova, Ph.D., Technical University of Liberec, Czech Republic. (Email id: daniela.lubasova@tul.cz)

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The nanofiber membranes used for further analysis were about 300 μm in thickness. For comparison, pure PVA nanofiber membrane without any antibacterial nanoparticles was also fabricated using the same procedure. After electrospinning, the samples were stored in a desiccator for further characterization.

C. SEM, EDS and EDX mapping of nanofiber membranes with incorporated nanoparticles

Fiber formation and morphology of the electrospun PVA nanofiber membranes with nanoparticles were determined using a scanning electron microscope (SEM) ULTRA PLUS (Carl Zeiss STM AG). Distribution of nanoparticles and their concentration in nanofiber membrane was detected by EDS detector (X-Max 20) with software Smart SEM Version 5.05, AZtec 2.1. SEM and EDX maps of nanofiber membranes were taken under nitrogen atmosphere, without any previous coating.

D. Antibacterial test of nanofiber membranes with incorporated nanoparticles

*Coli* and *S. Aureus* were cultivated in sterilized LB broth and then incubated overnight at 37 °C within a shaking incubator. The bacterial suspensions employed for the tests contained from 10² to 10³ colony forming units (CFU). For the antibacterial test, nanofiber membranes with nanoparticles were cut into circular swatches 4.8 cm in diameter and sterilized in an oven for 60 min at 80°C. A nanofiber membrane without any antimicrobial nanoparticles was used as a blank specimen. Sterilized nanofiber membranes were individually placed into a sterilized test tube and inoculated with 30ml of *E. coli* or *S. Aureus* bacterial suspension. In “0” contact time and after 24hours, 600 μL of bacterial suspension was extracted and quickly spread on Tryptic Soy agar plates. The number of viable *E. coli* or *S. Aureus* was determined by plating the extracted solution onto the Tryptic Soy agar plates and counting colonies after 24 hrs of incubation at 37 °C.

The percentage reduction of test microorganisms in test tubes with nanofiber membranes after 24 hours was calculated using the Eqn. 1.

$$R(\%) = 100 \cdot \frac{(B - A)}{B}$$  \hspace{1cm} (1)

where R is the reduction of test microorganism in percentage; A is the number of bacteria recovered from the inoculated nanofiber membrane with the nanoparticles in the test tube after 24 hours, and B is the number of bacteria recovered from the inoculated nanofiber membrane with the nanoparticles in the test tube at “0” contact time.

III. RESULTS AND DISCUSSION

A. Morphology of nanofiber membranes and homogeneity of incorporated nanoparticles

Fig. 1 shows the SEM and EDX (1st and 2nd line, respectively) maps of electrospun nanofiber membranes PVA with 1 and 10wt.% of SnO₂, TiO₂, ZnO, ZrO₂ nanoparticles. In all cases, nanofibers were elongated and straight with relatively homogenous diameters ranging from 150 to 350nm.

In case of 1wt.% of nanoparticles incorporated into PVA polymer solution, most of nanoparticles were not observed on the surface of nanofibers and therefore it was assumed that most of nanoparticles were encapsulated within the nanofibers. In contrast, SEM with nanofiber membranes containing 10wt.% of nanoparticles indicates that nanoparticles were encapsulated within the nanofiber and furthermore they created bigger clusters on the top of nanofibers. The biggest clusters created nanoparticles ZnO in comparison to the other tested.

![SEM and EDX maps of PVA nanofiber membranes with incorporated nanoparticles: (a) SnO₂, (b) TiO₂, (c) ZnO and (d) ZrO₂](image-url)

For better understanding of the homogeneity of nanoparticles inside nanofibers, EDX maps were taken, see Fig. 1 (the 1st line images). EDX mapping (also called element...
maps) provides, in addition to the conventional SEM image, a meaningful picture of the elements distribution in the nanofibers which are shown as colored maps representing elements distribution. All EDX maps confirmed that nanoparticles inside nanofibers were distributed homogenously. A higher color intensity of the EDX maps corresponds to the higher concentration of elements in the nanofiber membrane. From the maps it is clearly visible that the higher amount of nanoparticles (elements) incorporated into PVA solution before electrospinning corresponds to the higher amount of nanoparticles (elements) found in the nanofibers after electrospinning. Unfortunately, EDX mapping is entirely a qualitative method, therefore quantitative confirmation of nanoparticles amount in nanofiber membranes was analyzed by EDS.

B. EDS analysis of nanofiber membranes with incorporated nanoparticles

The EDS analysis of nanofiber membranes with a different concentration of incorporated nanoparticles (1, 5 and 10wt.%) was carried out, see Fig. 2.

The presence of Ti, Zn, Zr and Sn peaks in EDS spectra indicates the successful incorporation of nanoparticles in PVA nanofiber membrane through electrospinning technique. Additionally, a higher intensity of peaks corresponds very well with increasing concentration of incorporated nanoparticles.

Subtracting the background, the X-ray counts (y-axe in EDS spectrum, Fig. 2) of the elements Ti, Sn, Zn or Zr in PVA nanofiber membranes are proportional to the amount of TiO$_2$, ZnO, ZrO$_2$ and SnO$_2$ nanoparticles dispersed in the PVA solution before electrospinning process. Instance, the incorporation of 1, 5 and 10wt.% of SnO$_2$ into PVA polymer solution, the X-ray counts in EDS spectrum of nanofiber membrane reached for element Sn values 0.42, 2.4 and 4.8 cps/eV, respectively. Similar results were observed in case of nanofiber membranes with 1, 5 and 10wt.% of TiO$_2$, ZrO$_2$, ZnO$_2$ nanoparticles. All EDS spectra confirmed a semi-quantitative estimation of the amount of nanoparticles in the nanofiber membranes after electrospinning.

![Fig. 2 EDS spectra of PVA nanofiber membranes with incorporated nanoparticles: (a) SnO$_2$, (b) TiO$_2$, (c) ZnO and (d) ZrO$_2$.](image)

C. Antibacterial properties of nanofiber membranes with incorporated nanoparticles

Antibacterial properties of PVA nanofiber membranes with TiO$_2$, ZnO, ZrO$_2$ and SnO$_2$ nanoparticles were analyzed by a modified AATCC method 100-2004 (Antibacterial finishes on textile materials). Two tested microorganisms representing both gram positive (S. Aureus) and gram negative (E. Coli) were chosen. Fig. 3 shows the reduction of test microorganisms R caused by increasing concentration of TiO$_2$, ZnO, ZrO$_2$ and SnO$_2$ nanoparticles entrapped in PVA nanofiber membranes.

In both cases of tested microorganisms, number of bacteria recovered from the inoculated nanofiber membrane with the nanoparticles in the test tube after 24hours was reduced with increasing concentration of nanoparticles. However, the reductions of tested microorganisms R were different and depended on the type of nanoparticles in nanofiber membranes. The reduction of E. coli in bacterial suspension reached 100% when PVA nanofiber membrane with 10 wt.% of ZnO nanoparticles was in contact with tested suspension for 24 hrs. In addition, nanofiber membrane with 10 wt.% of ZrO$_2$ was able to reduce about 90% of bacteria E. Coli in bacterial suspension, whereas SnO$_2$ had a minimal impact on the reduction of bacteria E. Coli.

In contrast, nanofiber membrane with 10wt.% of SnO$_2$ reduced about 81% of bacteria S. aureus in bacterial suspension. The best antimicrobial activity against S. Aureus showed nanofiber membrane with 10wt.% of ZrO$_2$, where the reduction of bacteria in bacterial suspension reached almost 99% after 24hrs. Blank control, pure PVA nanofiber membrane without any nanoparticles did not evince any antimicrobial activity.

These results confirmed that nanofiber membranes with incorporated nanoparticles enhanced antimicrobial activity against bacteria E. Coli and S. Aureus compared to the pure PVA nanofibers without any nanoparticles. Both ZnO and ZrO$_2$ nanoparticles exhibit antibacterial properties against both tested microorganism.
IV. CONCLUSION

PVA nanofiber membranes with incorporated nanoparticles were prepared using electrospinning technique. The homogenous distribution of nanoparticles TiO$_2$, ZnO, ZrO$_2$, SnO$_2$ entrapped by nanofiber membranes was confirmed by EDX mapping along with the increasing concentration of incorporated nanoparticles. The PVA nanofiber membranes with all tested nanoparticles enhanced antibacterial activity against both gram-negative *E. Coli* and gram-positive *S. Aureus* bacteria based on the bactericidal properties of nanoparticles. It was found out that nanofiber membrane with SnO$_2$ exhibited antimicrobial properties only against *S. aureus* whereas nanofiber membranes with ZnO or ZrO$_2$, exhibited antimicrobial properties against both bacteria, particularly *E. Coli* and *S. aureus*. These results suggest that PVA nanofiber membranes with incorporated TiO$_2$, ZnO, ZrO$_2$ or SnO$_2$ nanoparticles have a potential to be used for many different environments where bacteria are harmful such as an air-conditioning system in hospitals, variable antibacterial products for medicine, sanitary and antibacterial textiles etc.”

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