SILICA NANOFIBERS WITH COMBINED BIOACTIVITY BASED ON GENTAMICIN SULPHATE WITH NATURAL BIOMOLECULES

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Abstract

The increasing prevalence of bacterial resistance poses a significant challenge to contemporary healthcare, representing a serious and potentially life-threatening complication in patients with bacterial infections of diverse origins, severities, and anatomical localizations. Strategies aimed at mitigating resistance and identifying novel substances or mechanisms able to suppress it are essential, particularly in the treatment of infected wounds. One promising strategy is the integration of traditional medicine and phytotherapy, which offers a wide spectrum of natural compounds with bioactive properties, including anti-inflammatory, antibacterial, antifungal, and regenerative effects. The synergistic combination of natural substances with conventional pharmaceuticals may enhance therapeutic efficacy, reduce required dosages, and accelerate healing, thereby minimizing systemic burden and shortening treatment duration. Moreover, certain phytochemicals have demonstrated the ability to modulate bacterial resistance mechanisms, such as efflux pump inhibition. However, the limited miscibility and biocompatibility of solvent systems used for natural compound extraction necessitate the development of optimal carriers that are biocompatible, biodegradable, solvent-stable, and capable of targeted delivery. In this context, micro- and nanomaterial-based carriers offer considerable potential. This study investigates the potential of biodegradable silica nanofibers (SiNFs) as carriers for the natural bioactive compounds carvacrol (CAR) and thymol (THY), as well as their combinations with the conventional antibiotic gentamicin sulphate (GEN). The functionalized carrier was evaluated for antibacterial activity and biocompatibility to murine fibroblasts.

Keywords: Silica nanofibers, gentamicin sulphate, carvacrol, thymol, combined antibacterial effect

1. INTRODUCTION

The growing resistance of pathogenic bacteria to conventional antibiotics and synthetic drugs presents a serious challenge to modern healthcare systems [1]. This issue is particularly critical in the treatment of chronic and infected wounds, where bacterial resistance significantly limits therapeutic options. Natural compounds derived from medicinal plants have emerged as promising candidates for antimicrobial therapy due to their diverse bioactive properties and ability to modulate bacterial resistance mechanisms [2, 3]. Numerous phytochemicals, including thymol and carvacrol, have demonstrated antibacterial activity through mechanisms such as membrane disruption, efflux pump inhibition, and interference with proton-motive force channels [4-6].

Detailed data on the mechanisms of action of various plant-derived compounds and their synergistic interactions with antibiotics are increasingly available [7]. These synergistic effects may enhance antibiotic

efficacy, restore sensitivity in resistant strains, and allow for dose reduction, thereby minimizing cytotoxicity and environmental impact.

Despite these advantages, the therapeutic application of phytochemicals is often limited by their volatility, hydrophobic nature, and cytotoxicity at effective concentrations [5]. Therefore, the development of advanced delivery systems is essential to ensure controlled dosing, stability, and biocompatibility. Nanomaterial-based carriers offer a promising solution, as they can improve the bioavailability of hydrophobic compounds and protect volatile components from degradation [8].

Among these, biodegradable silica nanofibers (SiNFs) represent an attractive platform for the immobilization and controlled release of bioactive molecules [9]. Their high surface area, structural stability, and tunable porosity enable efficient loading and sustained delivery of both natural compounds and antibiotics. Moreover, SiNFs can serve as physical barriers against secondary infections and support tissue regeneration due to their compatibility with skin cells. However, only a limited number of studies have explored the integration of defined mixtures of phytochemicals and antibiotics into advanced nanostructured carriers, despite their potential to enhance therapeutic outcomes [8].

This study aims to investigate the antibacterial efficacy and biocompatibility of SiNFs functionalized with gentamicin and selected phytochemicals – thymol and carvacrol, focusing on their synergistic potential and suitability for wound healing applications.

2. MATERIALS AND METHODS

Gentamicin sulfate salt, carvacrol, thymol, Alamar Blue dye and tetraethyl orthosilicate (TEOS) were purchased from Sigma-Aldrich (CZ). The remaining chemicals (acetic acid, ethanol, isopropyl alcohol) were supplied by Penta (CZ). Cell culture media components were purchased from iBiotech (CZ) and trypton soya agar (TSA) plates (BO03519351N) from Oxoid (Cambridge, UK). Biocompatibility testing was performed on murine skin fibroblasts 3T3-NIH purchased from ATCC (USA). Bacterial strain *Staphylococcus gallinarum* (CCM 3572) was obtained from Czech Collection of Microorganisms (Brno, CZ).

2.1 Nanofibers manufacturing, functionalization and characterization

Silica nanofibers (SiNFs) were fabricated by needle-less electrospinning using the NanoSpider NS 1WS500U device (Elmarco, CZ), followed by thermal stabilization at 180 °C [9]. Immobilization of the active compounds - (GEN), (CAR) and (THY) - was achieved through adsorption onto the surface of SiNFs from ethanol- or water-based solutions containing GEN alone or in combination with CAR or THY, concentration of the compounds being 0.01% and 0.1% for GEN (in water, when adsorbed alone)) and 0.01%, 0.1% and 2.5% for THY and CAR (in ethanol). Adsorption was conducted over a 2-hour period in sealed containers under continuous agitation at 50 rpm. After adsorption, the nanofibers were removed and air-dried at room temperature. The morphology of SiNFs before and after functionalization was characterized using scanning electron microscopy (SEM) with a Vega3 instrument (Tescan, CZ).

2.2 Disc diffusion method for evaluation of antibaterial potential of activated nanofibers

The minimum inhibitory concentration (MIC) of active molecules was determined by evaluating growth inhibition of *Staphylococcus gallinarum* (SG) in a multiwell plate assay following exposure to either the natural compound or the antibiotic. The antibacterial potential of the activated nanofibers was evaluated by disc diffusion method according to the standard AATCC Test Method 147-2004 [10]. To test the *in vitro* antimicrobial properties of treated SiNFs, bacterial suspension (10,000 bacteria per ml) of *SG* was seeded on TSA plates, nanofibrous discs (10 mm of diameter) were placed on the agar in triplicates and incubated for 24 hours at 37°C. After the exposition period, size of diffusion zone representing the inhibition of bacterial growth around samples was measured and photo documented.

2.3 *In vitro* cell viability

The biocompatibility of the nanofiber carrier with a combination of gentamicin sulfate and natural substances was evaluated *in vitro* on murine skin fibroblasts 3T3-NIH. The cells were seeded at a concentration of 40,000 cells/well (48-well plate). After 24 hours of pre-cultivation, the cells were exposed to activated SiNFs (28.3 mm^2). Cell viability was evaluated after 24 hours by fluorescence intensity measurement (AlamarBlue Assay) and compared with an untreated cell control (after subtracting the values measured in the non-cell control samples). Combinations of 0.01% and 0.1% GEN with 0.01–2.5% thymol or 0.01–2.5% carvacrol were tested. Samples with viability exceeding 70 % of the cell control are considered biocompatible.

3. RESULTS AND DISCUSSION

3.1 Morphology of nanofibers

The advantage of nanofiber carriers for potential use in wound dressings is their specific porosity, which prevents secondary infection from the surrounding environment while maintaining vapor permeability. The very small pore size prevents external microorganisms from penetrating the site of infection, while still allowing the wound to be ventilated and any exudate to be drained. We have previously demonstrated that the pore size of SiNFs does not exceed 0.5 µm (unpublished data), whereas the size of bacteria most commonly responsible for wound infections exceeds this dimension [11]. Verification of the preservation of specific porosity in SiNFs after the functionalization process was performed by microscopic analysis. Comparison of the morphologies prior and after the adsorption of active molecules is shown in **Figure 1**. Analysis of morphology showed fibrous structure with randomly oriented nanofibers and high porosity, which remained after the functionalization process.

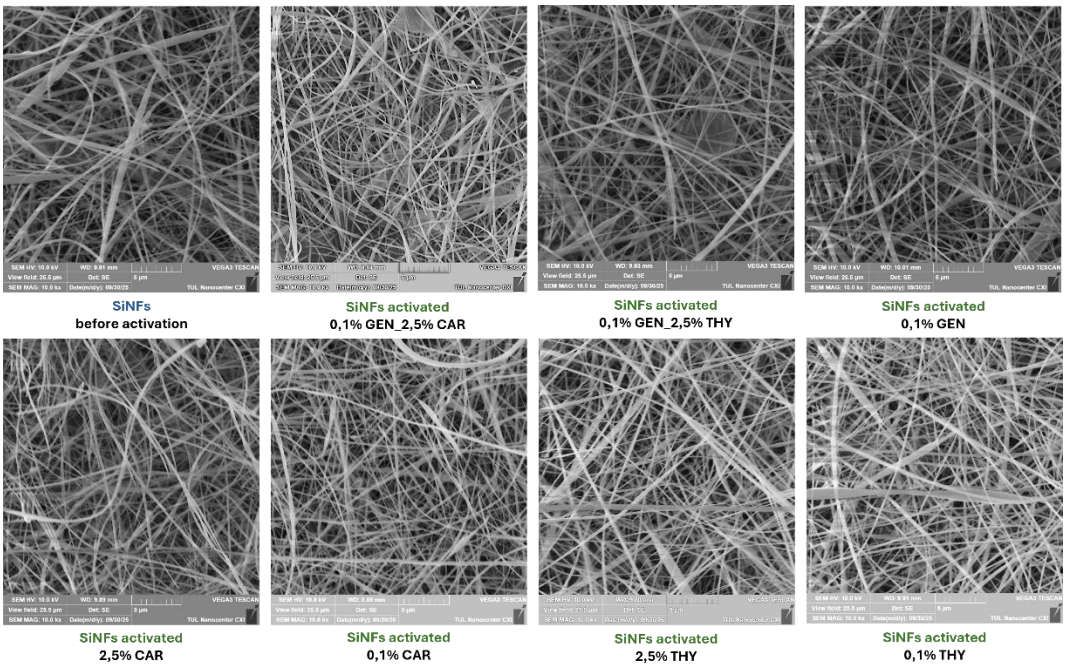

Figure 1 Morphology of SiNFs prior the activation and after the activation by adsorption of GEN, THY and CAR (concentration of solutions ranging from 0.1% to 2.5%). SEM under magnification 10 000 (bar = 5 µm).

3.2 Antibacterial activity

The susceptibility of *Staphylococcus gallinarum* to GEN, THY and CAR was assessed using a standard broth microdilution assay in a multiwell plate format. The minimum inhibitory concentrations (MIC) required to

achieve 80% inhibition of SG bacterial growth were determined to be 312.5 µg/mL for THY, 625 µg/mL for CAR, and 0.25 µg/mL for GEN (**Figure 2**).

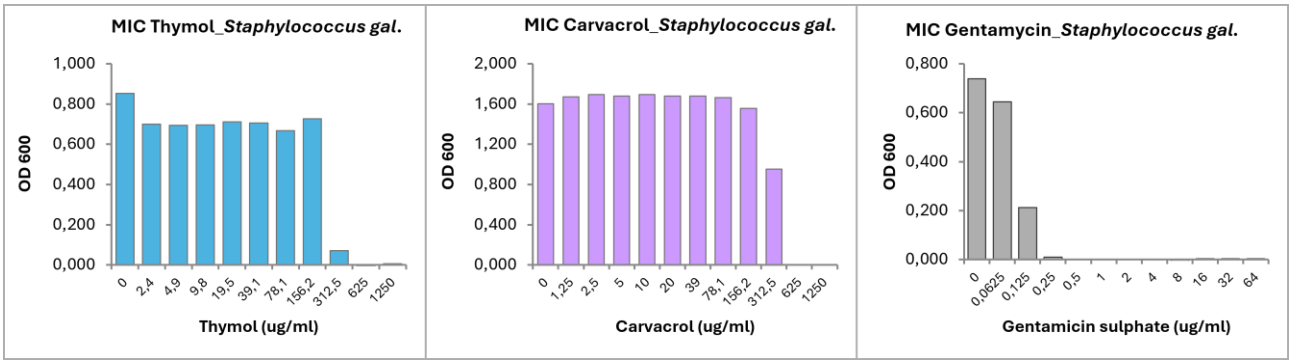

Figure 2 Minimal inhibitory concentration multiplate assay for THY, CAR and GEN. The rate of SG bacterial growth is directly proportional to optical density at 600 nm.

To evaluate the combined antibacterial effects of the synthetic antibiotic and natural compounds, GEN, THY, and CAR were individually or jointly adsorbed onto silica nanofibers (SiNFs). The antibacterial activity of the functionalized carriers was assessed by measuring the diameter of the inhibition zones surrounding the nanofiber samples. When applied individually at concentrations equivalent to those used for GEN, only GEN produced measurable inhibition zones, whereas THY and CAR alone were ineffective. However, when THY or CAR were released from SiNFs at higher concentrations, antibacterial activity against *S. gallinarum* monocultures was observed (**Figure 3**).

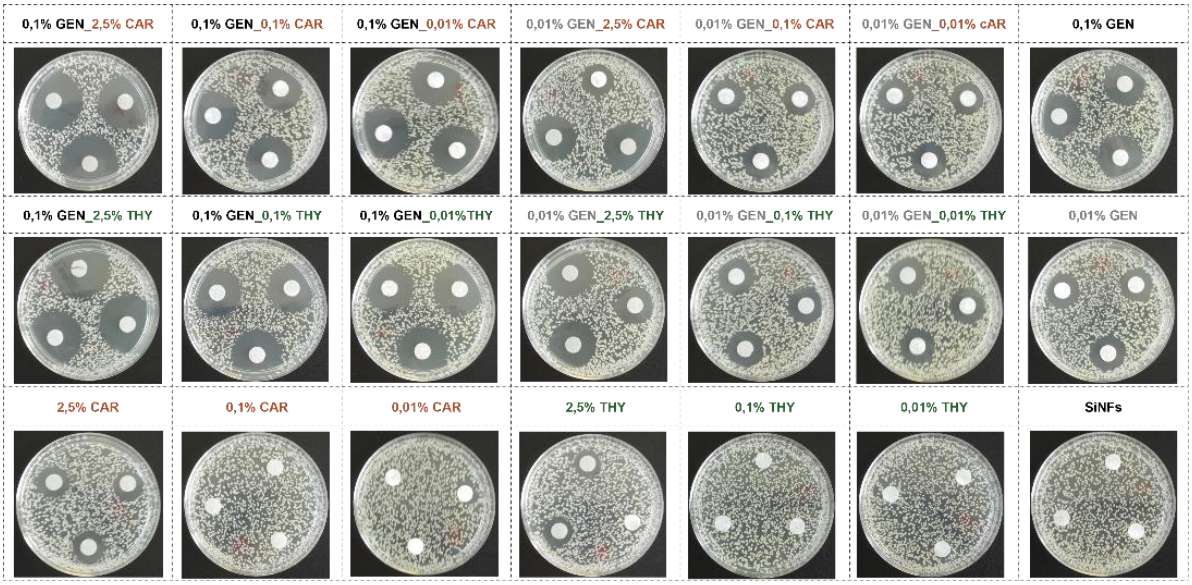

Figure 3 Disc diffusion zones – zones of bacterial growth inhibition formed around silica nanofibers (SiNFs) activated (or not) by (gentamicin sulfate) GEN, (thymol) THY, (carvacrol) CAR alone or in combination. Incubation of SiNFs with *Staphylococcus gallinarum* (10^5 cells/ml) on TSA plates after 24h incubation at 37°C).

Notably, the combination of GEN with either THY or CAR, even at lower concentrations, resulted in enhanced antibacterial effects, as evidenced by increased inhibition zone diameters compared to GEN alone. These findings suggest a synergistic interaction between the antibiotic and the natural compounds, contributing to the overall antibacterial efficacy of the composite system (**Figure 4**).

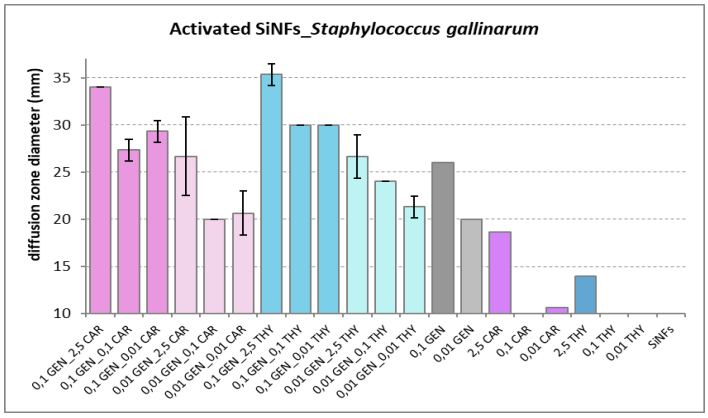

Figure 4 Comparison of disc diffusion zone diameters formed around SiNFs discs activated by GEN, THY, CAR alone or in combination, concentration of active compound ranging from 0,01% to 2,5% – size of the zone is directly proportional to the antibacterial activity of the tested nanofibrous carrier.

The bacterial strain *Staphylococcus gallinarum* was employed as a model organism for preliminary evaluation. To validate the broader applicability of the proposed system, further investigations involving multiple clinically relevant bacterial strains are warranted.

3.3 Evaluation of biocompatibility

Effective concentrations of natural extracts and phytochemical-based biomolecules often exceed cytotoxicity thresholds. Previous studies focusing exclusively on the antibacterial properties of pure natural substances have demonstrated that the concentrations required for antimicrobial efficacy are frequently several orders of magnitude higher than those considered safe for human cells [5]. To obtain preliminary insight into the biocompatibility of silica nanofibers (SiNFs) functionalized with active compounds, in vitro cytotoxicity assays were performed using murine skin cells.

None of the tested variants (GEN/THY, GEN/CAR) showed a significant decrease in cell viability after 24 hours of exposure. The lowest viability, 93.6 ± 11.8 %, was achieved with the combination of 0.1% GEN/2.5% CAR. Reducing the active substances dose to 0.01% GEN/0.01% CAR resulted in 109 ± 2.2 % viability while maintaining good antibacterial efficacy. A similar effect was observed with thymol. The viability of 100.4 ± 9.4 % achieved with the combination of 0.1% GEN/2.5% THY increased to 108 ± 8.8 % after adjusting the dose to 0.01% GEN/0.01% THY. A graphical summary of the results is shown in **Figure 5**. Increased cell viability indicates a positive effect of natural substances on cell proliferation and thus a potential improvement in wound healing.

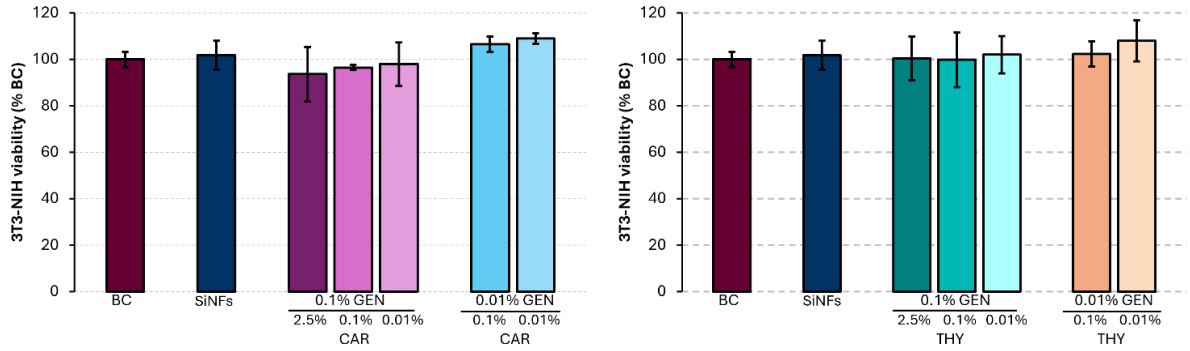

Figure 5 Biocompatibility evaluation in vitro of SiNFs prior or after activation by the tested compounds. Cells: 3T3-NIH murine fibroblasts. Exposure: 24 hours. Medium: DMEM, 10 % FBS, 1 % Penicillin-Streptomycin. BC: unterated cellular control. SiNFs: nanofibers before activation. The rest of the tested samples were SiNFs activated by different combination of 0,1% or 0,01% GEN with 0,1% or 0,01% THY.

4. CONCLUSION

The presented results demonstrate the potential of combining the antibacterial activity of conventional antibiotics with bioactive phytochemicals. Such combinations not only enhance the efficacy of antibiotics, which are increasingly compromised by bacterial resistance mechanisms, but also allow for a reduction in the required doses of sensitizing phytocompounds. The therapeutic effect of a given plant extract is often attributed to the synergistic action of its multiple constituents. Considering that thymol and carvacrol are principal components of several medicinal herbs (e.g., *Thymus vulgaris*), future studies may explore the functionalization of the proposed silica nanofiber carrier with complete herbal extracts. This approach could further expand the applicability of the system in the treatment of bacterial infections.

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