INITIAL SCREENING OF SILICA NANOFIBRES AS DRUG DELIVERY SYSTEM FOR ANTIBIOTICS IN WOUND HEALING

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Abstract

Over the last decade, drug delivery systems for wound management have undergone a significant evolution as novel nano-based dosage forms and wound dressing pads for active wound management have been developed. Significance of these materials is based on increasing demand for active wound dressing able to battle infection and inflammation in contaminated and slow-to-heal wounds. Nanofibres represent nanomaterial able to act simultaneously as wound dressing protecting the wound and drug delivery system for *in situ* drug release. Bioavailability of the drug and its release kinetics strongly depend on the drug nature, nanofibrous matrix characteristics and mechanism of drug incorporation. In bacterial infection treatment, fast reaching of minimal inhibitory concentration (MIC) is crucial and can be achieved by mediated surface drug adsorption. In this work, we explored interactions of electrospun silica nanofibres with five model antibiotics from tetracycline family (tetracycline, tetracycline hydrochloride, oxytetracycline hydrochloride, chlortetracycline hydrochloride and doxycycline hydrochloride) in order to evaluate robustness of this novel nanofibrous drug delivery system. The drug adsorption was mediated by surface functionalization with amino groups. Drug functionalized nanofibres were studied in terms of morphology and total drug content. Antibacterial activity against bacteria *E. coli* was tested *in vitro*. Overcoming the MIC value was achieved for all tested antibiotics. Biocompatibility was tested and confirmed on human dermal fibroblasts *in vitro*. Presence of all tested antibiotics led to increased cellular metabolic activity. Based on these results we assumed that silica nanofibres represent a robust system able to bind and release variety of tetracycline antibiotics for contaminated wounds management.

Keywords: Silica, nanofibres, drug delivery, antibiotics, wound healing

1. INTRODUCTION

Development of contaminated non-healing wounds, ulcers and decubitus ulcers represents one of serious issues related to deceases of affluence - diabetes mellitus primarily. According to statistics, the risk of developing foot ulcers is 25 % and leads to one lower limb amputation every 30 seconds among diabetic patients around the world. It is estimated that of all amputations (diabetes related), 85 % are contributed by foot ulceration which further deteriorates to chronic infection and severe forms of gangrene. Overall, the prevalence rate of diabetic foot ulcers is estimated to 5.1 % in Europe with cost demand increasing every year [1]. In 2005, for instance, the average cost of contaminated foot ulcer treatment in Germany was $ 2,957 [2]. This cost doesn´t involve the social impact or any emotional effects on the patient. Under given circumstances, if the ulcer formation can´t be avoided, the most suitable therapy is demanded to prevent the amputation and

to minimize the long term impact on patient. The *in situ* therapy seems to be the most effective and the most sensitive treatment and application of a nanofibrous dosage form may bring further benefits in form of wound protection and stimulation [3,4]. Among all materials used for nanofibres production, silica shows unique properties including fast biodegradation [5] and active wound stimulation [6]. Silica nanofibres combine traditional properties of nanofibres such as small fibre diameter and high porosity with unique properties given by their bioactive nature. Silica and organosilane nanofibres formed by sol-gel method and subsequent electrospinning were confirmed as suitable matrix for enzyme conjugation for active wound debridement [7], able to suppress bacterial colonization [8], and stimulate wound healing [9]. Their easy-to-modify surface can be grafted with functional groups introducing surface charge for further drug of choice adsorption. In the era of personalized medicine approach, robustness of such active wound dressing is required in order to meet the needs of individual patients and their specific bacterial profile [10,11].

2. MATERIALS AND METHODS

Tetracycline (TET), tetracycline hydrochloride (TET.HCl), oxytetracycline hydrochloride (OxyTET), chlortetracycline hydrochloride (ChlorTET), doxycycline hydrochloride (DOXY), tetraethyl orthosilicate (TEOS) and (3-aminopropyl)triethoxysilane (APTES) were purchased from Sigma-Aldrich (CZ). The remaining chemicals (acetic acid, ethanol, isopropyl alcohol) were supplied by Petr Švec – Penta (CZ). Cell culture media components and TSA agar were purchased from Fisher Scientific (CZ) and iBiotech (CZ).. Biocompatibility testing was performed on adult normal human dermal fibroblasts (aNHDF) purchased from ATCC (USA). *Escherichia coli* (K-12 CCM 7929) was obtained from Czech Collection of Microorganisms (Brno, CZ).

2.1. Nanofibres manufacturing and functionalization

Spinning solution was synthetized by sol-gel method in water/ ethanol mixture from TEOS as a precursor. Silica nanofibres was obtained by subsequent needle-less electrospinning on NanoSpider NS 1WS500U device (Elmarco, CZ)) and thermal stabilization (180 °C). Surface silanization was realized in 3 % APTES solution in water and ethanol mixture with pH adjusted to 4.5 - 5.5. Modification was performed overnight under constant shaking at laboratory temperature. Excess of the silanization solution was washed out and nanofibres were dried at 110 °C for 30 min. Antibiotic immobilization on the surface of APTES treated silica nanofibres was performed in 0.5 % antibiotic solution in ethanol or water for 2 hours in sealed container and under constant agitation (50 rpm). Subsequently, nanofibres were removed from the solution and dried in dark at room temperature.

2.2. Characterization of antibiotic functionalized nanofibres

Morphology of silica nanofibres prior and after the antibiotic immobilization was analysed using scanning electron microscopy (SEM) on device Vega3 (Tescan, CZ). Fibre distribution was performed based on 100 fibre diameter measurements for each sample. Absolute quantity of antibiotic immobilized per sample area was analysed spectrophotometrically using Multi-Mode Microplate Reader (BioTek Instruments, USA). Discs of nanofibres (12 mm diameter) were dissolved in 0.2 M NaOH. Each antibiotic was quantified by its specific absorption maximum values and calculation from calibration curve.

2.3. Biocompatibility and antibacterial activity evaluation

Antibacterial activity of functionalized nanofibres was tested by diffusion test according to the standard AATCC Test Method 147-2004 using Escherichia coli as model bacterial strain. Bacterial suspension (10,000 bacteria per ml) was seeded on trypton soya agar (TSA) plates. Nanofibrous discs (10 mm in diameter) were placed on the agar in triplicates and incubated for 24 hours at 37 °C. After the exposition period, size of diffusion zone inhibiting bacterial growth around samples was measured and photo documented.

Biocompatibility of antibiotic functionalized nanofibres was tested in compliance with ISO 10993-5 on adult NHDF cells. Cell suspension in concentration 10,000 cells per ml was seeded on 24 well plate (10,000 cells per well) and pre-cultured for 24 hours prior the test. After this period, nanofibrous discs (6 mm in diameter) were placed to each well into direct contact with cell population, covered with fresh medium, and incubated for 24 hours (5 % CO_2, 37 °C). After the exposition, cellular viability was tested using MTT assay. 1 mg/ml MTT solution in serum-free medium was placed into each well and incubated for 2 hours in 37 °C. In this widely used assay, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is converted by active oxidoreductase present in living cells onto its insoluble form MTT formazan. After incubation period, unreacted MTT solution was discarded and produced MTT formazan was solubilized in isopropyl alcohol. Produced MTT formazan was quantified spectrophotometrically by absorbance intensity measuring at 570 nm (and 650 nm for background). Cell viability was calculated as percentage share of living cells exposed to the sample in comparison to living cells present in untreated cell control (CC). DMSO solution (8 %) was used as positive control (PC). Each sample was tested in four repeats.

3. RESULTS AND DISCUSSION

3.1. Nanofibres morphology and immobilized antibiotic quantity

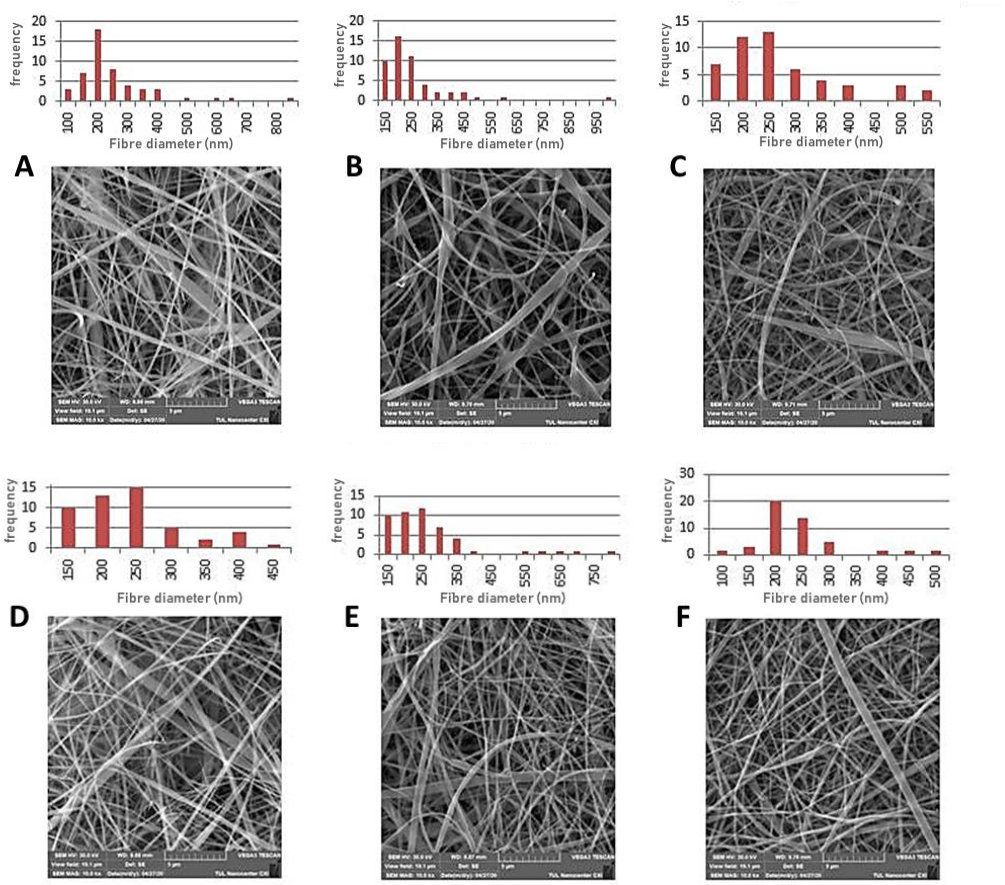

Figure 1 Morphology of SiO_2 nanofibres A. prior the treatment and treated with B. Tetracycline, C. Tetracycline hydrochloride, D. Oxytetracycline hydrochloride, E. Chlortetracycline hydrochloride and F. Doxycycline hydrochloride (Scale 5 μm)

Morphology of silica nanofibres prior and after the silanization procedure and antibiotic immobilization is shown in **Figure 1**. The silica nanofibres show broader fibre diameter distribution in comparison to conventional nanofibres electrospun from commercial synthetic linear polymers. This effect is caused by chain branching

and binding at the beginning of sol-gel transition. Comparison of fibre distribution histograms presented in **Figure 1** shows that silanization procedure and subsequent antibiotic immobilization have no significant impact on fibre diameter. For all the samples, the most populated histogram classes lie between 150 nm and 250 nm. All SEM micrographs show opened porous structure without loss of porosity or significant change in fibrous morphology. Functionalization in ethanol or aqueous solution didn´t cause changes in fibres structure, swelling or breaks. This proves good stability of silica nanofibres in variable solvents (except of strong bases), which gives opportunity for functionalization under various conditions and with various molecules. Treatment of nanofibres with 0.5 % antibiotic solution led to homogenous distribution of all model drugs over individual nanofibres.

Quantification of adsorbed antibiotics showed differences in their quantity suggesting differences in interactions between the silanized nanofibres surface and antibiotics molecules. The highest average amount was obtained in the case of doxycycline 244.22 μg/cm^2. The lowest amount was demonstrated in the case of chlortetracycline (51.36 μg/cm^2) and then tetracycline hydrochloride (77.39 μg/cm^2). All the obtained results are shown in **Figure 2**.

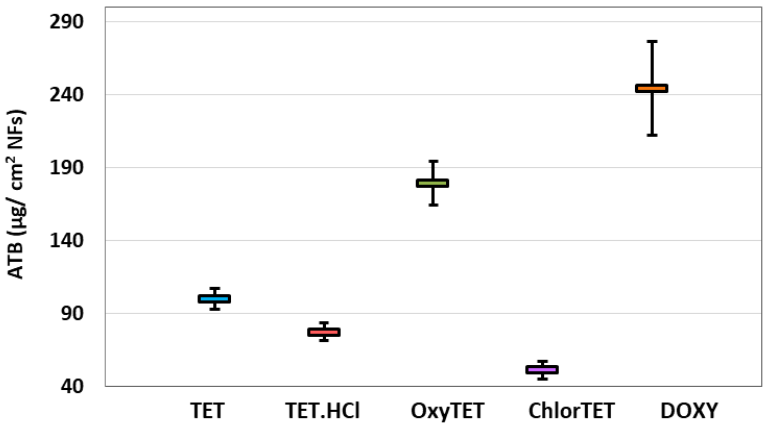

Figure 2 Quantity of tetracycline antibiotics immobilized in silanized silica nanofibres (mean ± S.D.)

3.2. Antimicrobial activity

All the tested tetracycline antibiotics immobilized on silica nanofibres proved antibacterial efficacy against *E. coli* monoculture on TSA substrate. Obtained results are shown in **Figure 3**. Each sample was able to form diffusion zone inhibiting bacterial growth around the nanofibres, The biggest zone was observed in the case of oxytetracycline (33.33 mm) and the smallest zone in the case of chlortetracycline (20.67 mm). These results correlate only partially with the absolute quantities of immobilized antibiotics. This discrepancy can be explained by different specifity and MIC values of tested antibiotics against *E. coli*. This bacterial strain was only used as model bacteria, which is commonly found in contaminated wounds and further investigation against multiple bacterial strains is needed.

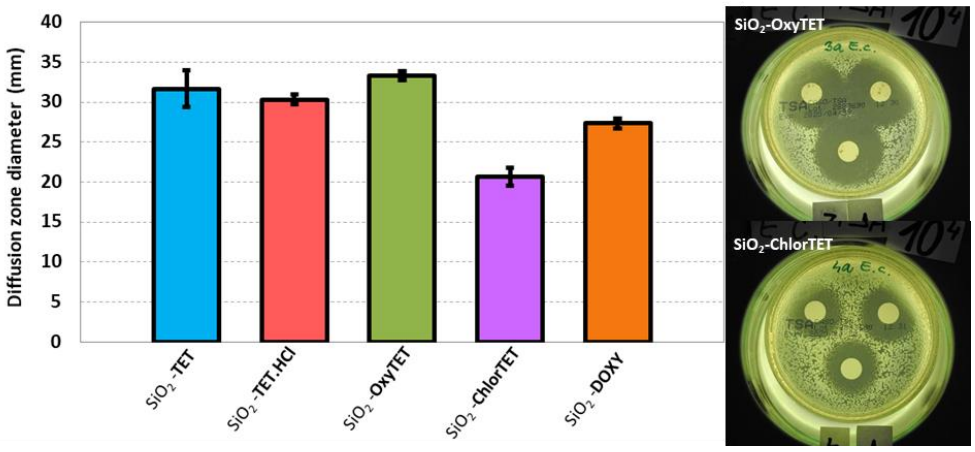

Figure 3 Diffusion zone diameters on *E. coli* monoculture expressed as graph (mean ± S.D.) and photographs of selected samples

3.3. Biocompatibility

Investigation on biocompatibility proved that exposition of human dermal fibroblasts to all the tested samples didn´t result in cell viability drop below 70 %. Therefore, all the tested samples can be declared as biocompatible in compliance to ISO 10993-5. Exposition to silica nanofibres with immobilized antibiotics led to increased metabolic activity compared to cell control (CC). All results are shown in **Figure 4**. Even though the results are presented as cellular viability, it is not possible to determine clearly if presence of antibiotics leads only to increased metabolic activity in NHDF cells or if number of cells increased. Such effect could be very beneficial for wound healing progress after contamination elimination and further instigation is needed.

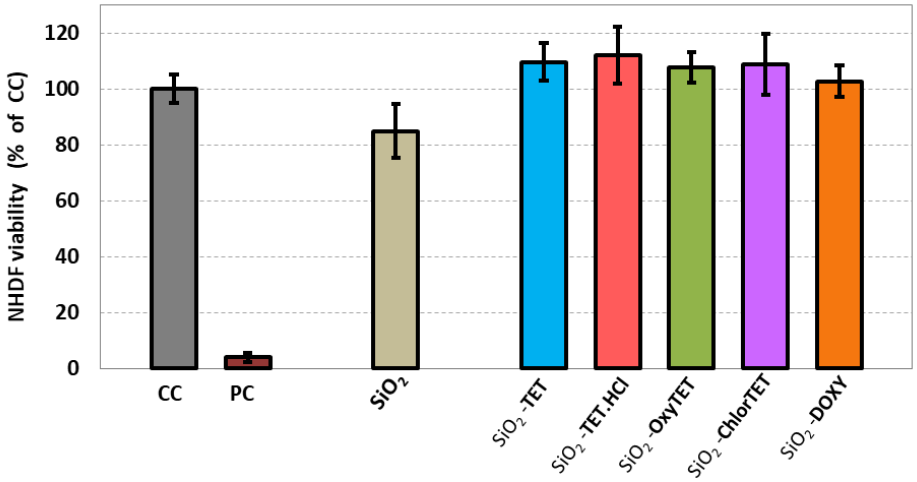

Figure 4 Normal human dermal fibroblasts (NHDF) viability after 24 hours exposure to silica nanofibres with immobilized tetracycline antibiotics *in vitro* (mean ± S.D.)

4. CONCLUSION

In this paper we presented results on initial study of silica nanofibres evaluation as potentially robust system for antibiotics immobilization and *in situ* release for contaminated wounds therapy. Silica nanofibres immobilized with five model antibiotics from tetracycline family were proven be antibacterial against *E. coli* and biocompatible to human dermal fibroblasts *in vitro*. The immobilization procedure in ethanol and aqueous solution didn´t cause changes in the fibrous morphology. Therefore, silanized silica nanofibres represent an interesting candidate for broad range antibiotics immobilization and potential application in personalized wound therapy. To confirm this assumption, further investigation will be needed.

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