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# Formation and Antibacterial Property Analysis of Electrospun PVA Nonwoven Material with a Small Amount of Silver Nanoparticles

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#### Abstract

Electrospinning is a process which produces nonwoven material from nano/microfibres. The aim of this study was to electrospin poly(vinyl alcohol) nano/microfibres material with small amount of silver nanoparticles (Ag-NPs), and estimate the influence of Ag-NPs on the structures and antibacterial properties of nonwoven materials. It was found that the Ag-NPs concentration does not have a significant influence on the viscosity and morphologies of electrospun materials. The addition of Ag-NPs causes the formation of thinner PVA nano/microfibres. The antibacterial activity test showed that a small amount of Ag-NPs provides antibacterial properties to electrospun PVA nano/microfibres. Antibacterial properties were tested with different bacteria: Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis ATCC 12228, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Proteus mirabilis ATCC 12453, Bacillus cereus ATCC 11778 and Candida albicans ATCC 10231. It was also noticed that the concentration of Ag-NPs in nonwoven materials affects antibacterial properties. Clearly superior antibacterial properties were obtained from nonwoven materials with a higher concentration of Ag-NPs.

Key words: electrospinning, Ag nanoparticles, antibacterial materials.

### Introduction

Electrospinning is a process which produces nonwoven materials from nano/microfibres. Electrospun nonwoven materials from polymer solution have unique properties such as a large surface area of fibres, high porosity, diameters of nanoscale, and small pore size. Due to these properties electrospun nonwoven materials are broadly applied in biomedical applications, such as tissue engineering scaffolds, wound healing, drug delivery, filtration, protective materials, sensors and e.c. [1 - 4].

In electrospinning, many factors are manipulated in order to generate material with different geometrical and structural properties. These include electrospinning parameters such as electric field strength, the distance of the electric field generated, the length and radius of the spinneret, the solution flow rate and solution parameters such as concentration, viscosity, ionic strength and conductivity [5].

Polymer nanocomposites have attracted a great deal of attention due to their unique optical, electrical, catalytic and antimicrobial properties, which enable them to be used in novel applications such as sensors, catalysts and antibacterial materials [6]. Nanoparticles exhibit completely new or improved properties based on specific characteristics, such as size, distribution, and morphology. New applications of nanoparticles and nanomaterials appear

to be emerging rapidly. Applications have been found for silver nanoparticles (Ag-NPs) in the high-sensitivity fields of biomolecular detection and diagnostics, antimicrobials and therapeutics, as well as catalysis and microelectronics [7, 8]. Silver nanoparticles (Ag-NPs) represent one of the most extensively studied nanomaterials, due to their unique optical, catalytic, sensing and antimicrobial properties [9]. Ag-NP has been widely used in different kinds of textile materials in order to provide antibacterial properties. It has been formed from chitosan (CS) fibres with Ag-NPs [10], cellulose and N-methylmorpholine-N-oxide (NMMO) (ALCERU) fibres [11], woven fabric from cotton yarns with silver finishing [12], polyester (PES) and polyamide (PA) fabrics with loaded Ag-NPs [13], as well as wool and silk fibres with Ag-NPs [14, 15]. Also Ag-NPs has been inserted in the following electrospun nonwoven materials: chitosan-gelatin fibres, poly (methyl methacrylate) (PMMA) nanofibre, poly(ε-caprolactone)-based polyurethane (PCL-PU), cellulose acetate, poly(N-vinylpyrrolidone) (PVP), poly lactic-co-glycolic acid (PLGA), polyurethane (PU), silicon dioxide (SiO<sub>2</sub>) and poly(vinyl alcohol) (PVA) nanofibres containing Ag-NPs [16 - 25].

Abdelgawad A.M. and et al. [8] investigated the production of antimicrobial nanofibre materials by means of typical electrospinning equipment, which could be used as materials for wound dressing

via the green method, which consisted of two steps: (1) preparation of Ag-NPs in high yields and highest possible concentration of enveloping chitosan with a green reducing agent and, (2) electrospinning of the chitosan-Ag-NPs/PVA blend. The authors noticed that the presence of Ag-NPs in the polymer blend solution of PVA and CS improved the electrospinnability of the blend. It was also noticed that Ag-NPs in the blend solution resulted in a reduction in the diameter of the fibres. The antibacterial experiment indicated that materials electrospun from PVA/CS and PVA/CS-Ag-NPs blends had good bactericidal activity against bacteria E. coli. Better antibacterial activity against E.coli was obtained from PVA/CS-Ag-NPs material [8].

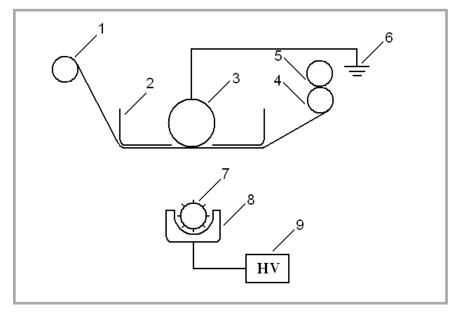
Zhijun Ma with co-authors [21] investigated the morphology, structure, texture, environmental stability and antibacterial ability of Ag-NPs decorated SiO2 nanofibres. In the preparation of SiO2 nanofibres (SNFs) using electrospinning, no polymer was used. The SiO2 sol was synthesized with the sol-gel technique, thus the calcination for removal of the polymer was circumvented. Ag-NPs were loaded on the fibre surface simply by incubating the SNFs in silver nitrate solution to obtain antibacterial ability. The SiO<sub>2</sub> nanofibres with Ag exhibited efficient inhibitory ability against proliferation of E. coli. Cytotoxicity investigation indicated that the SiO<sub>2</sub> nanofibres with Ag not only have no toxicity to human cells, but also can promote the growth of human cells in a wide concentration range [21].

Khalil with co-authors [22] fabricated and characterised biodegradable PLGA nanofibres containing Ag-NPs polymeric matrix scaffolds. The results showed that PLGA/Ag-NPs nanofibre can be easily electrospun to form smooth nanofibres [22].

A preparation of 12 wt% PVA nanofibres [24] containing various amounts of AgNO<sub>3</sub> (0; 0.5; 1; and 2 wt% of AgNO<sub>3</sub>) was investigated for use in antibacterial applications. Nanofibres were electrospun from refluxed PVA/AgNO<sub>3</sub> aqueous solutions. It was estimated that average diameters decreased as the conductivities of the PVA solution increases an increase in AgNO<sub>3</sub> concentration in the polymer solution [24]. The antibacterial activity of electrospun materials in this study was not investigated.

H. G. Kim with co-authors [25] investigated PVA/Ag-zeolite nanofibre. PVA/Ag-zeolite blend solutions were prepared by mixing bulk PVA (7 wt%) and different amounts of Ag-zeolite (0.5, 1, 3, and 5 wt % by weight based on PVA). According to literature [25], such electrospun PVA materials with Ag particles may be used for medical application in wound dressing as well as filters in medical devices.

Poly(vinyl alcohol) (PVA) polymer is water soluble, non-toxic and biocompatible. The incorporation of nanoparticles in polymer nanofibres has a great deal of attention because nanoparticles could provide new properties such as optical, electronic, catalytic and antimicrobial. From other studies, it is known that the incorporation of metal nanoparticles into polymer nanofibres can be achieved by incorporating metal nanoparticles into polymer solution, or reducing metal salts or complexes in electrospun polymer nanofibres. The aim of this study was to form nonwoven material from PVA nanofibres containing antibacterial Ag-NPs by means of roller type "Nanospider TM" electrospinning equipment and to investigate the influence of a small amount of Ag-NPs concentration on the structure and antibacterial properties of electrospun PVA materials. In this study a simple method was used for the preparation of nano/microfibres containing silver nanoparticles (Ag-NPs). The polymer



**Figure 1.** "NanospiderTM" electrospinning equipment: 1, 4, 5 – rollers of support material; 2 – frame of grounded (6) electrode; 3 – grounded electrode; 7 – electrode with tines; 8 – tray with polymer solution; 9 – high voltage supply.

solution was prepared by mechanically mixing water-based silver colloidal and PVA solution. The novelty of this method is that we do not need an additional process, such as precipitation—redissolution and photoreduction, which were used in other investigations [24, 25, 27], or any external reducing agents. Nonwoven materials with antibacterial properties formed by this type of equipment allows them to be applied in biomedical applications such as antibacterial wound dressing and antibacterial filter for medical devises.

### Experimental

#### Materials and methods

Poly(vinyl alcohol) (PVA) nonwoven materials containing Ag-NPs were produced by the electrospinning process. Electrospinning solution was prepared by dissolving PVA (Mw = 61000 g/mol) (Sigma Aldrich, UK) polymer in distilled water and stirring for 4 hours by magnetic stirring equipment - Yellow Line MSH basic (Germany) with heating (80 °C).

In this study two different concentrations of Ag-NPs suspensions were mixed with PVA polymer solutions. Colloidal Ag-NPs suspensions were prepared by dissolving and heating different concentrations (0.02 wt% and 0.04 wt%) of AgNO<sub>3</sub> (Girochem, Slovenia) and 5 wt% sodium citrate (C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> Na<sub>3</sub>·2H<sub>2</sub>O) (Lach-Ner, Czech Republic) in distilled water. In this way colloidal suspensions

with Ag-NPs (the diameter range of the particle produced was about 60 nm) were prepared. The diameter of nano particles was estimated using Malvern ZetaSizer Nano S equipment (Malvern Instruments, Ltd., Great Britain). Six types of electrospun solutions were prepared: (A) pure PVA solution (concentration of PVA in solution C = 15 wt%), (B) PVA with 0.02 wt% Ag-NPs (PVA C = 15 wt%), (C) PVA with 0.04 wt% Ag-NPs (PVA C = 15 wt%), (D) pure PVA solution (concentration of PVA in solution C =11 wt%), (E) PVA with 0.02 wt% Ag-NPs (PVA C = 11 wt%), (F) PVA with 0.04 wt% Ag-NPs (PVA C = 11 wt%). The PVA concentrations were chosen according to literature sources [24, 25]. The amount of PVA in the polymer solution varies from 5 to 12 wt%. According to these values, we chose the following concentrations of PVA polymer: 11 wt%, 15 wt%. From our experience, this concentration of PVA is optimal to form nonwoven materials from nano/microfibre without bead and polymer solution spots. The viscosity of the electrospun solution was estimated by a viscometer -BROOKFIELD DV-II+ Pro Viscometer.

Nonwoven materials from nano/microfibres were formed using 'Nano-spider<sup>TM</sup>' (Elmarco, Czech Republic) electrospinning equipment (*Figure 1*). Nano/microfibre from PVA solution were formed at the applied voltage U = 70 kV, the distance between electrodes L = 13 cm, the temperature of the envi-

**Table 1.** Average of viscosity  $(\eta)$  of PVA solutions with different concentrations: \*- the viscosity varies within margins of error. Because of this reason the results are not discussed.

Code of the samples	Polymer solution	Viscosity ± ∆, mPa·s
A	15% PVA without Ag-NPs	656 ± 69
В	15% PVA/0.02% Ag-NPs	680 ± 138
С	15% PVA/0.04% Ag-NPs	792 ± 0*
D	11% PVA without Ag-NPs	272 ± 34
E	11% PVA/0.02% Ag-NPs	240 ± 69
F	11% PVA/0.04% Ag-NPs	184 ± 34

ronment  $T = 20 \pm 2$  °C, and the air humidity was  $\varphi = 42 \pm 4$  %.

The structure of the electrospun non-woven materials was determined using a scanning electron microscope - SEM S-3400N (Hitachi, Japan). Experiments were carried out consistently at the same time, and if there was an environmental impact of a change, it would be the same for all samples. The diameter of nano/microfibres was evaluated using SEM

images and software Lucia Image 5.0. The infrared spectrum of absorption was obtained using a Fourier transform infrared spectrometer - FT-IR Spectrum GX (Perkin Elmer, USA).

Fourier transform infrared spectroscopy (FT-IR) was used in order to determine the existence of functional groups of PVA nano/microfibres. Spectra of the fibre were obtained using an IR spectrometer (FT-IR Spectrum GX, Perkin Elmer).

About 1 mg of the sample, which was in powder form, was mixed with 200 mg of potassium bromide (KBr) and vacuum pressed into tablet form. FT-IR spectra of the sample were collected in the range of 4000 - 630 cm<sup>-1</sup>.

Antimicrobial activity of the nonwoven materials from PVA nano/microfibres with Ag-NPs was determined at the Institute of Microbiology and Virulogy of the Lithuanian University of Health Sciences.

### Antimicrobial susceptibility tests

The antibacterial and antifungal activity was tested in vitro using the agar diffusion method in Mueller-Hinton II agar medium (BBL, Cockeysville, USA). Antimicrobial activity of nonwoven materials from PVA nano/microfibres with AgNPs was tested in vitro in these standard bacterial cultures: *Staphylococcus au*-

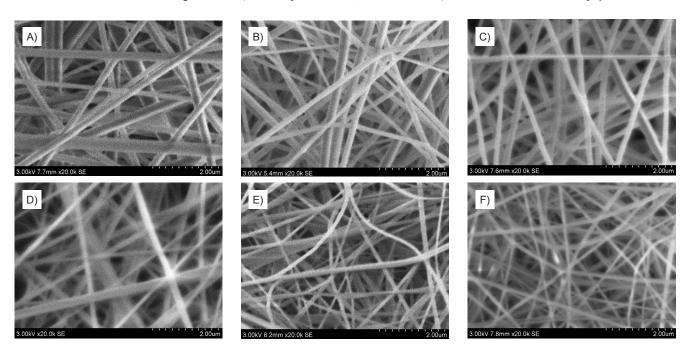
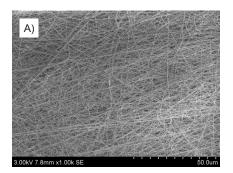
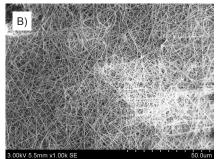


Figure 2. SEM images (scale 2  $\mu$ m) of electrospun PVA materials at applied voltage U=70~kV, distance between electrodes L=13~cm, concentration of PVA and PVA/Ag-NPs solutions C, %: A - 15% PVA/B - 15% PVA/D0.02%; C - 15% PVA/D0.04% Ag-NPs; D - 11% PVA/D0.04% Ag-NPs; D0.04% Ag-NPs.





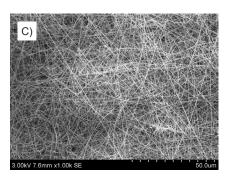


Figure 3. SEM images (scale 50  $\mu$ m) of electrospun PVA materials at applied voltage U=70~kV, distance between electrodes L=13~cm, concentration of PVA and PVA/Ag-NPs solutions C, %: A-15% PVA; B-15% PVA/0.02%; C-15% PVA/0.04% Ag-NPs.

reus ATCC 25923, Staphylococcus epidermidis ATCC 12228, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Proteus mirabilis ATCC 12453, Bacillus cereus ATCC 11778 and fungal culture Candida albicans ATCC 10231.

### Preparation of standard microorganism cultures

Standard cultures of nonsporic bacteria *Staphylococcus aureus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis* were cultivated for 20 - 24 h at a temperature of 35 °C in Mueller-Hinton Agar medium (Mueller-Hinton II Agar, BBL, Cockeysville, USA). A bacterial suspension was prepared from bacterial cultures cultivated in physiological solution according to turbidity standard 0.5 McFarland.

Standard culture of sporic bacteria *Bacillus cereus* was cultivated for 7 days at a temperature of 35 - 37 °C in Mueller-Hinton II Agar medium. After sporic bacteria culture had grown, it was washed away from the surface of the broth with sterile physiological solution, then heated for 30 min at a temperature of 70 °C and diluted till the concentration of spores in 1 ml ranged from 10×106 to 100×106. Sporic suspension can be kept for a long time at a temperature below 4 °C.

The standard fungal culture *Candida albicans* was cultivated for 20 - 24 h at 30 °C in Mueller-Hinton II Agar medium (Mueller-Hinton II Agar, BBL, Cockeysville, USA). A fungal suspension was prepared from the fungal cultures in cultivated physiological solution according to the turbidity standard 0.5 McFarland.

### Preparation of compounds investigated for antimicrobial activity

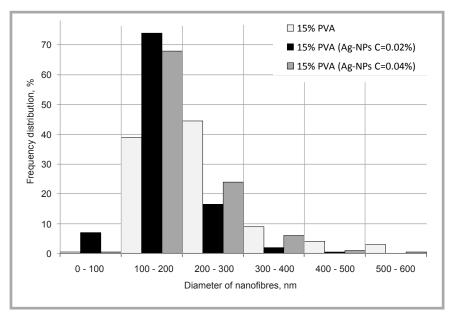
A sample of nonwoven materials from PVA nano/microfibres with and without Ag-NPs was cut to small 8 × 10 mm sized sheets. All samples were sterilised by ultraviolet rays. Microbial cultures (cell suspension of 1×108 cells/ml) were inoculated with a sterile swab onto the surface of Mueller-Hinton agar on a Petri dish. Later on the sterilised nonwoven materials from PVA nano/microfibres with Ag-NPs samples were gently placed on Mueller-Hinton agar with microbial culture sown in sterile conditions. Samples in Muller agar were incubated at 36 °C for 24 h (for bacteria) and at 30 °C for 24 h (for fungi). Samples from nonwoven materials of PVA nano/microfibres with Ag-NPs antimicrobial activity was determined from the sterile area of the sample.

#### Results and discussion

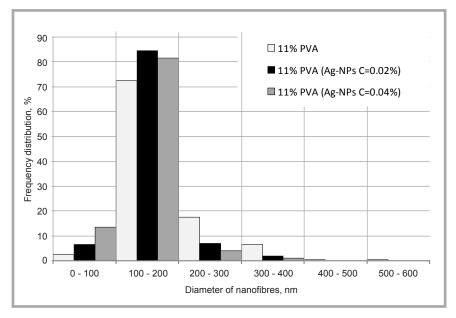
### Structure analysis of electrospun materials from PVA with a small amount of Ag-NPs

In this study the influence of Ag-NPs on the structure and properties of electrospun PVA materials was investigated. The viscosity of the polymer solution with different concentrations analysed is presented in *Table 1*. From the data presented in *Table 1* it is possible to notice that the amount of Ag-NPs concentration (compare sample B with C & E with F) does not have a significant influence on the viscosity of the electrospinning solutions. Naturally only the amount of PVA concentration has an influence on the solution viscosity, which increases 3 - 4 times when the concentration of PVA polymer in the solution increases from 11% to 15% (compare B with E & C with F).

Figures 2 & 3 presents the structure of the electrospun nonwoven materials. In all cases analyzed materials consist of uniform fibres. From the histogram pre-



**Figure 4.** Distribution of 15% PVA and 15% PVA/Ag-NPs nano/microfibers diameter at applied voltage U = 70 kV, distance between electrodes L = 13 cm.



**Figure 5.** Distribution of 11% PVA and 11% PVA/Ag-NPs nano/microfibers diameter at applied voltage U = 70 kV, distance between electrodes L = 13 cm.

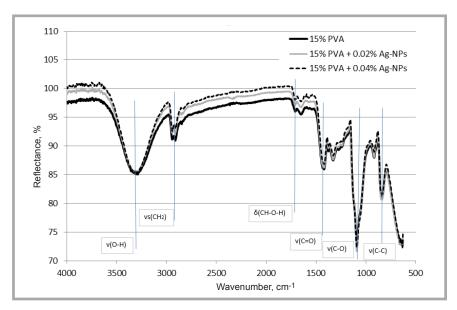


Figure 6. FT-IR spectra of PVA and PVA/Ag-NPs nano/microfiber materials.

sented in Figures 4 and 5 we can notice that the addition of Ag-NPs causes the formation of thinner (fibres with a diameter up to 200 nm) PVA fibres, but the morphology of electrospun nonwoven materials (Figure 2, Figure 3) were quite similar. From pure PVA of 15% concentration, the polymer solution (sample A) 39.5% of fibres with a diameter up to 200 nm were measured, and from PVA (C = 15%) solutions with 0.02% and 0.04% Ag-NPs, consequently, 81% and 75% of fibres with a diameter up to 200 nm were measured (Figure 4). When the concentration of pure PVA solution was 11% (D) 75% of fibres with a diameter up to 200 nm were measured, and from PVA (C = 11%) solutions with 0.02% and 0.04% Ag-NPs, consequently, 91% and 95% of fibres with diameter up to 200 nm were measured (Figure 5). Analogical results were presented in others scientific works [18, 24]. The addition of Ag-NPs in PVA solution causes an increase in solution conductivity, i.e, increases the charge density in the ejected jets and, thus, stronger elongation forces are imposed on them due to the self-repulsion of the excess charges under the electrical field, resulting in the electrospun fibres having smaller diameter [18, 25]. The concentration of Ag-NPs (0.02% and 0.04%) in electrospun PVA solutions does not have a significant influence on the diameter and structure of the electrospun materials.

From the results presented it is possible to notice that from PVA of 15% concentration almost two times fewer fibres with a diameter up to 200 nm were

formed (39.5% of fibres up to 200 nm) nor from pure PVA of 11% concentration (75% of fibres with a diameter up to 200 nm). These results are influenced by the different viscosity of the PVA solutions analysed. When PVA solution concentration C = 15%, the viscosity of the solution was  $\eta = 656 \pm 69$  mPa·s, and when PVA concentration C = 11%,  $\eta = 272 \pm 34$  mPa·s. The same tendency can be observed in the other authors' research [1, 22, 25]. The viscosity directly affects the diameter of nanofibres. With decreasing concentration of the solution, the viscosity and surface tension of the polymer solution also decrease. Lower viscosity and surface tension affect the formation of thinner fibre.

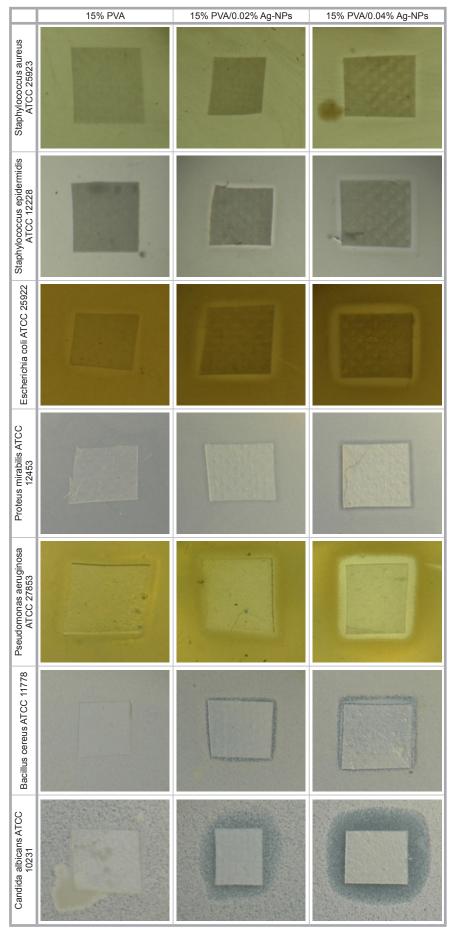
## FTIR analysis of electrospun materials of PVA with a small amount of Ag-NPs

FTIR analysis allows to identify functional groups of manufactured PVA nano/microfibres. FT-IR spectra (Figure 6) of nano/microfibres of PVA, PVA/ Ag-NPs (Ag-NPs C = 0.02 wt%) and PVA/Ag-NPs (Ag-NPs C = 0.04 wt%) showed dominant absorption peaks at 3305, 2940, 1420, 1709, 1092 and 838 cm-1, which were respectively attributed to v(O-H),  $vs(CH_2)$ ,  $\delta(CH-O-H)$ , v(C=O), v(C-O) and v(C-C) of pure PVA [26]. The PVA/Ag-NPs (Ag-NPs C =0.02 wt%) and PVA/Ag-NPs (Ag-NPs C = 0.04 wt%) nano/microfibre materials exhibited, in general, the same peaks as pure PVA nano/microfibre materials. The similarity of these peaks could be due to the low amount of Ag-NPs and sodium citrate (C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> Na<sub>3</sub>·2H<sub>2</sub>O). Citic acid was used in the preparation procedure for silver nanoparticles. Citric acid was used in a very small amount and may be the reason why FTIR does not show additional functional groups in nonwoven material with silver particles.

# Antibacterial properties of electrospun materials of PVA with a small amount of Ag-NPs

The results of antimicrobial activity showed that nonwoven materials from PVA nano/microfibres with even a small amount 0.02% and 0.04% Ag-NPs can be characterised as having antimicrobial and antifungal activity (Figure 7). PVA/Ag-NPs (Ag-NPs C = 0.04%) is antimicrobially effective compared with PVA/Ag-NPs (Ag-NPs C = 0.02%) when tested against prokaryotic microorganisms such as Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis **ATCC** 12228, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Proteus mirabilis ATCC 12453, Bacillus cereus ATCC 11778 and eukaryotic microorganism fungal culture Candida albicans ATCC 10231. Staphylococcus aureus ATCC 25923 and Staphylococcus epidermidis ATCC 12228 are gram-positive bacteria that have a cell wall with a thick layer of peptidoglycan. Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and Proteus mirabilis ATCC 12453 are gram-negative bacteria with two layers of the cell wall. Bacillus cereus ATCC 8035 is a spore-forming bacterium. Characteristics of these microorganisms suggest that antimicrobial activity of the preparations tested may differ. The highest antimicrobial activity was observed for nonwoven materials from PVA nano/microfibres with 0.04% Ag-NPs against gram-negative bacteria - Escherichia coli and Pseudomonas aeruginosa. Antimicrobial activity of nonwoven materials from PVA nano/ microfibres with 0.04% Ag-NPs against gram-positive bacteria Staphylococcus aureus and Staphylococcus epidermidis was significantly lower compared with that against gram-negative bacteria. Nonwoven material of PVA nano/microfibres with 0.04% Ag-NPs effectively inhibits growth of spore-forming bacteria Bacillus cereus and fungi Candida albicans.

In future research the release of antibacterial nanoparticles from stabilised electrospun nonwoven materials will be analysed.



**Figure 7.** Antimicrobial and antifungal activity of PVA, PVA/0.02% Ag-NPs and PVA/0.04% Ag-NPs nonwoven materials from nano/microfibers.

### Summary and conclusions

During this study, PVA nonwoven materials were electrospun with a small amount of Ag particles. It was found that the insertion of Ag nano particles into the solution does not affect the viscosity of the electrospinning solution. Ag-NPs affected the electrospinning process and the addition of Ag-NPs into the polymer solution allows to form more nano/microfibres with a diameter up to 200 nm. The concentration of Ag-NPs in the polymer solution has no significant influence on the structure or diameter of electrospun PVA.

FT-IR analysis allows us to identify functional groups of PVA and PVA/Ag-NPs nano/microfibres materials. PVA/Ag-NPs spectra showed the same peaks as pure PVA. The insertion of colloidal Ag-NPs suspensions prepared by dissolving and heating AgNO<sub>3</sub> and sodium citrate in distilled water does not form any additional functional groups in antibacterial PVA electrospun material, as confirmed by FTIR analysis.

The antibacterial activity test showed that electrospun nonwoven materials from nano/microfibres with a small amount of Ag-NPs have antimicrobials and antifungal activity according to our assumptions. Consequently materials electrospun with a higher concentration of Ag nanoparticles exhibit better antibacterial properties.

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### Institute of Textile Engineering and Polymer Materials



The Institute of Textile Engineering and Polymer Materials is part of the Faculty of Materials and Environmental Sciences at the University of Bielsko-Biala. The major task of the institute is to conduct research and development in the field of fibers, textiles and polymer composites with regard to manufacturing, modification, characterisation and processing.

The Institute of Textile Engineering and Polymer Materials has a variety of instrumentation necessary for research, development and testing in the textile and fibre field, with the expertise in the following scientific methods:

- FTIR (including mapping),
- Wide Angle X-Ray Scattering,
- Small Angle X-Ray Scattering,
- SEM (Scanning Electron Microscopy),
- Thermal Analysis (DSC, TGA)

Strong impact on research and development on geotextiles and geosynthetics make the Institute Institute of Textile Engineering and Polymer Materials unique among the other textile institutions in Poland.

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