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Comparative study of the Antibacterial Properties of Modified Textile Materials with Different Metal Nanoparticles

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Abstract

A number of metal particles have proven antibacterial properties. In this study, a comparative analysis was made of two types of metal particles: zinc oxide and titanium oxide nanoparticles respectively, which were deposited on cellulose material and were fixed by means of a cross-linked gelatin hydrogel. Three methods were applied under different reaction conditions for obtaining the biocomposite materials. The biomaterials were investigated by means of scanning electron microscopy, transmittance spectral analysis, antibacterial analysis, air permeability. A different morphological structure was observed in the different samples. The titanium oxide nanoparticles were covered by gelatin film and were dispersed into smaller structures. Zinc oxide nanoparticles from a spherical shape have transitioned into newly formed crystal structures with needle-like ends. The modified cotton samples were tested against Gram-positive and Gram-negative bacteria. Biocomposite materials with TiO_2-NPs exhibited better antibacterial activity towards Bacillus cereus and Pseudomonas aeruginosa than materials containing ZnO-NPs. Therefore, such materials containing these two types of metal particles can find application in medical practice.

Keywords: Cotton fabric, crosslinking, gelatin, medical, metal nanoparticles

INTRODUCTION

To protect the human body from the effects of various microorganisms, it is extremely important to select the appropriate antibacterial agent. Many metal particles and their oxides exhibit antibacterial effect against various bacteria and viruses. Some dressings for wound healing contain metal nanoparticles and oxides [1]. Metal oxides such as TiO_2, ZnO, MgO, Cu_2O are of particular interest due to their stability and are generally considered safe materials for humans and animals. Nanoparticles based on metal oxides have revolutionized all major industrial fields, from drug delivery to agriculture, the food industry, the textile industry, and more [2–4]. Nanoparticles have a larger contact surface, as a result of which they dissolve faster in a given solution compared to larger particles, releasing therefore a greater amount of metal ions, and hence their antimicrobial effect is stronger. They also have the benefit of being easily integrated into the fibers' polymer matrix. Antibacterial activity was found to be inversely proportional to the size of the nanoparticles, i.e. the smaller the nanoparticles, the higher their

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antibacterial activity. Different metal oxides have been developed as active ingredients to impart antimicrobial properties to textile materials [5–15].

Silver is the most widely used antimicrobial material incorporated in the form of nano silver in textiles and wound dressings. ZnO nanoparticles (ZnO-NPs) are one type of metal oxides that is frequently employed as an antibacterial agent because of its potent bactericidal activity against both Gram-positive and Gram-negative infections, as well as bacteria resistant to heat and high pressure [12, 14, 16–21].

It has been proven that TiO_2 exhibits antibacterial and antiviral effects, has UV protective and self-cleaning properties. A number of methods of synthesis of titanium oxide nanoparticles have been developed [3, 10, 15, 22]. There are studies on the deposition of such particles on textile materials, but the disadvantage is their poor bonding with textile fibers [23–25]. New research is aimed at modifying nanocomposite polymer materials with graphene-based TiO_2. Emerging graphene-based composites have proven to be good additives due to their excellent mechanical, optical, electrical, thermal and electronic properties. Graphene oxide/TiO_2/cotton nanocomposites have been shown to have excellent self-cleaning and antibacterial activity against *S.aureus* and *E.coli* compared to cotton fabrics treated only with TiO_2 [5]. Research has been conducted on the incorporation of nanomaterials, such as TiO_2 and silver, to textile substrates and results have been achieved in improving functional properties such as self-cleaning, UV protection and antimicrobial properties [26].

Cellulose textile fibers are widely applied in medical textiles: wound dressings; for transdermal drug delivery; also as cosmetic textiles, etc. However, cotton products have a disadvantage, they provide a favorable environment for the development of microbes (bacteria, fungi, algae, viruses, yeast, etc.) due to their large surface area and ability to retain moisture. This requires modification or finishing antimicrobial treatment of the cotton textile materials [6, 27]. One method is the incorporation of metal particles into the cellulose matrix [17, 18, 28].

For fixing the metal particles on the surface of the cellulosic matrix, the cross-linked hydrogel method would be suitable. Collagen hydrogels have been shown to provide excellent extracellular matrix conditions for cell attachment and proliferation. To improve the physico-mechanical properties, collagen cross-linking is applied, most often with glutaraldehyde [29]. Gelatin is very amenable to chemical cross-linking because of its abundance of functional side groups. This property is critical to its potential application as a biomaterial for medical textiles [30–32]. Composites of cotton fabric based on collagen and ZnO-NPs have shown good antibacterial properties in a number of recent studies [12, 14, 19, 27, 33].

In our previous studies, cross-linked gelatin hydrogel biocomposites containing ZnO [34] and TiO_2 particles were synthesized. Three *in situ* synthesis methods of ZnO and TiO_2 were investigated by varying the components and processing conditions. For the first time, titanium nanoparticles obtained by reduction of TiO_2 with oxalic acid were used to modify cotton fabrics. The biocomposites showed very good morphological characteristics, which also give grounds for good antibacterial performance.

The present study aims to obtain, characterize and compare the antibacterial properties of biocomposite materials containing different metal nanoparticles.

MATERIALS AND METHODS

Materials

The used textile material is made from 100% cotton, plain-woven, bleached and unmercerized, surface weight of 145±5 g/m^2. $Zn(NO_3)_2{\cdot}6H_2O$ (CAS:10196-18-6) and NaOH were purchased from Sigma-Aldrich (Darmstadt, Germany); TiO_2 from Sigma-Aldrich (Darmstadt, Germany); Oxalic acid ($H_2C_2O_4$) from Merck KGaA (Darmstadt, Germany); Gelatin (CAS: 9000-70-8), from Merck KGaA

(Darmstadt, Germany); Glutaraldehyde (25% aqueous solution GA) from Sigma-Aldrich (Darmstadt, Germany). All solutions were prepare with distilled water.

Methods for Preparation of Composite Materials

Methods for Preparation of Composite Materials with ZnO [34]

The functionalization of the cotton materials with zinc oxide nanoparticles was carried out by means of three methods. According to method 1, the first step is the preparation of a gelatin hydrogel, which is cross-linked by glutaraldehyde, followed by the immersing of the textile samples in this solution. The next step is addition of $Zn(NO_3)_2{\cdot}6H_2O$ solution and NaOH solution and *in situ* synthesis of ZnO nanoparticles. According to method 2, first is the immersing of the textile samples in the aqueous gelatin solution, the next step is the addition of the $Zn(NO_3)_2{\cdot}6H_2O$ solution, then the crosslinking agent solution and finally the sodium hydroxide solution. As per method 3, the first step is the immersing the cotton samples in a gelatin solution. The solutions of $Zn(NO_3)_2{\cdot}6H_2O$ and NaOH solution were premixed. Next is the immersion of the cotton samples in the solution with the already formed ZnO. And the last step is to add the crosslinkg agent. The processes are carried out under heating.

Methods for preparation of Composite Materials with TiO_2

Three techniques for modification: methods 1, 2, and 3 were used. The samples were modified by immersion in the different solutions in different sequences and at different temperatures. For each sample, 5% gelatin solution, 2,5% GA solution, 0.1 M TiO_2 solution, and 0.1 M $C_2H_2O_4$ solution were prepared in advance.

The functionalization of textile substrates with TiO_2 particles according to method 1 involves the following stages: cross-linking of gelatin with glutaraldehyde; immersing the textile samples in a solution of the cross-linked gelatin; addition of TiO_2 solution and oxalic acid solution and *in situ* synthesis of titanium oxide nanoparticles. In method 2, the initial step is the immersing of the cotton substrates in a gelatin solution; addition of TiO_2 solution and oxalic acid solution; then finally adding the crosslinking agent. In method 3, the first stage is the immersing of the cotton substrates in a gelatin solution. The next stage is preparing in advance a solution of TiO_2 and oxalic acid solution and mix. The last stage is the immersing of the textile materials in the solution with the already obtained particles of titanium oxide with the addition of glutaraldehyde. The processes are carried out under heating.

Analyses

Air Penetration Analysis

The analysis of the air penetration of the modified samples was made using Air-penetration–DVT HG DLC, DEVOTRANS, Turkey, according to standard method BDS EN ISO 9237 under standard atmospheric conditions.

pH Analysis of the Textile Samples

The change in the *pH* values of an aqueous solution of the modified samples was carried out using a pH meter-Portamess 913 pH, "KNICK", Germany.

UVA–VIS-NIR Transmittance Spectral Analysis

Analysis was done using a spectrophotometer UVA/VIS/NIR Lambda 750S, Perkin Elmer, USA, in the range of the spectrum from λ-2000 nm to λ-250 nm.

Morphological Analysis

The surface morphology of the modified cotton fabric was analyzed with a Philips ESEM XL30 FEG SEM.

Antibacterial Analysis of the Treated Cotton Fabrics

Test Microorganisms

Gram-positive *Bacillus cereus* and Gram-negative *Pseudomonas aeruginosa* were employed as model organisms in the investigation of the cotton samples' antibacterial activity (Collection of the

Institute of Microbiology, Bulgarian Academy of Sciences, Sofia). Microbial cultures were transferred once a month and kept at 4°C on meat-peptone agar slants.

Testing the Antibacterial Properties of the Modified Textile Samples

Cotton samples were tested in meat-peptone broth (MPB) for antibacterial activity against model microorganisms. A sterile MPB test tube and cotton specimens measuring 10 mm were inoculated with a bacterial culture's cell suspension. Control tubes were also prepared, one with an untreated cotton sample and the other without any specimens. Following an 18-hour shaking incubation period, the specimens were taken out and the medium's turbidity at 600 nm (OD600) was measured. After the incubation period, the antibacterial activity of the modified cellulose substrates compared to the control sample (i.e. the untreated sample) was determined by decrease in OD600. All tests was run three times, and the average (standard deviations under five percent) was calculated.

RESULTS AND DISCUSSION

Air Penetration

Air penetration is an indicator of the porosity of the impregnated material. The established decrease in air permeability proves the formation of a film on the surface of the samples treated with metal oxides. The obtained results of the analysis are shown in a Table 1. The used area was 5 cm^2, standard for fabrics atmospheric pressure P_{atm}= 100 Pa).

The calculations are made using a formula:

$$R = \frac{Q_v}{A}.167 \text{ [mm/s]}$$

where: R–air permeability [mm/s]; Q_v -the arithmetic mean flowrate of air [dm^3/min];

A-the area of the textile [cm^2]; 167-the conversion factor from [dm^3/min] to [mm/s].

Table 1. Air penetration values of the modified textile samples

Samples	Air Volume /dm^3	Air penetration mm/s	% difference compared to cotton
Cotton (control sample)	5,7	196,4	0
Method 1: cotton_ TiO_2 NPs	0,7	80,9	- 58,8
Method 2: cotton_ TiO_2 NPs	6,6	169,8	- 13,5
Method 3: cotton_ TiO_2 NPs	2,9	97,3	- 50,5
Method 1: cotton_ ZnO NPs	3,1	96,7	- 50,8
Method 2: cotton_ ZnO NPs	3,2	104,2	- 46,9
Method 3: cotton_ ZnO NPs	4,6	154,5	- 21,3

Each method gives a different distribution of ZnO or TiO_2 particles and where their density is greater, air permeability is less. As a result of the formed film of the investigated metal particles, the air permeability properties of the fabric are largely preserved.

The reduced ventilation capacity, i.e. reducing air permeability, respectively, can result in less opportunities for penetration of microorganisms through the fabric, but it could have the inverse effect. Figure 1 shows the air penetration of modified textile samples obtained by investigated methods and the changes for the studied methods. As a result for samples with ZnO-NPs, the air permeability properties of the formed film are largely preserved. In Method 2: Cotton_ TiO_2 NPs, an increase is observed. The biggest reduction is observed at samples modified by Method1: Cotton_TiO_2 NPs. In conclusion there might be reduction of possibility of penetration of microorganisms carried by the air, but the movement in the opposite direction of fluids is also reduced, that is, the breathability of the composite material.

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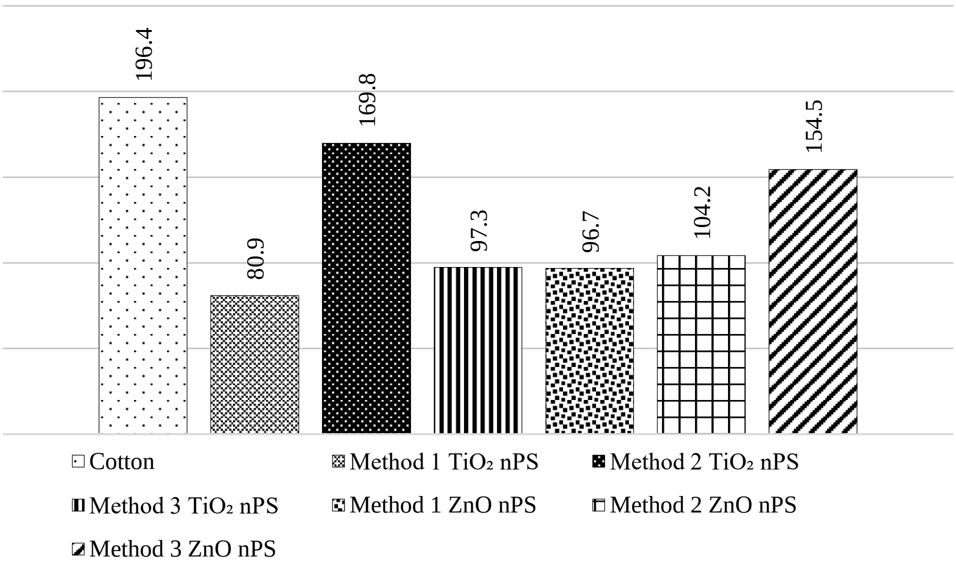

Figure 1. Air penetration of modified textile sample, mm/s.

Table 2. pH values of an aqueous solution of the composite materials

Sample	***pH***	**± U**
Cotton (control sample)	6,5	0,1
Method 1: Cotton_ TiO_2 NPs	3,5	0,1
Method 2: Cotton_ TiO_2 NPs	4,4	0,1
Method 3: Cotton_ TiO_2 NPs	4,8	0,1
Method 1: Cotton_ ZnO NPs	10,5	0,1
Method 2: Cotton_ ZnO NPs	11,8	0,1
Method 3: Cotton_ ZnO NPs	8,6	0,1

Results *pH* for Textile Composites

Table 2 shows the established change in the *pH* values during the different treatments and is the result of three measurements made according to the standard method BDS EN ISO 3071.

The specific *pH* of the textile fabric is 4,8÷7,5. A significant change in *pH* values was observed after the impregnation. As a result of alkalization (*pH* above 7), the concentration of OH^- ions increases, this indicates the growth of crystals, the formation of a dense coating of nanoparticles on the surface of the sample. The most significant increase is at Method 2: Cotton_ ZnO NPs. For the modified samples with TiO_2 NPs, the *pH* value is below 7, indicating acidic properties. This is most likely due to the presence of oxalic acid that was not bound during the synthesis of the titanium nanoparticles.

UVA–VIS-NIR Transmittance Spectral analysis

Analysis was done using a spectrophotometer UVA/VIS/NIR T/% in the UV, VIS and the near IR region were investigated. The results are shown in Figures 2 and 3.

The T% values indicate increased transmittance at the expense of the reflectivity of the composites, i.e. a significant change in the structure of the composite material was observed as a result of the nanoparticle impregnation. Changes in transmittance are observed in the investigated methods. This is related to asymmetric and symmetric vibrations. The appearance of new peaks in the VIS area of the spectrum up to 1200 nm are associated with the absorption of the glycosidic parts of cellulose, the groups responsible for the bond, respectively: -OH, -CH, -C-OH. As a result, there are changes in the structure of the cellulose and a rearrangement of the molecule, which confirms the presence of bonds between the cotton fabric and the investigated ZnO-NPs and TiO_2-NPs.

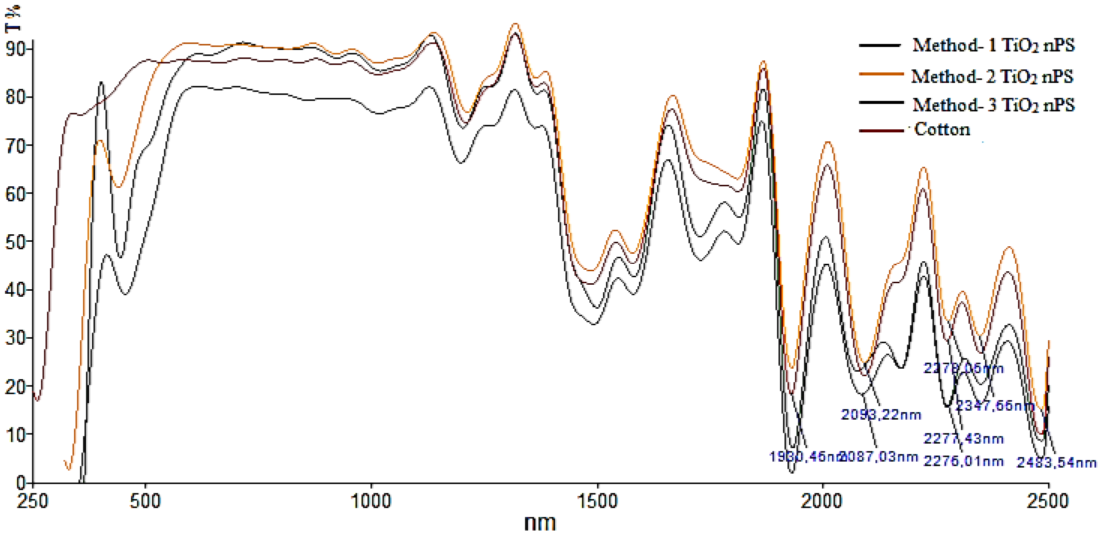

Figure 2. Spectral analysis of modified Cotton_TiO_2 NPs.

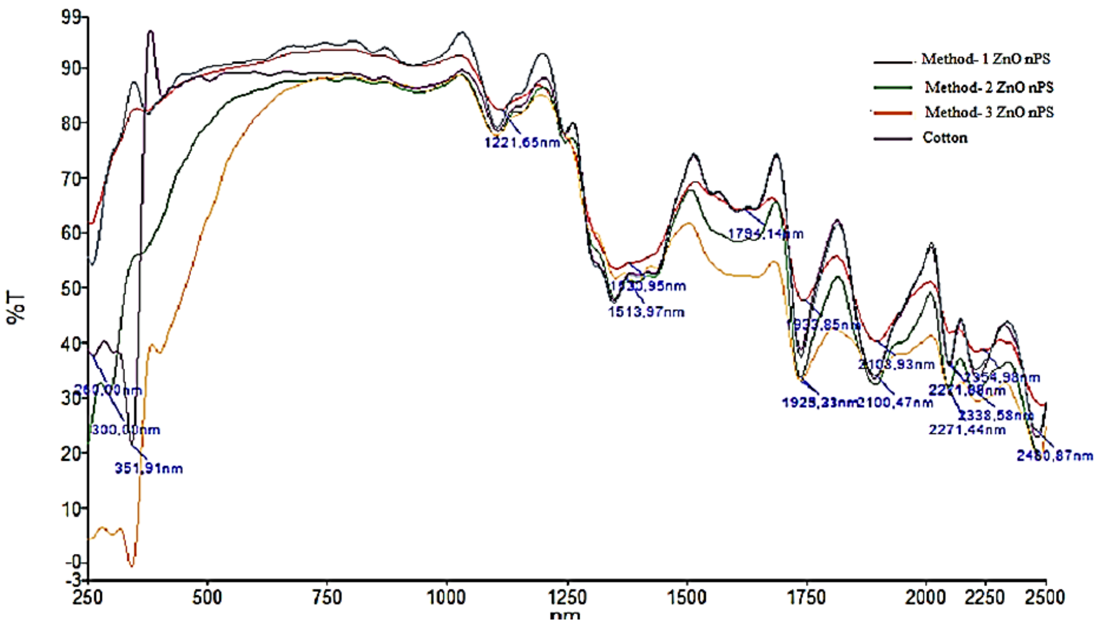

Figure 3. Spectral analysis of modified Cotton_ZnO NPs.

Morphological Properties

Micrographs of the composite with ZnO obtained by Method 1 are presented on Figure 4*a*. As can be seen the spherical shape of the particles have transitioned into newly formed crystal structures with needle-like ends [34].

From the micrographs on Figure 4*b*, it can be seen that the TiO_2 NPs are impregnated into the hydrogel structure on the cotton fabric and distributed into small film-forming structures on the cotton fabric surface.

This indicates that different metal particles are differently distributed in the gelatin hydrogel.

Antimicrobial Properties

The antimicrobial activity of the modified textile samples was evaluated by reducing bacterial growth in MPB. The results showed different activity depending on the strain and the type of the compounds

(Table 3). Gram-negative *P. aeruginosa* was found less resistant to the samples than Gram-positive *B. cereus,* and TiO_2-samples showed higher activity than ZnO-samples (Figure 5). As can be seen, cotton fabrics with TiO_2-by method 1 and TiO_2-method 2, inhibited about 90% *P. aeruginosa* (Figure 6). Since the analyzed metal particles are insoluble in water, they are firmly attached to the surface of the cellulose substrate. The direct contact with bacterial cells contributes to the antimicrobial effect of the treated cotton fabrics in liquid broth.

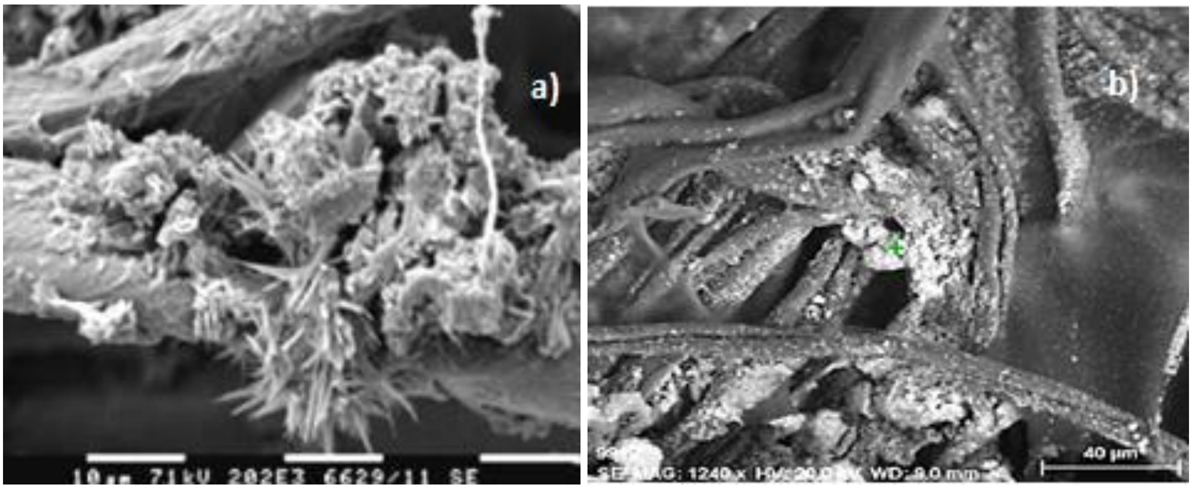

Figure 4. SEM micrographs of composite materials with a) ZnO b) TiO_2 [34].

Table 3. Growth of model strains P. aeruginosa and B. cereus on composite materials.

Composite materials	**Gram-negative P. aeuginosa, %**	**Gram-positive B. cereus, %**
Cotton (control sample)	100	100
Cotton–crosslinked Gelatin	69	92
with ZnO–method 1	18	31
ZnO–method 2	25	41
ZnO–method 3	36	61
with TiO_2–method 1	9	19
TiO_2–method 2	11	29
TiO_2–method 3	22	52

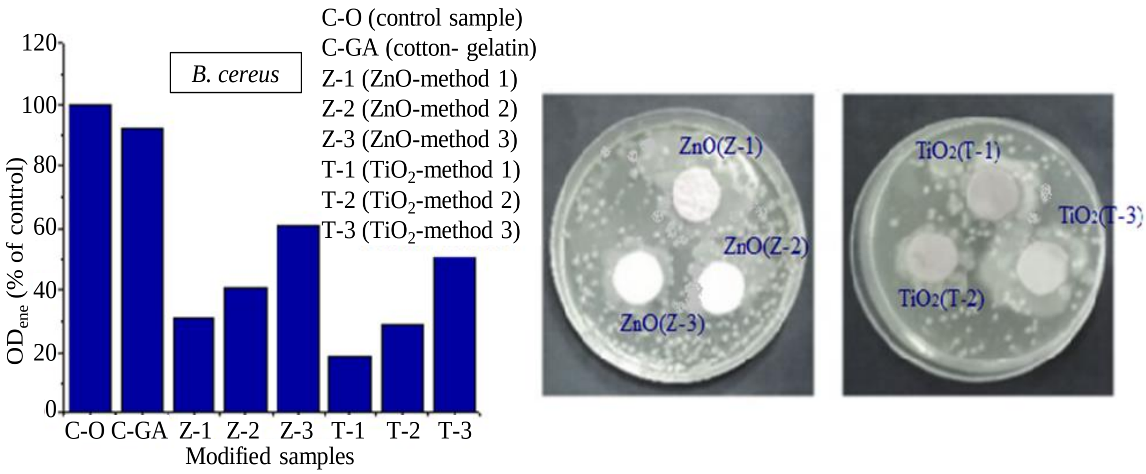

Figure 5. Antibacterial activity of functionalized cotton materials with ZnO-NPs and TiO_2-NPs against B. cereus.

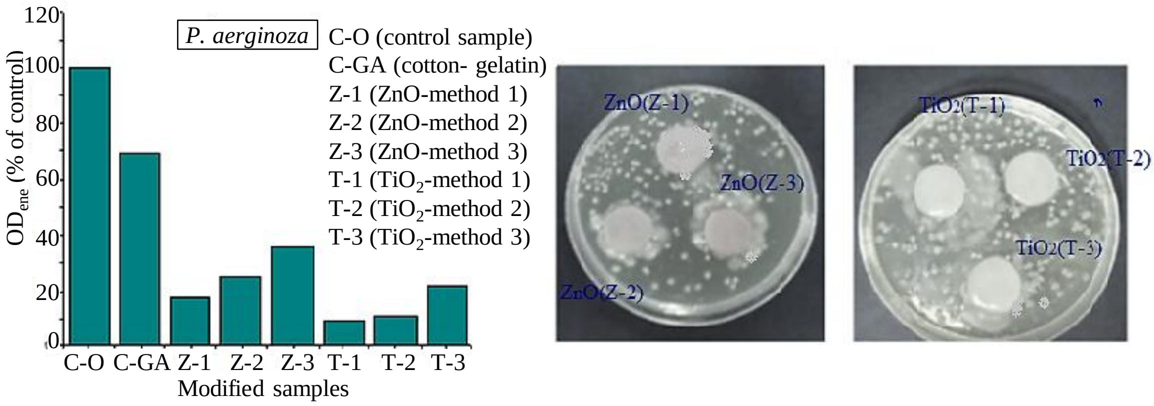

Figure 6. Antibacterial activity of functionalized cotton materials with ZnO-NPs and TiO_2-NPs against P. aeruginosa

CONCLUSIONS

Already known antibacterial agents have disadvantages, such as the development of bacterial resistance, insufficient long-term textile modification and in some cases deterioration of properties and the invention of new modification methods is a promising innovation. Titanium oxide and zinc oxide nanoparticles were synthesized and incorporated into the cellulose matrix by means of cross-linked gelatin. Biological analyses prove excellent activity of the modified textile biomaterials with included TiO_2 nanoparticles against the bacteria used. The TiO_2-treated cotton samples were found slightly more active than ZnO treated cotton samples, and better effect was observed in Gram-negative *P. aeruginosa.* As an outcome, biocomposites based on Cotton-Gelatin-TiO_2 NPs or respectively ZnO NPs can be successfully applied in medical practice as wound dressings.

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