MODIFIED INORGANIC-ORGANIC NANOFIBERS FOR REGENERATIVE MEDICINE

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Abstract

This study deals with modifications of inorganic-organic nanofibers to optimize the manufacturing process and to ensure long-term antibacterial activity of the nanofibers. The nanofibers material is combination of poly(vinyl alcohol) and silica. There are presented two methods of nanofibers´ structure modifications: i) surface functionalization of the nanofibers by silver and copper nanoparticles, and ii) modification of initial sol by antiseptic additive. The both modifications methods ensure significant antibacterial activity of inorganic-organic nanofibers, which was proven by *in-vitro* antibacterial tests. This novel material shows a big potential for wound dressing applications.

Keywords: Silica nanofibers, antibacterial activity, wound dressing, silver ions, copper ions

1. INTRODUCTION

For successful tissue regeneration, the living human cells should be effectively proliferated and organized into a special support structure - scaffold with physiological and morphological features mimicking their natural environment. Electrospun nanofibers have this potential; they are very similar to extracellular matrix with their structure. Nanofibrous scaffolds are produced from organic or inorganic materials, and a few studies considering use of inorganic-organic materials combination [1, 2, 3].

Nanofibers produced by electrospinning have found application in many biomedical areas, e.g. drug delivery, tissue engineering, or wound dressing. Regarding wound dressing application, an important role of the nanofibers is to prevent bacterial growth or infection. Suitable modified nanofibers provide possibility of functionalization by metal particles or various types of biomolecules (enzymes, peptides, and antibiotics) to adjust its properties for specific application [4].

This research presents two methods of inorganic-organic nanofibers modifications to ensure long-term antibacterial activity of the nanofibers. As the nanofibrous mat material, combination of silica and poly(vinyl alcohol) was chosen. Silica has a big potential for production of nanofibers for medical applications because it is able to meet a number of strict criteria (low toxicity, biodegrability, and biocompatibility) and provide suitable surface for functionalization thanks to the Si-O bonds on the surface.

As antibacterial agents, silver nanoparticles and antiseptic etyltrimethylammonium bromide (CTAB) were chosen. Today, silver ions are widely used to control bacterial growth in number of medical devices and materials. Silver ions and nanosilver are able to kill a wide range of bacteria including those which are resistant to antibiotics [5]. CTAB contains the cetrimonium (hexadecyltrimethylammonium) cation which is an effective antiseptic agent against bacteria and fungi. It is one of the components of the topical antiseptic cetrimide [6].

Copper was described as an essential component of the angiogenesis process in skin layer and plays a critical role in the cells formation and differentiation leading to blood vessel formation [7]. Based on these facts, copper nanoparticles were also immobilized onto PVA/silica nanofibers and their effect is assumed to support the cell proliferation in the next phase of material testing. In this research, there is also observed the influence of copper nanoparticles presence on the antibacterial activity of nanofibrous material modified by silver ions and CTAB.

2. EXPERIMENT

2.1. Material

The PVA/silica nanofibers were prepared from tetraethyl orthosilicate (TEOS, ≥ 99%, Sigma Aldrich), hydrochloric acid (HCl, min. 35%, Penta chemicals), (3-mercaptopropyl)trimethoxysilane (MPS, 95%, Sigma Aldrich) and poly(vinyl alcohol) (PVA, 16%, Sloviol R). Ethanol (Penta chemicals) was used as a solvent. Cetyltrimethylammonium bromide (CTAB, Acros Organics), silver nitrate ($AgNO_3$, p.a., Penta chemicals), and copper nitrate trihydrate ($Cu(NO_3)_2$ $3H_2O$, p.a. 99-104%, Sigma Aldrich) were used for different methods of antibacterial modification of the nanofibers.

For antibacterial tests, the Gram-negative *Escherichia coli* (*E. coli*, ATCC 9637) and the Gram-positive *Staphylococcus Aureus* (*S. aureus*, ATCC 12600) were purchased from the Czech Collection of Microorganisms, Masaryk University in Brno. The base medium for antibacterial tests was nutrient agar (Nutrient Agar No. 2, Himedia).

2.2. Preparation of Nanofibers

Preparation of the nanofibers was processed as described in [8]. The PVA/silica nanofibers were produced from silica sol prepared by sol-gel method, the sol was electrospun. The electrospinning was performed on laboratory instrument Nanospider (Elmarco Liberec Company). Prior to nanofibers surface antibacterial functionalization, the PVA/silica nanofibers were thermally stabilized at 150 °C, respectively 180 °C for 2 hours.

2.3. Functionalization of Nanofibers

Antibacterial modification of the PVA/silica nanofibers was performed by two methods: modification of initial sol by antiseptic additive and functionalization of the nanofibers surface by silver and copper nanoparticles.

The first modification method was based on modification of initial sol. CTAB was added into the initial silica sol and the mixture was gently stirred for 60 min. Consequently, the sol was electrospun as described and prepared PVA/silica nanofibers were thermally stabilized at 150 °C, respective 180 °C for 2 hours - samples i-t150, i-t180.

The second method involves functionalization of the prepared PVA/silica nanofibers´ surface by only silver nanoparticles or silver and copper nanoparticles together. $AgNO_3$ and $Cu(NO_3)_2{\cdot}3H_2O$ were dissolved in a solution of ethanol and distilled water. The samples of nanofibers (cca 10 x 10 cm) were immersed into the solution with silver ions for 60 min - samples A-t150, A-t180. Specified samples with silver ions were then immersed into the solution with copper ions for next 60 min - samples CA-t150, CA-t180. Finally, all samples were rinsed in distilled water and dried in thermostat at 35 °C.

2.4. Characterization

The morphology of the electrospun nanofibers was observed by field emission scanning electron microscopy (FE-SEM) Zeiss Ultra Plus. Prior to the analysis, the samples were coated with 2 nm of platinum to achieve sustainable surface conductivity. An InLens secondary electron detector operated at accelerating voltage of 2 kV was used for the imaging of topographical contrast. For a local chemical analysis was used EDS detector Oxford X-MAX on SEM; applied accelerating voltage was 15 kV.

2.5. Antibacterial Activity Test

The antibacterial tests were carried out by standard test method of bacterial spreading on the nutrient agar plate (according to CSN EN ISO 20645 - antibacterial activity testing of fabrics), where the inhibitory zone H is measured and evaluated. Antibacterial activity of modified PVA/silica nanofibers were tested against Gram-negative bacteria *E. coli* and Gram-positive *S. aureus*. 1 ml of inoculum in concentration of 10^8 CFU/ml was

applied on the nutrient agar plate and sterile sample (1.8 x 1.8 cm) was placed on this nutrient agar plate. The samples were incubated in a thermostat at 37 °C for 24 hours [8]. Pure silica nanofibers were considered as control sample, where no antibacterial activity was expected. The testing was processed in triplets and results were averaged.

3. RESULTS AND DISCUSSION

3.1. Morphology of PVA/silica Nanofibers

The influence of thermal stabilization temperature on the PVA/silica nanofibers´ morphology was not observed. The nanofibrous layer as well as single fibers keeps their morphology after thermal stabilization at 150 °C, respectively 180 °C. The nanofibrous layers were compact and uniform, the mean fiber diameter was 362 nm and the mean nanoparticles diameter was 88 nm.

3.2. Antibacterial Modification of PVA/silica Nanofibers by Metal Nanoparticles

PVA/silica nanofibers thermally stabilized at 150 °C, respectively 180 °C were functionalized by silver and copper nanoparticles. Samples A-t150 and A-t180 were functionalized only by silver nanoparticles. Samples AC-t150 and AC-t180 were functionalized both silver and copper nanoparticles. Nanoparticles were attached on the surface of PVA/silica nanofibers in constant density thorough the bulk of the sample, as shown in **Figure 1**. That is very important factor for long-term antibacterial activity of the samples corresponding with the nanofibers degradation and gradual releasing of Ag.

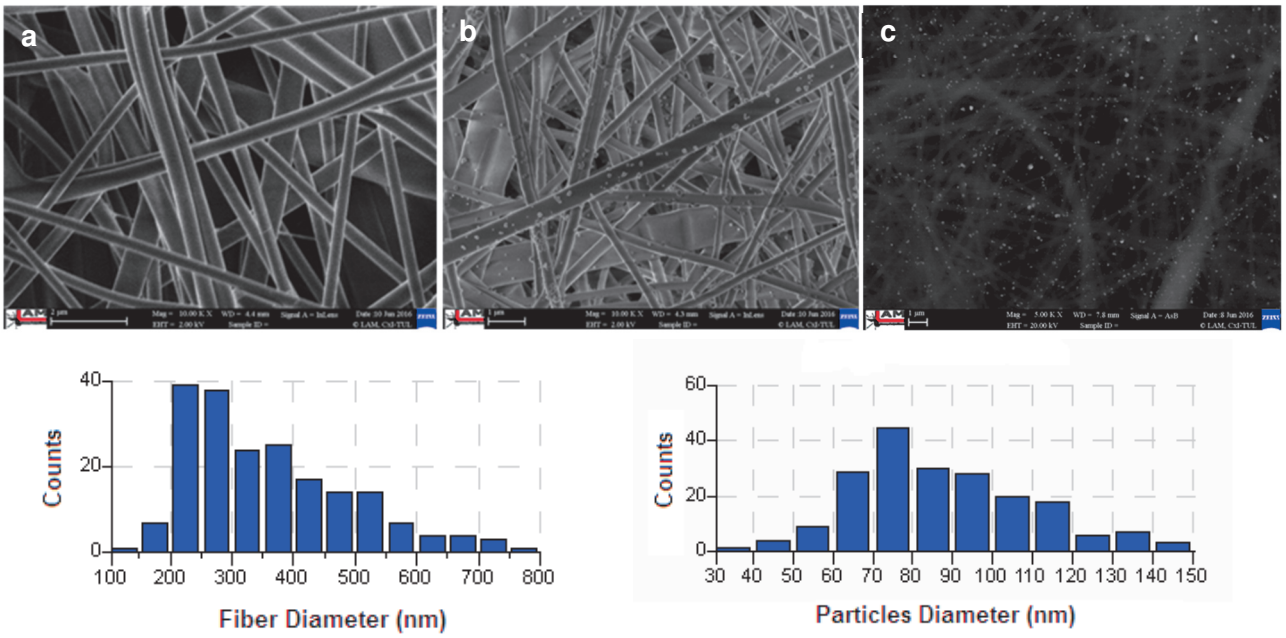

Figure 1 SEM pictures of PVA/silica nanofibers, fiber and particles size distribution - samples a) i-t150 (given scalebar 2 μm), b) AC-t150 (given scalebar 1 μm), c) AC-t150 - BSE detector imaging (given scalebar 1 μm)

According to EDS analysis results (**Table 1**), temperature of thermal stabilization does not significantly affect quantity of immobilized metal nanoparticles onto the nanofibers´ surface. The samples´ specification and EDS analysis results are presented in **Table 1**. The quantity of immobilized Ag nanoparticles for the samples A-t150 and A-t180 was 5.1 At%, respectively 5.4 At%. For the samples AC-t150 and AC-t180, the quantity of immobilized Ag nanoparticles was 2.3 At%, respectively 2.0 At%. The quantity of immobilized Cu nanoparticles

was determined identical for both AC-t150 and AC-t180 samples - 0.2 At%. Based on these facts, we can expect intensive antibacterial activity of all samples with Ag nanoparticles.

Table 1 PVA/silica nanofibers samples´ specification and EDS analysis results

Sample	Si	i-t150	A-t150	AC-150	i-t180	A-t180	AC-t180
Thermal stabilization temperature (°C)	180	150	150	150	180	180	180
Ag content (At%)	-	-	5.1	2.3	-	5.4	2.0
Cu content (At%)	-	-	-	0.2	-	-	0.2
Ø Halo zone (mm) *S. aureus*	0	8.6	1.7	4.1	5.6	1.8	5.1
Ø Halo zone (mm) *E. coli*	0	2.5	3.0	3.8	3.2	3.4	5.1

3.3. Antibacterial Activity Test Results

The antibacterial activity of the modified PVA/silica nanofibrous mats was tested against *E. coli* Gram-negative bacteria and *S. aureus* Gram-positive bacteria. The resulting diameters of halo zones for each sample are stated in **Table 1**. It was concluded that the inhibition zone diameters differ according to the type of antibacterial modification method, but very significant antibacterial activity is presented for all samples (**Figure 2**). The inhibition zone diameter of the samples is ≥ 1 mm; that is evaluated as a good antibacterial activity according to the CSN EN ISO 20645 standard evaluation. The both modifications methods ensure significant antibacterial activity of PVA/silica nanofibers. This novel material shows a big potential for wound dressing applications.

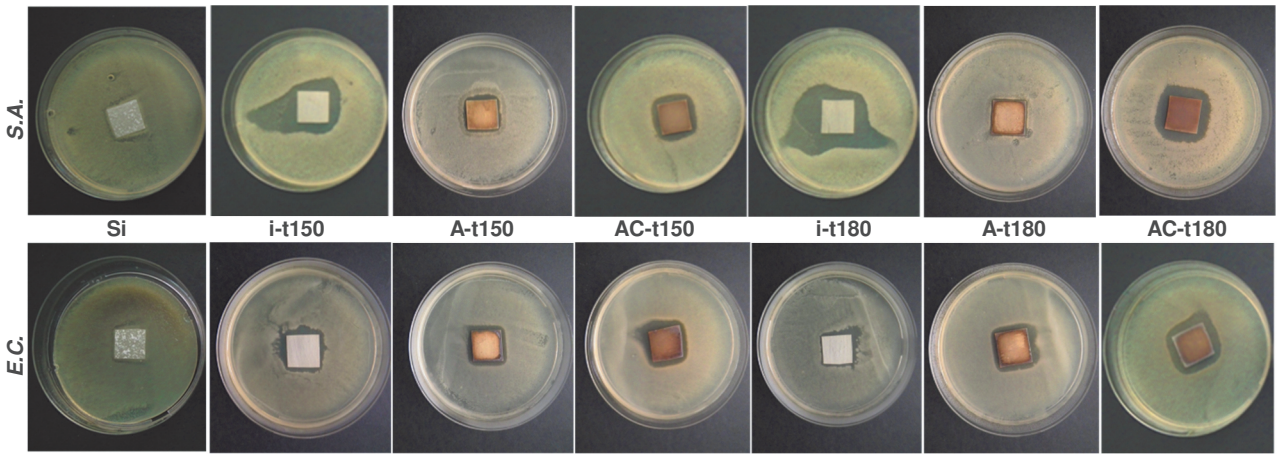

Figure 2 Antibacterial activity of the modified PVA/silica nanofibers against *S. aureus* (S.A.) and *E. coli* (E.C.)

The highest antibacterial activity against *S. aureus* had the samples i-t150 and i-t180. It was caused by CTAB, which exhibits an antibacterial effect. However, some degree of cytotoxicity is expected for this sample; it will be the subject of further research. The inhibition zone diameters highly above the limit in accordance with the standard were evaluated also for samples AC-t150 and AC-t180. These results are very promising in association with cytotoxicity tests, because copper nanoparticles do not affect negatively the antibacterial activity.

4. CONCLUSION

This research is focused on antibacterial modification of PVA/silica nanofibers. The first modification method was using antiseptic additive which was added into the initial sol prior to electrospinning. The second modification method was aimed at immobilization of silver and copper nanoparticles onto PVA/silica nanofibers´ surface. Both methods were proven as very effective, the antibacterial activity of all tested samples was highly above the limit in accordance with the CSN EN ISO 20645 standard evaluation. The antibacterial activity of the samples is caused by CTAB and silver nanoparticles application. It was verified that copper nanoparticles do not affect negatively the antibacterial activity. Copper nanoparticles´ effect is assumed to support the cell proliferation in the next phase of material testing.

ACKNOWLEDGEMENTS

This research was supported by the student grant SGS 2016, Technical University of Liberec.

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