Hindawi Publishing Corporation
Journal of Nanomaterials
Volume 2016, Article ID 2485173, 6 pages
http://dx.doi.org/10.1155/2016/2485173

Research Article

Silica Nanofibers with Immobilized Tetracycline for Wound Dressing

Irena Lovětinská-Šlamborová,[1] Petr Holý,[2] Petr Exnar,[3] and Ivana Veverková[4]

[1]*Institute of Health Studies, Technical University of Liberec, Studentská 1402/2, 46117 Liberec, Czech Republic*
[2]*Faculty of Science, Humanities and Education, Technical University of Liberec, Studentská 1402/2, 46117 Liberec, Czech Republic*
[3]*Institute for Nanomaterials, Advanced Technologies and Innovation, Technical University of Liberec, Studentská 1402/2, 46117 Liberec, Czech Republic*
[4]*Faculty of Textile Engineering, Technical University of Liberec, Studentská 1402/2, 46117 Liberec, Czech Republic*

Correspondence should be addressed to Ivana Veverková; ivana.veverkova@tul.cz

Received 24 March 2016; Revised 3 June 2016; Accepted 12 July 2016

Academic Editor: Antonio Villaverde

Local antibiotic treatment has its justification for superficial infections. The advantage of this treatment is that the antibiotic has effects on bacterial agent directly at the application site. Skin infections which are intended for the local antibiotic treatment are superficial pyoderma, some festering wounds, burns of second and third degree, infected leg ulcers, or decubitus of second and third degree. Tetracyclines are available topical antibiotics with a broad bacterial spectrum. At present, ointments containing tetracycline are also used for the treatment, which rarely can lead to skin sensitization. In this paper, a development of novel nanofibrous material with immobilized tetracycline is presented. Two different methods of immobilized tetracycline quantification onto silica nanofibers are employed. It was proven that the prevailing part of tetracycline was bound weakly by physisorption forces, while the minor part was covalently bound by NH_2 groups formed by the preceding functionalization. The silica nanofibers with immobilized tetracycline are promising material for wound dressing applications due to its antibacterial activity; it was proved by tests.

1. Introduction

In recent years, the properties of nanofibers are intensively studied due to their suitability for biomedical applications [1, 2]. The advantage of this nanomaterial is the surface chemical modification possibility and subsequent immobilization of suitable biomolecules.

In literature, many methods of biomolecules immobilization onto a silica matrix are described. Primarily, it is the formation of covalent bond between the biomolecule and the silica substrate; the noncovalent interactions and adsorption are also often utilized [3, 4]. For biomedical applications, these nanomaterials are studied as effective drug delivery systems for a wide group of substances such as the drugs for the diabetes and cancer treatment or drugs for bones and tendons regeneration [5].

Pure silica nanofibers are convenient for medical applications because they are able to satisfy a number of very stringent criteria, such as low toxicity, relatively high porosity, and a suitable surface for subsequent functionalization. With formed Si-OH bonds on the surface, this material provides an attractive matrix for the binding and a controlled release of biomolecules, this for several reasons. First, the highly porous silica matrix is stable in an aqueous solution for a period which is identical to the normal short-term administration of the drug (from 20 minutes to several days). Furthermore, the silica nanofibers are prepared without using of stabilizing agents [6]; they do not exhibit any toxicity and immunogenicity. When longer stability in the aqueous medium is required (to prolong drug release, e.g.), the matrix is stabilized at higher temperatures (about 500°C) [7].

The gradual release of the drug is a key aspect to increase efficiency and reduce potential side effects of drugs such as antibiotics. In the first phase, the noncovalently attached biomolecules are released; in the second phase, the covalent bonds between biomolecules and the substrate are broken and the remaining drug is gradually released. Therefore, the silica nanofibers with immobilized broad-spectrum antibiotic appear to be the ideal wound dressing material for the chronic wounds treatment. The material completely adheres to the wound bed; it copies its surface and thus no air bubbles are created between the nanomaterial and the surface of the wound. In the wound bed, gradual release of an antibiotic occurs and the antibiotics directly affect the bacterial microflora in the wound. Relatively small concentration of antibiotics leads to cleaning of the wound and healing process is initiated. Very important fact is that the silica nanofibers are gradually dissolved in body fluids; this phenomenon also occurs in the wound and it is not necessary to remove wound dressing residues [6].

Tetracyclines belong to a group of broad-spectrum bacteriostatic antibiotic. Good to medium sensitivity to this antibiotic exhibits streptococci, *Listeria*, *pneumococci*, *Vibrio cholerae*, *Campylobacter jejuni*, *Treponema pallidum*, and others. Variable sensitivity is reported for enterococci, staphylococci, *Escherichia coli*, *Klebsiella*, *Salmonella*, or *Clostridia*. Their antibacterial mechanism consists in affecting the protein synthesis; binding of transfer-RNA at the m-RNA-ribosome complex is averted in a bacterial cell. This leads to blockage of protein synthesis [8].

Another side effect of tetracycline is irritation of the digestive tract mucosa and inhibition of intestinal and pancreatic enzymes. Tetracyclines have negative influence on the normal microflora in the oral cavity, in the vagina, or in the intestine. pathogenic bacteria, fungi, or yeasts can settle. Sometimes this can lead to rare infections disease that can occur as a septic disease ending in death for some patients [9].

Pharmaceutical forms of tetracyclines (Doxycycline and Minocycline) are tablets, suspension, or syrup for children or injection. Established Doxycycline per os dosage is 200 mg/24 hours in the first day and then 100 mg/24 hours. Intravenous dosage is 1×200 mg/24 hours (initial dose), followed by 100–200 mg/24 hours (maintenance dose) [10]. Bacteriostatic activity of tetracycline does not depend on concentration, but on the time of exposure. Doxycycline elimination half-life $t_{1/2}$ is approximately 20 hours in favor of biliary excretion; Minocycline half-life $t_{1/2}$ is approximately 16 hours with the same ratio of biliary and renal excretion. Locally, tetracyclines are applied in concentrations of 2-3% (lower concentration can cause resistance). Moreover, it can also lead to photosensitization of the skin (pigmentation). Doxycycline and Minocycline drawbacks are an increasing number of resistant gram-negative rods and gram-positive cocci bacterial strains and the absence of bactericidal activity when using pharmacotherapeutic concentrations in plasma [9].

Silicon dioxide (SiO_2) or silica exhibits properties which make it potential ideal material for scaffolds or grafts. SiO_2 is widely considered as basic material in a form of nanoparticles, nanofibers, or thin films for biomedical applications [11, 12]. Direct physical adsorption of bioactive factors onto inorganic scaffolds such as mesoporous silica scaffold [13] is a commonly utilized method for bone tissue engineering.

2. Materials and Methods

2.1. Materials of Experiment. The materials used in this research were tetraethoxysilane (TEOS ≥ 98%, Sigma Aldrich), (3-aminopropyl)triethoxysilane (APTES ≥ 98%, Sigma Aldrich), isopropyl alcohol (p.a., Penta, Czech Republic), tetracycline (TC ≥ 98%, Sigma Aldrich), absolute ethanol (Penta, Czech Republic), fluorescein 5(6)-isothiocyanate (≥90% for HPLC, Sigma Aldrich), sodium hydroxide (NaOH, p.a., Penta, Czech Republic), hydrochloric acid (HCl, p.a., 35%, Penta, Czech Republic), hydrofluoric acid (HF, p.a., 40%, Penta, Czech Republic), ammonium-acetate (p.a. Penta, Czech Republic), methanol (p.a. Penta, Czech Republic), and trifluoroacetic acid (99%, Sigma Aldrich).

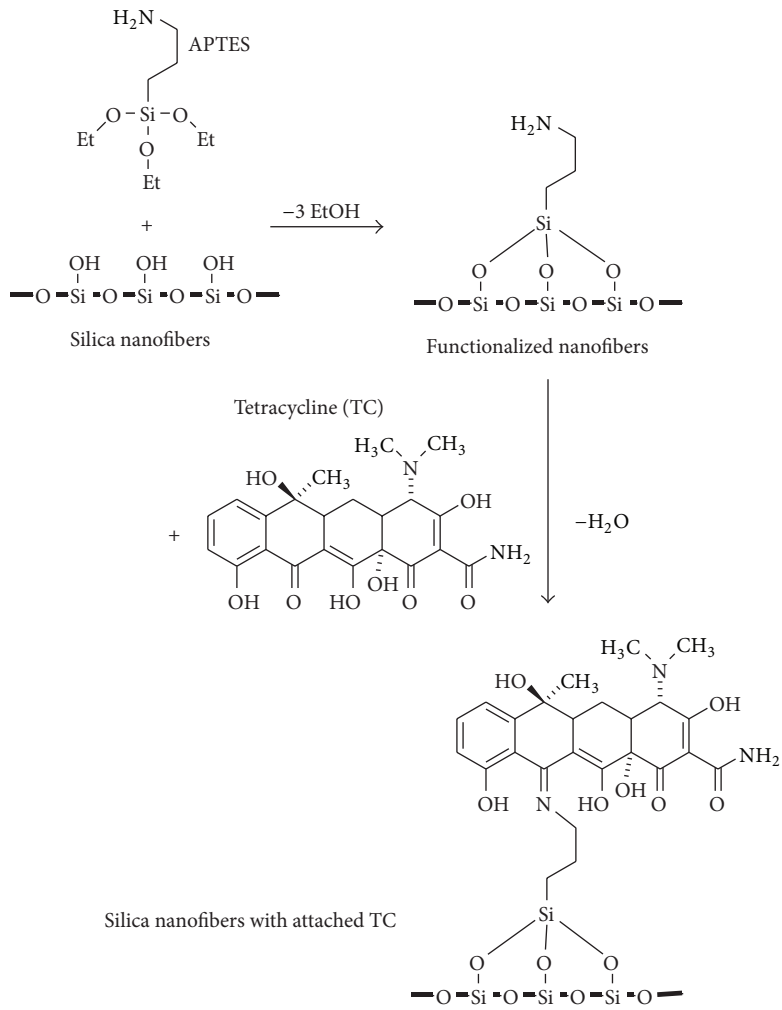
For antibacterial tests, the gram-negative *Escherichia coli* (*E. coli*, ATCC 9637) and the gram-positive *Staphylococcus aureus* (*S. aureus*, ATCC 12600) were purchased from the Czech Collection of Microorganisms, Masaryk University in Brno. As a solid base for antibacterial tests, the blood agar (Biorad s.r.o., Prague) and Müeller-Hinton agar (OXOID CZ s.r.o.) were used. Antibiotic discs were purchased from ITEST plus s.r.o., Hradec Králové (tetracycline content 10 μg/piece).

2.2. Silica Nanofibers Preparation. Preparation of the silica nanofibers is fully described in patent [3]. The initial sol was prepared by the sol-gel method; controlled hydrolysis and polycondensation of tetraethoxysilane (TEOS) are carried out, where isopropyl alcohol was used as a solvent and HCl as a catalyst. TEOS was dissolved in isopropyl alcohol, and water and HCl were added so that the molar ratio k = [H_2O]/[TEOS] was $k = 2.3$ and the molar ratio m = [HCl]/[TEOS] was $m = 0.01$. After the hydrolysis and polycondensation reaction, the sol was concentrated by evaporation of the solvent to a final content of 36 wt% SiO_2. No auxiliary polymer or wetting agents were used for the electrospinning.

Silica nanofibers were produced by electrospinning of the sol using a technology called Nanospider in the pilot plant Superlab of the Elmarco Liberec Company [15] and on the pilot plant laboratory device at the Department of Nonwovens, Technical University of Liberec. For the electrospinning, strings electrode in a length of 50 cm was used. By using this system, the nanofibrous layer is wider and the yield is higher; it is favorable for mass production. Prior to use, the silica nanofibers were thermally stabilized at 180°C for 2 hours.

The stabilized silica nanofibers comprise analytically more than 90% of SiO_2. The rest are retained alcohols from preparation process (isopropyl alcohol and ethanol) and water (in the form of Si-OH groups and adsorbed water). Alcohol content is gradually decreased with storage time, but it does not affect other properties of the nanofibers.

2.3. Characterization of Silica Nanofibers. The prepared silica nanofibers were visualized and characterized by a scanning electron microscope (SEM) Carl Zeiss ULTRA plus. The measurement of specific surface area was carried out by the



Scheme 1: Reaction of silica nanofibers with APTES and the subsequent coupling reaction with tetracycline [14].

method of krypton adsorption on the Autosorb IQ-KR/MP instrument.

2.4. Silica Nanofibers Functionalization by Amino Groups. Amino groups on the silica nanofiber matrix were introduced by reaction with (3-aminopropyl)triethoxysilane (APTES) (Scheme 1). A sample of the nanofibrous sheet was dipped in 0.08% APTES solution in aqueous isopropyl alcohol (6% H_2O v./v.) for 1 hour. After removal from APTES solution, the samples were washed twice with water and once with 0.1% acetic acid and dried at 30°C for 3 hours.

2.5. Quantification of Amino Groups on Silica Nanofibers. Determination of formed amino groups on the surface of the functionalized nanofibers in APTES was performed according to the method developed by Ritter and Brüwiller [16]; the method was slightly modified for our purpose.

Samples of silica nanofibers (weight approximately 5 mg) were incubated with 2 mL of stock solution of fluorescein isothiocyanate in absolute ethanol (1 mg/mL) overnight in the dark, under gentle shaking. After removing from fluorescein isothiocyanate solution, the samples were exhaustively washed with ethanol and dried. Afterwards, dried nanofibers were dissolved in 0.2 M NaOH and the absorbance of the resultant solution was measured by spectrophotometric analysis. Measurements were performed on the double-beam UV-Vis spectrophotometer Cintra 202 in 10 mm quartz cuvettes; used wavelength was 490 nm ($\varepsilon = 75000\ \text{mol}^{-1}\cdot\text{cm}^{-1}$).

These measurements of reactive (accessible) amino group quantity were confronted with elemental analysis of functionalized nanofibers. Elemental analysis of the samples was performed on the PE 2400 Series II CHNS/O Analyzer.

2.6. Silica Nanofibers Functionalization by Tetracycline. Amino groups on the surface of functionalized nanofibers are usable for immobilization of tetracycline by formation of a covalent bond (see Scheme 1). Samples of functionalized nanofibers were left in the action of tetracycline solution in absolute ethanol (0.1% or 1% w./v.); the reaction was carried out in a covered Petri dish placed in the dark for 24 hours at

room temperature. Subsequently, the samples were eluted in absolute ethanol and dried at 35°C for 30 min.

2.7. Tetracycline Quantification. The quantity of tetracycline (TC) entrapped in nanofibrous net was determined by spectrophotometric analysis (see Section 2.5). The quantity of adsorbed TC was calculated from measurement of ethanolic extracts (absorbance at max. 365 nm, $\varepsilon = 16600\ \text{mol}^{-1}\cdot\text{cm}^{-1}$).

Total quantity of TC was analyzed separately by elution with 0.1 M HCl followed by absorbance reading at max. 356 nm ($\varepsilon = 13900\ \text{mol}^{-1}\cdot\text{cm}^{-1}$). The differences of these values correspond to the portions of covalently bound TC, but with an inferior accuracy. The covalently bounded TC was determined more precisely by using a larger amount of sample. In this experiment, the adsorbed TC was removed at first by extraction with ethanol, and then the remaining (covalently bounded) amount of TC was released due to the incubation with 0.1 M HCl.

Extraction of TC from the nanofibers was done as follows: the sample (5–10 mg) was immersed in 4–10 mL of an eluent and placed for at least 8 hours under gentle shaking in the dark. The extinction coefficients were calculated from the plots of absorbance values of standard TC samples series in concentration range from $1{\cdot}10^{-6}$ to $5{\cdot}10^{-5}\ \text{mol}\cdot\text{dm}^{-3}$. Peaks maxima in ethanolic and hydrochloride acid extracts were found the same as in standard TC solutions.

The reliability of the results from spectrophotometric measurements was confirmed by HPLC analysis of selected samples. The adsorbed quantity of TC was determined by measurements of ethanolic samples extracts (calculated from area of the peak with max. 365 nm). After dissolving of ethanol-washed nanofibers in HF/HCl mixture, a covalently bonded TC was detected (area of the peak with max. at 356 nm was measured).

The HPLC analysis of selected samples were made on Dionex Ultimate 3000 HPLC Systems, equipped with the Diode Array Detector and a reverse-phase column Phenomenex Kinetex PFP 2.6 μm 100 R, 150 mm × 4.6 mm, and using a mobile phase 0.05 M trifluoroacetic acid and 0.01 M ammonium-acetate in water and/or in 20% methanol for gradient elution.

2.8. Antibacterial Activity Tests. For testing of the immobilized tetracycline antibacterial efficacy, modified microbiological quantitative method AATCC 147 was chosen. Onto a Petri dish with blood agar, 1 mL of bacterial inoculum at a concentration of 10^8 CFU/mL was pipetted, and the inoculum was triturated (the excess of inoculum was aspirated). The tested material (size of 18 × 18 mm) was placed in the middle of the Petri dish, the plate was closed, and samples were incubated at 37°C for 24 hours. After incubation time, the size of inhibition zones (IZ) of both samples was evaluated.

As a comparative method, the quantitative method of the disc diffusion test was utilized; this method is commonly used for determining of bacterial strain sensitivity to the tested antibiotic. The antibiotic (TC) diffuses from the disc to the surroundings; bacterial growth is inhibited; and a zone of inhibitions is created (IZ) around the disk. Subsequently, the diameter of the inhibition zone is measured and it must be compared with the reference inhibition zone specified by the manufacturer. The result is sensitive or resistant bacterial strain to the antibiotics, according to the inhibition zone diameter in comparison with manufacturer data. For this test, Müeller-Hinton agar was used.

Table 1: Analysis results of samples after functionalization by APTES: [a]elemental analysis, [b]calculation from % N, and [c]data from spectrophotometric measurements.

Sample	Content of N[a] (wt%)	APTES captured[b] (μmol/g)	Accessible NH_2 groups[c] (μmol/g)
1	0.13	93	4.46
2	0.10	71	3.76
3	0.11	79	9.83
4	0.13	93	4.52

3. Results and Discussion

3.1. Silica Nanofibers Morphology. The silica nanofibers produced by electrospinning by applying a string electrode (Figure 1) have a standard bimodal distribution with diameter about 250 nm and 650 nm. The silica nanofibrous layer includes both nanofibers (diameter of 70–500 nm) and microfibers (diameter of 500–1500 nm). This material shows better mechanical properties for the manipulation required in medical applications compared with nanomaterial without thick silica fibers [7]. Final nanofibrous layer has the specific surface area $8.8\ \text{m}^2\cdot\text{g}^{-1}$; it was determined by the krypton adsorption method.

3.2. Results of Amino Groups and Tetracycline Quantification on Silica Nanofibers. Elemental analysis of silica nanofibers after the treatment with APTES showed the nitrogen content in the range 0.10–0.13 wt%. If the silica nanofibers before functionalization did not contain any nitrogen, the calculations based on wt% N gave the amount of APTES entrapped on nanofibers. These values were much higher than the amounts of accessible (reactive) amino groups (assigned with fluorescein isothiocyanate) in consequence of the known multilayer deposition of the reagent. The full utilization of amino groups should give the load of tetracycline from 0.17 to 0.4 wt%. Summarized data for samples 1–4 (taken from four different batches of the silica nanofibers) are in Table 1.

Table 2 summarizes the results of the TC attachment on the functionalized samples 1–4. Elemental analysis results of all samples demonstrated an increased content of nitrogen in comparison with values in Table 1. These differences served for rough calculations of TC content. In most cases, estimations were in accordance with the results of the subsequent spectrophotometric analysis and HPLC analysis. The analysis of the samples in Table 2 showed the content of tetracycline within 0.6% to 3.7%, which is a good range for an intended medical application. The prevailing quantity of tetracycline was bound weakly by physisorption forces, while the minor quantity was covalently bound. As the adsorbed TC quantity went up more rapidly than covalent

Table 2: Results of tetracycline analysis: [a]concentration of TC solution in immobilization process, [b]increase of % N after TC immobilization, [c]calculation from rising of N content, [d]not determined, and [e]value from separate analysis of larger sample.

Sample	Concentration of TC (wt%)[a]	N[b] (wt%)	Estimated wt% TC[c]	Spectrophotometric measurements			HPLC Analysis	
				Total TC (wt%)	Adsorbed TC (wt%)	Bound TC (wt%)	Adsorbed TC (wt%)	Bound TC (wt%)
1	1	0.21	2.86	2.88	2.71	0.17	2.61	0.05
2	0.1	0.04	0.63	0.63	0.54	0.09	—[d]	—[d]
2	1	0.20	3.33	3.69	3.52	0.17	—[d]	—[d]
3	1	0.23	3.65	3.69	3.58	0.11	3.59	0.04
4	1	0.26	4.13	3.73	3.68	0.05/0.06[e]	—[d]	—[d]

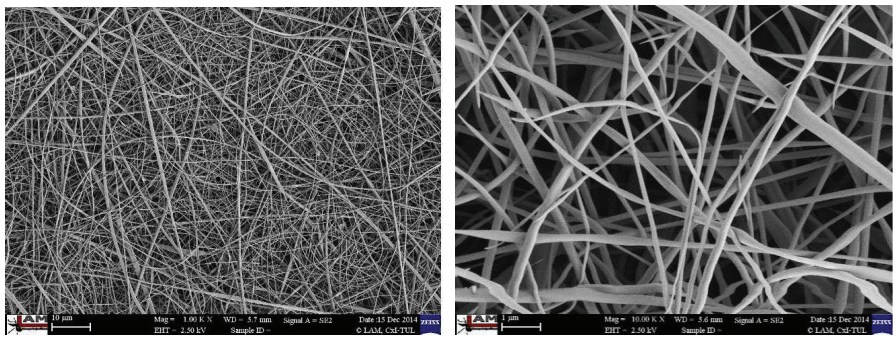


Figure 1: SEM pictures of pure silica nanofibers at various magnifications.

bonding with an increased concentration of TC solution, this proportion can be influenced in this way (see sample 2). The content of covalently bound TC is determined 0.04–0.17%. These percentages correspond to 0.9 to 3.83 μmol TC/g. The comparison with data in Table 1 implies that not all accessible amino groups were involved in TC covalent bonding.

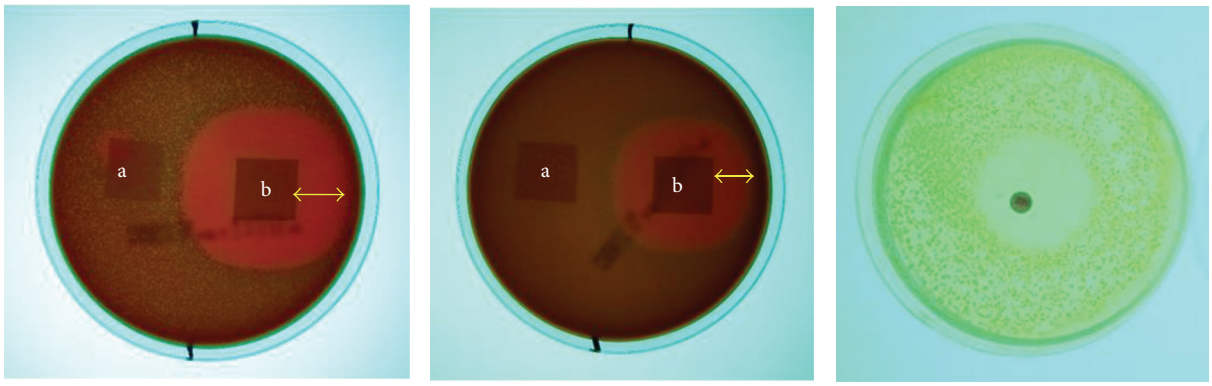
By calculation of the number of amino groups per surface area from the elemental analysis results (0.10–0.13 wt% N) and the surface area determined by krypton sorption (8.8 m^2g^{-1}), we can determined the range of 4.9–6.4 molecules/nm^2. These values correspond to multilayer binding of APTES.

3.3. Antibacterial Activity of Silica Nanofibers with Tetracycline. Newly developed dressing material—silica nanofibers with tetracycline—shows a large number of advantages which does not provide any other commercial wound dressing of this type. The advantage of this dressing is that it closely adheres to wound bed; the silica nanofibers are gradually dissolved and the gradual release of the immobilized and anchored TC occurs simultaneously. The total content of tetracycline (i.e., covalently and noncovalently bonded) for nanofibers with specific weight of 49.3 g·dm^{-2} was determined to be 17.0 mg·dm^{-2} (on average). The size 10 × 10 cm is standard size of commercial wound dressing. Compared with TC tablets form, where the TC dosage is 400 mg/24 hours (later 200 mg/24 hours), using of this nanofibrous material is dosage decreased to 17 mg/72 hours. The human organism is not overloaded by the drug and no resistance to the antibiotics occurs.

The tests clearly demonstrated the effect of tetracycline on testing bacterial strains. Tests proved that the pure silica nanofibers exhibit no inhibitory effect on the tested bacterial strains; there is no obvious IZ. In contrast, for bacterial strain *E. coli* 14.6 mm is the size of IZ and for the bacterial strain *S. aureus*, the size of IZ is measured 10.6 mm (Figure 2). As a control sample, a TC disc was used. Dual method of TC binding (covalently and noncovalently) can offer an advantage in the longer-term effects of the substance. First, noncovalently bound TC is released, and subsequently depending on the silica nanofibers degradation, covalently bound TC is released. TC thus affects the long term and high intensity in the wound bed, which cannot be achieved with systemic administration of antibiotics.

4. Conclusion

This material represents a new and very convenient application form of wound dressing. The nanofibrous material itself is nontoxic (based on a silica which does not irritate) and after suitable surface treatment, it may be immobilized a wide range of different biomolecules on its surface to cause the same effect of action as tetracycline in this study. Here presented silica nanofibers with determined quantity of tetracycline are a potential novel type of wound dressing. The quantity of tetracycline was determined by two different methods, spectrophotometric analysis and HPLC analysis to compare. Antibacterial tests showed that the specific quantity of tetracycline on the silica nanofibers was sufficient for intended biomedical application. Moreover, tested dosage of the antibiotics is much lower in comparison with commercially used tetracycline drugs, which is highly desirable. The human organism is not overloaded by the drug and resistance to the antibiotics does not occur.



S. aureus *E. coli* *S. aureus*

FIGURE 2: Inhibition zones sizes of pure silica nanofibers (a), silica nanofibers with immobilized tetracycline (b), and control disc with 10 μg of tetracycline (right picture).

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

Acknowledgments

The authors thank the Analytical Laboratory of the Institute of Organic Chemistry and Biochemistry, Academy of the Sciences of the Czech Republic, Prague 6, for the results of elemental analysis. The research presented was supported by the Ministry of Education, Youth, and Sports in the framework of the targeted support of the "National Programme for Sustainability I" LO 1201 and the OPR&DI project "Centre for Nanomaterials, Advanced Technologies, and Innovation," CZ.1.05/2.1.00/01.0005.

References

[1] H. S. Yoo, T. G. Kim, and T. G. Park, "Surface-functionalized electrospun nanofibers for tissue engineering and drug delivery," *Advanced Drug Delivery Reviews*, vol. 61, no. 12, pp. 1033–1042, 2009.

[2] Y. Gao, Y. B. Truong, Y. Zhu, and I. Louis Kyratzis, "Electrospun antibacterial nanofibers: production, activity, and in vivo applications," *Journal of Applied Polymer Science*, vol. 131, no. 18, pp. 9041–9053, 2014.

[3] G. Wang, A. N. Otuonye, E. A. Blair, K. Denton, Z. Tao, and T. Asefa, "Functionalized mesoporous materials for adsorption and release of different drug molecules: a comparative study," *Journal of Solid State Chemistry*, vol. 182, no. 7, pp. 1649–1660, 2009.

[4] S. Hashemikia, N. Hemmatinejad, E. Ahmadi, and M. Montazer, "Optimization of tetracycline hydrochloride adsorption on amino modified SBA-15 using response surface methodology," *Journal of Colloid and Interface Science*, vol. 443, pp. 105–114, 2015.

[5] C. Fu, T. Liu, L. Li, H. Liu, D. Chen, and F. Tang, "The absorption, distribution, excretion and toxicity of mesoporous silica nanoparticles in mice following different exposure routes," *Biomaterials*, vol. 34, no. 10, pp. 2565–2575, 2013.

[6] I. Šlamborová, V. Zajícová, P. Exnar, and J. Studničková, "Nanofiber structure with immobilized organic agens and the method of its preparation," (Patent), CZ 303 911, 14.8.2012, 9.5.2013; WO 2014026656, 20.2.2014; EP2884968A1, 24.6.2015; US2015240411A1, 27.8.2015.

[7] I. Lovětinská–Šlamborová, P. Exnar, I. Danilová, and I. Veverková, "Medical and biochemical applicability of silica nanofibers," in *Proceedings of the Nanofibers, Applications and Related Technologies (NART '15)*, pp. 263–269, Liberec, Czech Republic, 2015.

[8] S. Saivain and G. Houin, "Clinical pharmacokinetics of doxycykline and minocykline," *Clinical Pharmacokinetics*, vol. 15, no. 6, pp. 355–366, 1988.

[9] H. Lüllmann, K. Mohr, and M. Wehling, *Farmakologie a Toxikologie*, Grada Publishing, Praha, Czech Republic, 2nd edition, 2002.

[10] C. Simon and W. Stille, *Antibiotika v Současné Lékařské Praxi*, Grada Publishing, Praha, Czech Republic, 1st edition, 1998.

[11] J. A. Sowjanya, J. Singh, T. Mohita et al., "Biocomposite scaffolds containing chitosan/alginate/nano-silica for bone tissue engineering," *Colloids and Surfaces B: Biointerfaces*, vol. 109, pp. 294–300, 2013.

[12] Z. Luo, Y. Deng, R. Zhang et al., "Peptide-laden mesoporous silica nanoparticles with promoted bioactivity and osteodifferentiation ability for bone tissue engineering," *Colloids and Surfaces B: Biointerfaces*, vol. 131, pp. 73–82, 2015.

[13] T.-H. Kim, M. Kim, M. Eltohamy, Y.-R. Yun, J.-H. Jang, and H.-W. Kim, "Efficacy of mesoporous silica nanoparticles in delivering BMP-2 plasmid DNA for in vitro osteogenic stimulation of mesenchymal stem cells," *Journal of Biomedical Materials Research Part A*, vol. 101, no. 6, pp. 1651–1660, 2013.

[14] R. K. Sharma, S. Mittal, S. Azami, and A. Adholeya, "Surface modified silica gel for extraction of metal ions: an environment friendly method for waste treatment," *Surface Engineering*, vol. 21, no. 3, pp. 232–237, 2005.

[15] Nanofiber Equipment, http://www.elmarco.com/nanofiber-equipment/nanofiber-equipment/.

[16] H. Ritter and D. Brühwiler, "Accessibility of amino groups in postsynthetically modified mesoporous silica," *The Journal of Physical Chemistry C*, vol. 113, no. 24, pp. 10667–10674, 2009.