THE ANTIBACTERIAL ACTIVITY OF NANOFIBER BASED ON POLY-VINYL-ALCOHOL (PVA) DOPED BY METAL

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Abstract
The nanofiber is prepared by using electrospinning on Nanospider LB 500 (Elmarco, Czech Republic). We used rotating cylindrical electrode, width 50 cm. The nanofiber is based on polyvinyl alcohol and it has been doped with antibacterial solution e.g. metal stock (AgNO₃, CuSO₄•5H₂O). The final concentration of supplement solution is 1%, 0.5% and 0%. The samples have rectangle shape, with size approximately 15 × 20 cm or cycle shape with 10 cm in diameter and weight per unit area between 5 - 15 g/m² and it stabilized 10 minute at 140 °C. The antibacterial activity of nanofibers doped by antibacterial supplement we evaluated by specialized method of direct contact of nanofibers and bacteria as a growth curve in a liquid medium. The experiment is prepared at constant temperature 25 °C. We used Escherichia Coli (member of Gram negative bacteria) as model organisms. The nanofiber textile based on PVA is decreased the bacterial growth in the first stage. The nanofiber textile based on PVA with addition of silver to final concentration 0.5 or 1 wt% decrease bacterial growth during first 180 minute or more. The nanofiber textile based on PVA with addition of copper to final concentration 0.5 or 1 wt% has not antibacterial activity. The results showed that the antibacterial properties are dependent on concentration and type of the antibacterial supplement, but it is probably independent of the bacteria type nanofiber

Keywords: Antibacterial properties, metal, Polyvinyl alcohol, nanofibre membrane

1. INTRODUCTION
The polyvinyl alcohol (PVA) is colorless, water-soluble synthetic substance and non-toxic, it can dissolve in another solvent too. It has been used in practical application for excellent chemical resistance, physical properties and good fibre forming, e.g. medicine, cosmetic, food, pharmaceutical and packing industry [1]. The recent time it is tested as a engineering scaffold and drug delivery system [2-6]. Ultrafine PVA fibres, which may have different potential applications, cannot be produced by conventional spinning techniques [1]. The electrospinning process has gain much attention due to its effectiveness in producing ultrafine fibres or fibrous structure from many polymers which a diameter in the range from several micrometers down to a few nanometers. The great nanofibre membrane can be produced by electrospinning on machine Nanospider (Elmarco, Czech Republic). The initial characterization of fabricated nanofiber membrane based on polyvinyl alcohol (PVA) showed high water dissolving, that the crosslinking and stabilization is necessary for next testing. There are two methods in used, the first is chemical crosslinking by immersion in methanol or in combination of methanol and glutaraldehyde. [7] The second one is physical stabilization by heat treatment at 140-150 °C.

Electrospun webs from ultrafine polymer fibers are attracting great attention because of their unique properties such as high surface-to-volume ration, high porosity and diameters in nano-scale. In recent time, it has also increased interest in the incorporation of functional nanoparticles or substances in the polymer nanofiber due to their uniquely promising properties and applications. The metals are one of the functional
substances which is incorporated into nanofiber membrane for addition of antibacterial properties. Metallic ions, such as a copper or silver, can be used as a sanitizing agent, ensuring hygiene, due its antimicrobial properties \[8\]. The antimicrobial study in literature showed that his metal, as a silver and copper, has antibacterial effect for many bacteria. \[8\].

2. MATERIALS AND TESTED SAMPLES

2.1. PREPARATION OF NANOFIBER MEMBRANE

The standard solution stock of PVA is used from 375 g 16% PVA (Sloviol R 16%, Eurošarm, Slovak Republic), 4.4 g Glyoxal (Sigma Aldrich, USA), 3 g H\(_2\)PO\(_4\) (Sigma Aldrich, USA) and distillate water up to 0,5 L. The nanofiber membrane based on PVA is prepared by electrospinning at lab machine Nanospider (Elrmarco, Czech Republic). The spund bond for electropinning is made from polypropylene with weigh per units area around 23 g/m\(^2\) and it is antistatic adjusted. The standard set up is distance from 120 to 140 mm, high voltage is from 76 to 80 kV. We used cylindrical tube electrode with width 600 mm. The produced membrane has rectangular shape, width is 600 mm and length is more than 500 mm.

The antibacterial membrane is prepared by addition of metal ion to standard solution with final concentration 0.5 or 1 % (wt/V). The addition of silver is by aqueous solution of AgNO\(_3\) and copper is by CuSO\(_4\)•2 H\(_2\)O. The samples is named according supplement, for silver is A, for copper is C, and according set up method working. The sample I and IV is single-layer membrane and the set up is in tab 1. The other samples are double-layer and the set up is in tab 1. The distance of electrode was around 120 mm in first layer; the second layer is prepared with distance 130 mm. The sample PVA is prepared as a double-layer membrane; the distance of electrode was 140 mm during the whole of electrospinning process, the voltage was 76 kV during preparing the first layer, the second layer was making at 80 kV, rotation of electrode was 5 Hz and 10 Hz. Whole PVA is used as a comparative sample for next studies.

Tab 1 The samples name and set up preparation

<table>
<thead>
<tr>
<th>Name of the sample</th>
<th>Rotation of electrode [Hz]</th>
<th>Movement of spund bond [Hz]</th>
<th>High voltage [kV]</th>
<th>Final concentration of supplement [wt %]</th>
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<tbody>
<tr>
<td>Number of layer</td>
<td>I</td>
<td>II</td>
<td>I</td>
<td>II</td>
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<tr>
<td>A I</td>
<td>5</td>
<td>none</td>
<td>10.87</td>
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<tr>
<td>A II</td>
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<td>10</td>
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<td>A III</td>
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<td>A IV</td>
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<td>10</td>
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<td>A VI</td>
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<td>C I</td>
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2.2. ANTIBACTERIAL ASSAY
The antibacterial assay is used with *Eschelichia Coli* as modelling gram negative bacteria. The storage culture is prepared by over night cultivation in 50 ml liquid glucose – yeast medium (Bi media 001A, SY-LAB VGMPH, Austria) at 37°C with horizontal shaking 300 round per minute. The testing culture is prepared from standard O/N culture dissolving in ratio 1 : 500 into 20 ml fresh glucose – yeast medium. The bacterial concentration is measured by optical density at 640 nm (OD$_{640}$). When the OD$_{640}$ was around 0.1, the nanofiber membrane is put in. The growth of concentration of bacteria (bacterial masses) is measured as a changing of OD$_{640}$ during time. The period of measuring was around 30 minutes.

The samples is prepared from central part of membrane with cycle shape, diameter was 100 mm. The study sample is used as a membrane together with spund bond.

3. RESULTS
We prepare nanofiber membrane based on PVA, PVA/Ag and PVA/Cu. The samples for antibacterial assay are prepared with circle shape with 10 cm in diameter. The weights per unit area for alone PVA sample is 9.11 g/m$^2$, the sample designated PVA. It is prepared as a double layer membrane with set up as a sample A III. The nanofiber membrane based on PVA/Ag has weights per unit area from 5.89 to 10.78 g/m$^2$. The sample based on PVA/Cu had weights per unit area from 6.21 to 14.65 g/m$^2$. This value was calculated from sample with rectangle shape, the size is 130 x 25 mm. The weight is measured to accuracy $10^{-4}$.

The storage over night bacterial culture had OD$_{640}$ 1.986. The bacterial assay started when the OD$_{640}$ of refresh culture is from 0.077 to 0.099. The value of OD$_{640}$ is measured by the time with period around 30 minutes.

The Fig. 1 is showed growth curve with membrane based on PVA/Ag. The alone PVA membrane reduces bacteria reproduction at the first stage. The bacteria growth was in normal progress after 40 minutes. The antibacterial membrane based on PVA/Ag showed stopping of reproduction during 180 minutes. The sample A VI showed the highest antibacterial activity, the reduction of reproduction was during whole experiment.

The Fig. 2 is showed growth curve with membrane based on PVA/Cu. This type membrane is showed any reduction of bacteria reproduction. The whole curve have same progress as the alone bacteria. The first stage stresses is visible in all experimental set up.

![Fig. 1 Bacterial growth during time with supplement of antibacterial membrane doped by silver](image-url)
4. DISCUSSION

The nanofiber membrane based on PVA was prepared with two potential antibacterial supplement, silver as a AgNO$_3$ and copper as a CuSO$_4$ $\cdot$ 5H$_2$O. The weight per unit area is from 5.89 to 10.78 g/m$^2$ for sample A I – A VI and 6.21 to 14.65 g/m$^2$ for sample C I - C VI. The alone PVA membrane has 9.11 g/m$^2$.

The results confirmed antibacterial effect of silver according [8 -10]. The antimicrobial effect of silver is increasing when the silver is as nanoparticles or dispersed in volume. In the case, when the nanofiber of PVA is used as a scaffold for silver agent, the materials have increased specific area and porosity and the silver is accessible for more number of bacteria. [11-13]. The effectiveness and persistence of antimicrobial effect of PVA/Ag is dependence on concentration of the supplement. The sample A I - A V is effective during first 180 minute, after that the bacteria is adhered on the surface. The surface of nanofiber is covered by layer of bacteria and next one has not direct contact to membrane. In this time, the antibacterial activities are happened by diffusion of ion of silver. The effective diffusion distance for silver is dependence of concentration. The sample A VI is double – layer membrane with the highest concentration of silver. The approximate weigh of silver by sample is 0.029 g by sample.

The result of membrane based on PVA/Cu showed interesting conclusion. The copper and their complexes is used as an antibacterial agents, it is caused to destroyed bacterial membrane [8, 14, 15]. In the case of the copper and their substance is into polymer scaffold, the antibacterial activity is not. The nanofiber membrane based prepared on supplement CuSO$_4$ $\cdot$ 5H$_2$O has not accessible Cu for bacteria. The metal ion is probably inside of fiber and it can release, there is not free diffusion.

5. CONCLUSION

We prepared two potential antibacterial nanofiber membrane based on PVA and doped by metal ions. The membrane is prepared as a single and double layer with weight per unit area is from 5.89 to 10.78 g/m$^2$. The metal ion is added to electrospinning solution to final concentration 0.5 and 1 wt%. The concentration of supplement in the alone membrane is 20.8 and 41.7 wt% in the PVA. The real concentration is unknown for small weight per unit area and for not exist method for nanoscale.
PVA/Ag membrane showed antibacterial activities during 180 minutes, but only the highest concentration showed longer antibacterial activities. PVA/Cu membrane didn’t show antibacterial activities. The alone PVA membrane only cased stress during first stage of experiment.

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LITERATURE