Research Article

Modified Silica Nanofibers with Antibacterial Activity

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This study is focused on development of functionalized inorganic-organic nanofibrous material with antibacterial activity for wound dressing applications. The nanofibers combining poly(vinyl alcohol) and silica were produced by electrospinning from the sol and thermally stabilized. The PVA/silica nanofibers surface was functionalized by silver and copper nanoparticles to ensure antibacterial activity. It was proven that quantity of adsorbed silver and copper nanoparticles depends on process time of adsorption. According to antibacterial tests results, this novel nanofibrous material shows a big potential for wound dressing applications due to its significant antibacterial efficiency.

1. Introduction

In the past decade, the big attention is paid to tissue engineering, the wide field of nanomaterial application, leading to regeneration or replacement of damaged human tissue. One of the key factors in tissue engineering is the development of functional three-dimensional scaffold with suitable degradation rates. The scaffolds for tissue engineering should have high porosity to allow enough space for adhesion and proliferation of a large number of cells, as well as large interconnected pores to facilitate distribution of cells into the bulk of scaffold and the diffusion of oxygen and nutrients. Essential properties of scaffold for tissue engineering are its biocompatibility and biodegradability. The design of the scaffolds is very important; it should restore tissue structure as closely as possible to the native structure of extracellular matrix.

Typically, biodegradable scaffolds are fabricated using electrospinning. Electrospinning is a method that produces polymer fibers using electrically driven jet; starting material is polymer solution or melt. Despite the simplicity of the process, it produces ultrafine fibers (micro- or nanofibers) with high specific surface area with various pore sizes. Regarding the nanofibers composition, there is a possibility of scaffold modifications by metal particles or various type of

biomolecules (enzymes, peptides, and antibiotics) to adjust its properties for specific application [1]. The electrospun nanofibers are widely used for biomedical applications as mentioned tissue engineering involving also wound dressing [2, 3], drug delivery systems [4], cell culture, and others. There are many studies of research organic [5, 6] or inorganic polymers [7, 8] as a scaffold material for biomedical applications and a few studies considering use of inorganic-organic materials combination [9].

Poly(vinyl alcohol) (PVA) is a hydrophilic polymer with specific properties: inherent nontoxicity, noncarcinogenicity, good biocompatibility, and high degree of swelling in aqueous solutions. Due to these properties, PVA is presented in some frequently used technologies such as hydrogels and biomaterials [10] including soft contact lenses [11], implants, and artificial organs [12]. It is an inexpensive biocompatible material and it is easily electrospinnable.

Silica nanomaterials are a good candidate for medical applications because they are able to meet a number of mentioned strict criteria (low toxicity, high porosity, and relatively suitable surface for subsequent functionalization). Thanks to the Si-O bonds on the surface, these materials represent attractive matrix for binding and release of biomolecules. Localized and controlled release of additives is a crucial aspect to increase efficiency and reduce potential side effects

of additives (antibiotics, enzymes, and metal ions). Thanks to these properties, silica nanofibers appear to be the ideal material for tissue engineering as well as wound dressing for the treatment of chronic wounds [13].

As is generally known, silver (Ag) in form of ions or nanoparticles has a broad spectrum of antibacterial activity. This phenomenon is widely used in commercially available products for wound dressing. Presence of Ag ions in wound bed facilitates wound healing and it has a strong antibacterial activity at the site of damage. Silver ions and nanosilver are able to kill a wide range of bacteria including those which are resistant to antibiotics [14]. In recent years, there have been several published researches of possible antimicrobial mechanism of Ag [15], but the complete mechanism of action is not fully understood. According to available researches, Ag ions immobilized onto the surface of organic or inorganic nanofibers show significant antibacterial activity and cytocompatibility [16, 17].

It was proven that copper (Cu) is an essential component of the angiogenesis in skin layer [18]. It has been reported that copper ions enhance angiogenesis by imitating hypoxia, which plays a critical role in the cells formation and differentiation leading to blood vessel formation. In combination with specific growth factors (vascular endothelial growth factor, VEGF; basic fibroblast growth factor, bFGF), Cu^{2+} ions were shown to enhance the vascularization of an implant or regenerate tissue [19, 20].

In this study, development of combined polymer/silica nanofibers with silver and copper nanoparticles is presented. These hybrid nanofibers are intended to function as antibacterial wound dressing. There are discussed results of antibacterial activity tests of the PVA/silica nanofibers with various amounts of silver and copper nanoparticles.

2. Materials and Methods

2.1. Materials. The main chemical components of PVA/silica nanofibers were tetraethyl orthosilicate (TEOS, $\geq 99\%$, Sigma Aldrich), ethanol (Penta chemicals), cetyltrimethylammonium bromide (CTAB, Acros Organics), hydrochloric acid (HCl, min. 35%, Penta chemicals), (3-mercaptopropyl)trimethoxysilane (MPS, 95%, Sigma Aldrich), and poly(vinyl alcohol) (PVA, 16%, Sloviol R). For modification of the nanofibers surface, silver nitrate (AgNO_3 , p.a., Penta chemicals) and copper nitrate trihydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, p.a. 99–104%, Sigma Aldrich) were used.

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 composition of base for antibacterial tests was the blood agar supplied from Biorad s.r.o, Prague, and nutrient agar (Nutrient Agar number 2, Himedia).

2.2. Preparation of Nanofibers. The PVA/silica nanofibers were produced from sol prepared by sol-gel method. The initial sol was prepared from TEOS by controlled hydrolysis and polycondensation in ethanol as solvent and HCl as

catalyst. Into the initial sol, MPS and CTAB were added to improve conductivity and thus electrospinning process. The sol was heated to 60°C and mixed for 60 minutes. After that time, PVA solution was slowly dropped into the sol. The viscous mixture of PVA and silica sol was obtained.

The nanofibrous layer was produced by needleless electrospinning. The electrospinning was performed on laboratory instrument. The instrument consisted of an adjustable, regulated, high-voltage power supply (up to 80 kV), a rod electrode, and collector units. The prepared solution was applied on the rod electrode. The electrospinning was performed at room temperature; the applied high voltage was 40–45.7 kV. The electrospun nanofibers were assembled on a conductive plate as a collector, and the collector was placed 10 cm from the top of the rod electrode. The nanofibers were then thermally stabilized (180°C , 2 hours).

2.3. Functionalization of Nanofibers. AgNO_3 as well as $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ were dissolved in a solution of ethanol and distilled water. The prepared nanofibers samples (cca 0.005 g/l sample) were immersed into the solution. The functionalization of nanofibers by Ag and Cu nanoparticles was carried out in different process times of 30 min, 45 min, and 60 min. Subsequently, the samples were rinsed in distilled water and dried.

2.4. Characterization of Nanofibers. 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, EDS detector Oxford X-MAX on SEM was used; applied accelerating voltage was 15 kV.

2.5. Antibacterial Activity Test. The antibacterial tests against Gram-negative bacteria *E. coli* and Gram-positive *S. aureus* were carried out by standard test method of spreading on the agar plate (according to ČSN EN ISO 20645, antibacterial activity testing of fabrics). The nutrient agar (final pH = 7.2) was sterilized and inoculated with 1 mL of bacterial inoculum (10^8 CFU/mL). The prepared inoculate agar was mixed thoroughly and 5 mL of the agar was poured into Petri dishes on agar base: standard blood agar. Within each Petri dish, there was placed a sterile sample (2×2 cm). The samples were incubated in a thermostat at 37°C for 20 hours. After removal from the thermostat, the inhibitory zone *H* is measured and evaluated. Pure PVA/silica nanofibers were considered as control sample, where no antibacterial activity was expected.

The size of the inhibition zone *H* is calculated according to the formula $H = (D - d)/2$, where *D* is the overall diameter of the sample and inhibition in mm *d* is diameter of the sample in mm.

FIGURE 1: SEM images of PVA/silica nanofibers with fiber diameter distribution (magnification 25000x and 1000x).

TABLE 1: Quantitative EDS analysis results for PVA/silica nanofibers with Ag and Cu nanoparticles.

At%	C	O	Si	S	Cu	Ag
Cu-t30	41.54	38.33	15.81	2.95	1.37	—
Cu-t45	41.15	38.75	15.79	2.93	1.38	—
Cu-t60	38.84	40.44	16.24	2.91	1.57	—
Ag-t30	37.90	40.20	15.13	2.84	—	3.93
Ag-t45	36.84	40.22	15.57	2.93	—	4.44
Ag-t60	37.07	39.86	15.18	2.88	—	5.01

3. Results and Discussion

3.1. PVA/Silica Nanofibers Morphology. SEM images of PVA/silica nanofibers after thermal stabilization are depicted in Figure 1. The nanofibers retained their morphology after thermal stabilization; they do not break significantly. The nanofibrous layer was compact and uniform with mean fiber diameter of 362 nm (Figure 1).

3.2. Functionalization of PVA/Silica Nanofibers. The thermally stabilized PVA/silica nanofibers were functionalized by Ag (as presented in [21]) and Cu nanoparticles in different process times: 30, 45, and 60 min (samples labeled Ag/Cu-t30, Ag/Cu-t45, and Ag/Cu-t60).

For PVA/silica nanofibers functionalized by Cu nanoparticles, there were no significant differences between Cu nanoparticles amounts on samples processing for 30 and 45 min (Cu-t30, Cu-t45), as shown in Table 1. Because of that,

samples chosen for antibacterial testing were Cu-t30 and Cu-t60. Figure 2 gives the SEM images of PVA/silica nanofibers with Cu nanoparticles. There are lightened Cu nanoparticles presented in the nanofibrous layer. The presence of Cu nanoparticles was also proved by local quantitative EDS analysis results (Table 1); the quantity of Cu was determined 1.37 At% for sample Cu-t30 and 1.58 At% for sample Cu-t60. According to the researches of Cu ions and nanoparticles function [19, 20], significant antibacterial activity of the samples Cu-t30 and Cu-t60 had not been expected.

In Figure 3, there are displayed SEM images of PVA/silica nanofibers with Ag nanoparticles: samples Ag-t30, Ag-t45, and Ag-t60. As observed, Ag nanoparticles are attached on the nanofibers surface in constant density on the entire surface of single fibers. There are also irregularly presented Ag nanoparticles clusters. Ag nanoparticles are attached not only on the fibers in surface layer of the sample, but also on the fibers inside the bulk of samples. That is a very important factor for long-term antibacterial activity of the samples corresponding with the nanofibers degradation and gradual releasing of Ag.

EDS analysis results for these samples Ag-t30, Ag-t45, and Ag-t60 are given in Table 1. It should be concluded that process time of functionalization by Ag nanoparticles significantly influences the quantity of Ag nanoparticles on the PVA/silica nanofibers. According to Table 1, quantity of Ag nanoparticles for the sample Ag-t30 was 3.93 At%, for the sample Ag-t45 was 4.44 At%, and for the sample Ag-t60 was 5.01 At%. Based on these facts, we can expect intensive antibacterial activity of PVA/silica nanofibers with Ag nanoparticles.

FIGURE 2: SEM images of PVA/silica nanofibers with Cu nanoparticles: sample Cu-t60 (magnification 2500x and 5000x).

FIGURE 3: SEM images of PVA/silica nanofibers with Ag nanoparticles: samples Ag-t30 (a), Ag-t45 (b), and Ag-t60 (c).

3.3. Antibacterial Activity Test Results. The antibacterial activity of the fiber mats against *E. coli* Gram-negative bacteria and *S. aureus* Gram-positive bacteria, which is commonly found on burn wounds, was tested by using the test method of spreading on the agar plate, where the inhibition zone diameter is measured. The capability of the functionalized nanofibrous mats to inhibit the growth of the tested microorganisms on solid media is shown in Table 2. It was found that the diameter of inhibition zone varied according to the type and quantity of nanoparticles on the nanofibers surface.

Very significant antibacterial activity is presented for PVA/silica nanofibers with Ag nanoparticles. The inhibition zone diameter was 5 mm for Ag-t30 and Ag-t40 samples, 6 mm was measured for Ag-t60 sample (Figure 4).

TABLE 2: Diameters of inhibition zones H (mm) for given samples and the control sample against different test bacteria after 20 h by test method of spreading on the agar plate at 37°C.

Inhibition zone H (mm)	Control PVA/silica NF	Ag-t30	Ag-t45	Ag-t60	Cu-t30	Cu-t60
<i>E. coli</i>	0.5	5	5	6	0	0
<i>S. aureus</i>	1	1	1.5	1	0	0

The inhibition zone diameter of these samples is ≥ 1 mm; that is evaluated as good antibacterial activity according to the ČSN EN ISO 20645 standard evaluation. In fact, inhibition zone diameters are highly above the limit in

FIGURE 4: The antibacterial activity of the PVA/silica nanofibers with Ag nanoparticles against *S. aureus* (SA) and *E. coli* (EC).

accordance with the standard for samples Ag-t30, Ag-t40, and Ag-t60. Summarizing the results, it can be noted that PVA/silica nanofibers with Ag ions show significant antibacterial activity. Antibacterial activity is affected by quantity of Ag nanoparticles on the nanofibrous mats. How that was demonstrated, PVA/silica nanofibers with 5.01 At% of Ag nanoparticles (sample Ag-t60) exhibited the most effective antibacterial activity. It is associated with the highest Ag nanoparticles content of all tested samples.

As expected, PVA/silica nanofibers with Cu nanoparticles do not show any significant antibacterial activity. Bacterial colonies both of *E. coli* and *S. aureus* were reproduced after the incubation time; the samples Cu-t30 and Cu-t60 were overgrown by the bacteria. The efficiency of Cu nanoparticles is predicted in support of cell growth (fibroblasts, keratinocytes).

Moderate antibacterial effect is also recognized for control sample: pure PVA/silica nanofibers. It may be caused by presence of CTAB, which itself exhibits an antibacterial effect. The cetrimonium (hexadecyltrimethylammonium) cation is an effective antiseptic agent against bacteria and fungi. It is one of the components of the topical antiseptic cetrimide [22].

4. Conclusion

In the study, preparation of inorganic-organic nanofibers for antibacterial wound dressing is demonstrated. PVA/silica nanofibrous mats were prepared by electrospinning method. This nanofibrous mat is able to adsorb Ag and Cu nanoparticles on the nanofibers surface. Different quantity of Ag and Cu nanoparticles was successfully bonded on the PVA/silica nanofibers. The quantity of adsorbed Ag and Cu nanoparticles depends on the process time. Significant antibacterial activity of the nanofibrous material was proven for the PVA/silica nanofibers with Ag nanoparticles; it was not proven for PVA/silica nanofibers with Cu nanoparticles.

The presence of Ag nanoparticles in PVA/silica nanofibers enhanced the antibacterial ability of the electrospun mats giving the material potential as a good wound dressing material.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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