

# Antimicrobial activity of different wound dressing products treated with silver

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## Abstract

The main goal of this work was to optimize the method of processing wound dressing products (like gauzes, sanitary pads, cotton wool, compresses, and bandages) with a commercial silver colloidal solution (Koloid doo, Belgrade, Serbia) and then to examine the antimicrobial properties of the obtained items in order to potentially reach the market with new improved wound dressing products. The influence of different silver concentrations used for treatment on antimicrobial activity was investigated only against *Escherichia coli*. The antimicrobial activity of different types of materials treated with silver solutions of 30 ppm was investigated against Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecalis*, as well as the fungus *Candida albicans*. The microbial reduction of the tested materials loaded with a silver solution of 30 ppm (15-20 µg of Ag on 1 g of fabric) against the Gram-negative bacteria *E. coli* and *P. aeruginosa* was almost maximal after 2 h of contact (*i.e.* 95 and 99 %, respectively). In the case of Gram-positive bacteria *S. aureus*, *B. subtilis*, and *E. faecalis*, a longer time is needed to completely eradicate bacteria (over 99 %). Antifungal activity testing against the fungus *C. albicans* gave moderate antifungal activity results.

**Keywords:** process optimization; textile materials; cotton; microorganisms; silver colloidal solution.

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## 1. INTRODUCTION

Spreading infections by pathogenic microorganisms, especially in hospitals, is an actual and global problem. Also, due to the uncontrolled use of antibiotics, microorganisms have become more and more resistant to various antimicrobial agents known up to now. It is of great importance to find an antimicrobial agent that is non-toxic, easily available, and cheap. Also, preventing the spread of infection, especially in medical facilities, is crucial and is achieved precisely by using medical devices that already have antimicrobial properties. Medical textiles that are in constant use, provided that they exhibit antimicrobial properties, can greatly improve the situation in terms of preventing and reducing infections.

As the production of medical, healthcare, hygiene, and protective textile materials is steadily growing, interest in effective, non-toxic, long-lasting, and cost-effective antimicrobial finishing of these products is quickly rising [1,2]. In this respect, reduction in microbial survival on textile materials is crucial since germs can degrade both the fabric and the wearer's comfort. In other words, the microbial presence may have a variety of detrimental impacts, including the production of offensive odor, stains, material decolorization, and a reduction in the mechanical strength of the fabric [3,4]. Production of textiles for sports, leisure, medical non-implantables (such as bandages, plasters, gauze dressings, lint, wadding, and adsorbent pads) and healthcare/hygiene products (such as surgical gowns and hosiery, sheets, pillowcases, uniforms, and blankets) is particularly based on the use of cotton fibers [1]. However, cotton is

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particularly vulnerable to microbial infection because of its extraordinary capacity to absorb moisture. Thus, cotton may serve as a nutrient, acting as a good medium for bacterial and fungal development under specific humidity and temperature circumstances [3,4].

The growing issue of multi-antibiotic-resistant microorganisms has revoked interest in silver and its compounds, which have previously been known to be effective biocides for more than 650 different microbes [5,6]. Silver can be applied to textile fabrics to provide a desired level of antibacterial activity without significantly altering the fabric color [7-11]. Also, a small concentration of silver is needed to achieve satisfactory antimicrobial effects, while being below the toxicity level of 1 mg dm<sup>-3</sup> [12].

This work aimed to examine the method of processing different medical wound dressing products (gauze, sanitary pads, cotton wool, compress, and bandages) with a commercially available colloidal silver solution (company Koloid). After optimizing the process, the silver content was determined followed by examination of the antimicrobial properties of all obtained materials against Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecalis*, and the fungus *Candida albicans*.

## 2. EXPERIMENTAL

The cotton materials used as medical supplies in this work are gauze, sanitary pads, cotton wool, compresses, and bandages made by NIVA company (Zabalj, Serbia). Silver colloid solutions at different concentrations were made by the company Koloid doo (Belgrade, Serbia).

### 2. 1. Impregnation of dressing products with silver

Two processing methods were tested together with four different concentrations of the antimicrobial agent. For processing, we used a colloidal silver solution with concentrations of 2, 5, 10 and 30 ppm.

The cotton materials were processed by two methods:

- 1) "deep coating" - immersing 1 g of the material in the colloid solution for 30 min, then air-drying at room temperature, and
- 2) "micro-dispersion" – spraying the material with the colloidal silver solution in a precisely determined amount and ratio (2 cm<sup>3</sup> per 1 g of fabric).

### 2. 2. Determination of silver content

The silver content in cotton materials treated with Ag colloid solution at the concentration of 30 ppm was determined using inductively coupled plasma optic emission spectroscopy (ICP-OES Thermo Scientific iCAP 7400, producer, country). The amount of 1 g of material was immersed in 1 M nitric acid to disperse silver from the material. The concentration of silver in the solution was measured after dilution.

### 2. 3. Antimicrobial performance

Antimicrobial testing of cotton materials impregnated with Ag was performed by the contact method against pathogenic microorganisms, Gram-negative bacteria *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27833, Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, and *Enterococcus faecalis* ATCC 29812, and the fungus *Candida albicans* ATCC 10259.

The method used for antimicrobial testing is the standard method for textile materials. A 1 g cotton material sample was immersed in a 50 cm<sup>3</sup> saline solution containing around 10<sup>5</sup> CFU cm<sup>-3</sup> microorganisms in a sterile 300 cm<sup>3</sup> Erlenmeyer flask. After the contact of 2 h and 24 h under mixing in a rotary shaker (150 rpm), 100 mm<sup>3</sup> of aliquot is taken for further dilution and seeded on a tryptone soy agar medium in Petri dishes, which is then thermostated at 37 °C for 24 h (48 h for *C. albicans*). Grown colonies are counted and microbial reduction (*R* / %) is calculated according to the following formula:

$$R = \frac{C_0 - C}{C_0} 100 \quad (1)$$

where  $C_0$  is the initial number of colonies in  $1 \text{ cm}^3$  ( $\text{CFU cm}^{-3}$ ) in  $t = 0$ , while  $C$  is the number of colonies in  $1 \text{ cm}^3$  after the contact of the sample with the bacterial suspension.

### 3. RESULTS AND DISCUSSION

The main purpose of the experiments carried out in this work was to optimize the process of treating several wound dressing products with a commercial silver colloidal solution (Koloid company, Serbia) to achieve the best product that can be commercialized. First, it was necessary to determine an adequate method of processing the samples, which does not significantly change the external appearance of the product and leads to improved performance. Second, the antimicrobial properties of the obtained products were determined in order to evaluate improvement in the desired performance.

In order to establish the efficiency of the processing and to choose the best method, antimicrobial activity against a reference culture the Gram (–) bacterium *E. coli* was performed (Table 1).

**Table 1.** Antimicrobial activity against *E. coli* of samples treated with Ag colloid solutions by the "deep coating" method, after 24 h,  $C_0 = 290,000 \text{ CFU cm}^{-3}$

Sample	Concentration of Ag in the colloid solution, ppm			
	2		5	
	$C / \text{CFU cm}^{-3}$	$R / \%$	$C / \text{CFU cm}^{-3}$	$R / \%$
Gauze	40,000	86.21	1,200	99.59
Sanitary pads	4,000	98.62	1,400	99.52
Cotton wool	18,000	93.79	800	99.72
Compress	2,200	99.24	300	99.90
Bandage	9,400	96.76	4,700	98.38

Treatment of the samples by the "deep coating" method gave good results using colloid solutions with low concentrations of the antimicrobial agent. In specific, 99 % of bacteria were inhibited with all tested samples treated with 5 ppm colloid. Only the bacterial reduction by the bandage was slightly lower amounting to ~98 %. This result may be due to the bandage hydrophobicity compared to other samples, so the contact between silver and bacteria is difficult due to non-wetting. Also, the hydrophobicity of the bandage could be the reason for the lower impregnation of silver. The initial number of bacteria of  $10^5$  was reduced to  $10^2$  to  $10^3$  after just 2 h of contact between bacteria and the sample in saline solution. Samples treated with a colloid concentration of 2 ppm induced a lower reduction of bacteria but were still considered as a good antimicrobial activity with more than 85 % reduction for all tested samples.

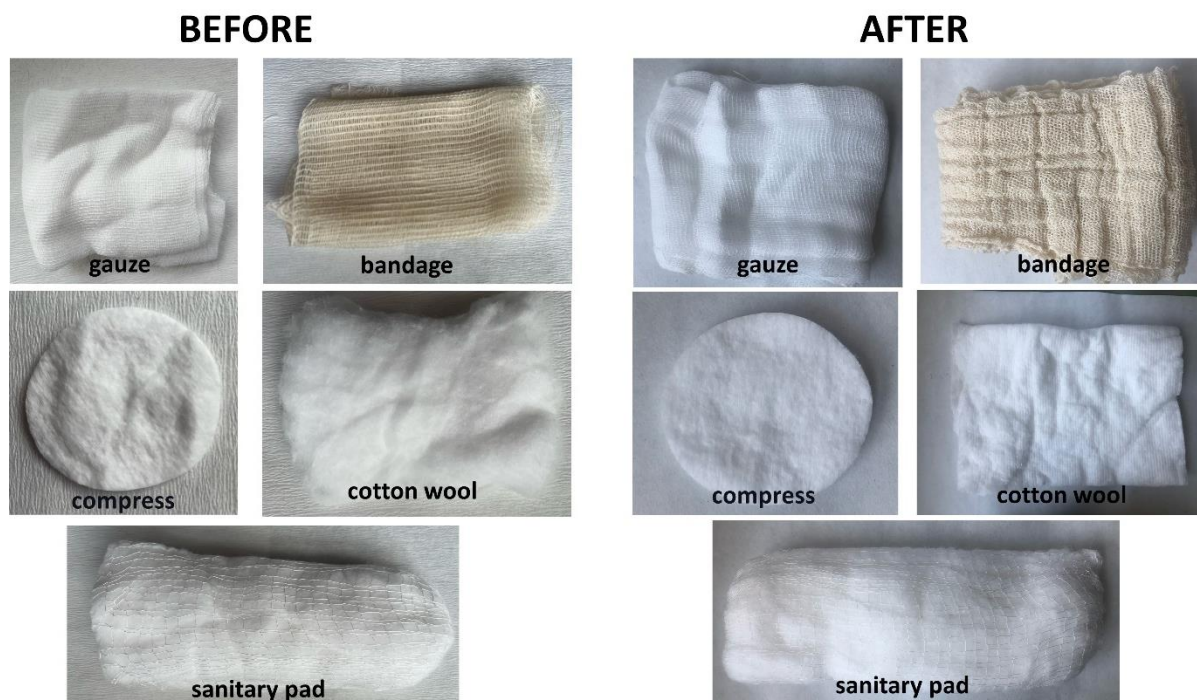
However, after the "deep coating" method, some of the samples lost their original shape (sanitary pads, cotton wool, and compresses). Also, this method would be slow and inadequate for the quantities of materials planned for industrial production. Because of these two reasons, another processing method was tested, that is spraying the sample with colloid solutions. The pilot process was created and owned by the company Koloid doo (Belgrade, Serbia). In the present work, the process was optimized so that 1 g of the material was sprayed with  $2 \text{ cm}^3$  of the Ag colloidal solution.

It can be seen from Table 2 that the antimicrobial activity of all tested samples treated with a colloid concentration of 10 ppm is unsatisfactory, while samples treated with a colloid concentration of 30 ppm show excellent antimicrobial properties against *E. coli* after 2 h of contact. All further tests of antimicrobial activity against selected strains will be done with samples treated with the Ag colloid of 30 ppm.

**Table 2.** Antimicrobial activity of samples processed by the micro-dispersion method against *E. coli* after 24 h,  $C_0 = 250,000 \text{ CFU cm}^{-3}$  for 10 ppm,  $C_0 = 300,000 \text{ CFU cm}^{-3}$  for 30 ppm

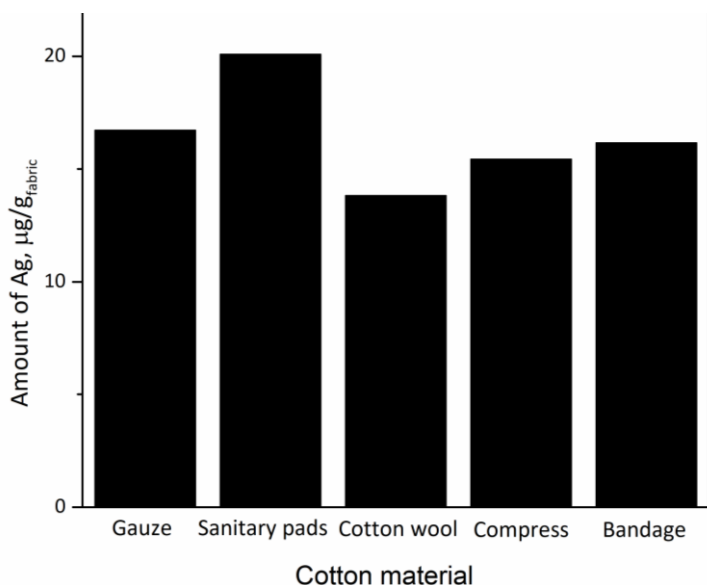
Sample	Concentration of Ag in the colloidal solution, ppm			
	10		30	
	$C / \text{CFU cm}^{-3}$	$R / \%$	$C / \text{CFU cm}^{-3}$	$R / \%$
Gauze	110,000	56.00	40,000	92.00
Sanitary pads	160,000	36.00	6,200	98.76
Cotton wool	40,000	84.00	1,200	99.76
Compress	10,000	96.00	1,200	99.76
Bandage	60,000	76.00	700	99.86

Figure 1 presents photographs of the samples before and after the treatment showing insignificant changes in the sample shape or color.



**Figure 1.** Photographs of samples before and after the treatment by the micro- dispersion method with a colloidal solution with the Ag concentration of 30 ppm

Therefore, the amount of silver that remained on 1 g of the material after treatment with the 30 ppm colloid solution was determined (Fig. 2).



**Figure 2.** The amount of silver in cotton material after micro-dispersion treatment with a 30 ppm colloidal solution

Figure 2 shows that the amount of silver in the materials is roughly three-fold lower than that anticipated based on the processing method ( $2 \text{ cm}^3$  of the 30 ppm colloidal solution per 1 g of the material). The calculated amount should be  $\sim 60 \text{ g}$  per 1 g of the material, but only  $13\text{-}20 \mu\text{g g}^{-1}$  was measured. Given that a certain amount of Ag is lost throughout the drying and squeezing steps of processing, the outcome is to be expected.

The amount of silver found on fabrics is far below the environmental regulatory limit: 1 g of fabric immersed in 50 cm<sup>3</sup> of saline solution results in a silver concentration of 0.3 to 0.4 mg dm<sup>-3</sup>, while the environmental toxic limit is 1 mg dm<sup>-3</sup> [13]. It is important that the amount of Ag found in the material provides good antimicrobial properties to selected microorganisms, and on the other hand, that it is not toxic to the environment after use and is within the limits according to the European standards of ecological toxicity [13].

All selected microorganisms are the most common causes of infections in humans and belong to the group of strains that are often resistant to many antibiotics. Gram (-) bacteria *E. coli* is a common cause of urinary and gynaecological infections, while *P. aeruginosa* is a common hospital infection and a bacterium that lives in ventilation openings and thus easily contaminates hospitals. Representatives of Gram (+) bacteria are *S. aureus*, which is very common in skin infections and resistant to most antibiotics, then *B. subtilis*, as an example of a sporogenous microorganism, and *E. faecalis* which are also very often resistant to antibiotics and occur in the intestines and gastrointestinal tract and there cause very severe infections. Finally, one fungus, *C. albicans*, was also examined, with the most used reference pathogenic microorganism, which often appears after the use of antibiotics, multiplies quickly, and causes problems mostly in the intimate region.

Antimicrobial activity against pathogenic Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* are presented at Tables 3 and 4, respectively.

**Table 3.** Antimicrobial activity of tested samples treated by the microdispersion method with the 30 ppm colloidal solution after 2 ( $C_0 = 500,000 \text{ CFU cm}^{-3}$ ) and 24 h ( $C_0 = 400,000 \text{ CFU cm}^{-3}$ ) of contact with *Escherichia coli*

Sample	Time of contact with <i>Escherichia coli</i> , h			
	2		24	
	C / CFU cm <sup>-3</sup>	R / %	C / CFU cm <sup>-3</sup>	R / %
Gauze	40,000	92.00	< 10	99.99
Sanitary pads	6,200	98.76	< 10	99.99
Cotton wool	1,200	99.76	1,300	99.68
Compress	1,200	99.76	< 10	99.99
Bandage	700	99.86	1,100	99.73

**Table 4.** Antimicrobial activity of tested samples treated by the micro-dispersion method with colloid concentration 30 ppm after 2 ( $C_0 = 900,000 \text{ CFU cm}^{-3}$ ) and 24 h ( $C_0 = 400,000 \text{ CFU cm}^{-3}$ ) of contact with *Pseudomonas aeruginosa*

Sample	Time of contact with <i>Pseudomonas aeruginosa</i> , h			
	2		24	
	C / CFU cm <sup>-3</sup>	R / %	C / CFU cm <sup>-3</sup>	R / %
Gauze	3,100	99.66	< 10	99.99
Sanitary pads	120	99.99	< 10	99.99
Cotton wool	500	99.94	22	99.99
Compress	320	99.96	< 10	99.99
Bandage	400	99.96	< 10	99.99

After 24 hours of contact between the tested Gram (-) bacteria and the samples, a complete reduction of bacteria occurred (Tables 3 and 4). There are practically no visible surviving bacteria after contact with gauze, pads, and compresses for both investigated bacteria, so the reduction is 99.99 %. About 1,000 surviving colonies remain on cotton wool and bandages also. The bandages most likely due to low wettability have a slower release of silver. The cotton wool due to high absorption and swelling makes mixing difficult and decreases the release of silver. After 2 h of contact, the reduction is slightly lower for both tested bacteria but satisfactory. The reduction of *P. aeruginosa* is over 99 % even after 2 h of contact, while the reduction of *E. coli* is over 90 %. We can conclude that the contact time affects the complete extinction of bacteria if the initial number of bacteria is over 10<sup>5</sup> CFU cm<sup>-3</sup>, which is the case with strong infections with these bacteria. If the materials are used preventively or in places where the infection is just beginning, the antimicrobial protection provided by the samples processed in this way is sufficient to prevent the further spread of the infection. The antimicrobial results obtained for this fabric against *E. coli* agree with our previous results [5,6,14-16].

The tests of antimicrobial activity were performed against the Gram (+) bacteria *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecalis*. Tables 5, 6, and 7 show the results of antimicrobial activity against these bacteria.

**Table 5.** Antimicrobial activity of tested samples treated by the micro-dispersion method with colloid concentration 30 ppm after 2 ( $C_0 = 400,000 \text{ CFU cm}^{-3}$ ) and 24 h ( $C_0 = 200,000 \text{ CFU cm}^{-3}$ ) of contact with *Staphylococcus aureus*

Sample	Time of contact with <i>Staphylococcus aureus</i> , h			
	2 h		24 h	
	C / CFU cm <sup>-3</sup>	R / %	C / CFU cm <sup>-3</sup>	R / %
Gauze	85,000	78.75	3000	98.50
Sanitary pads	50,000	87.50	< 10	99.99
Cotton wool	90,000	77.50	1	99.99
Compress	30,000	92.50	7	99.99
Bandage	300,000	25.00	6200	96.90

**Table 6.** Antimicrobial activity of tested samples treated by the micro-dispersion method with colloid concentration 30 ppm after 2 ( $C_0 = 200,000 \text{ CFU cm}^{-3}$ ) and 24 h ( $C_0 = 200,000 \text{ CFU cm}^{-3}$ ) of contact with *Bacillus subtilis*

Sample	Time of contact with <i>Bacillus subtilis</i> , h			
	2		24	
	C / CFU cm <sup>-3</sup>	R / %	C / CFU cm <sup>-3</sup>	R / %
Gauze	800	96.00	30	99.85
Sanitary pads	500	97.50	53	99.74
Cotton wool	300	98.50	40	99.80
Compress	200	99.00	165	99.18
Bandage	400	98.00	17	99.92

**Table 7.** Antimicrobial activity of tested samples treated by the micro-dispersion method with colloid concentration 30 ppm after 2 ( $C_0 = 600,000 \text{ CFU cm}^{-3}$ ) and 24 h ( $C_0 = 1,500,000 \text{ CFU cm}^{-3}$ ) of contact with *Enterococcus faecalis*

Sample	Time of contact with <i>Enterococcus faecalis</i> , h			
	2 h		24 h	
	C / CFU cm <sup>-3</sup>	R / %	C / CFU cm <sup>-3</sup>	R / %
Gauze	70,000	88.33	60,000	96.00
Sanitary pads	230,000	61.67	61	99.99
Cotton wool	70,000	88.33	2,600	99.83
Compress	120,000	80.00	1,100	99.93
Bandage	870,000	NA	5,900	99.61

The reduction of Gram (+) bacteria *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecalis* is at a satisfactory level even after 2 h, while after 24 h of the contact, the reduction is almost total (Tables 5, 6, and 7). The number of bacteria was reduced from  $10^6$  to  $10^4$  after 2 hours of contact between fabrics and *S. aureus*. The microbial reduction is over 75 % for all samples except for the bandage. On the other hand, after 24 h of contact, cotton wool, sanitary pad, and compress treated with colloid at a concentration of 30 ppm almost completely extinguish *S. aureus* bacteria, and after 24 h of contact, less than 10 bacteria survive per 1 cm<sup>3</sup> of suspension. With the gauze and bandage samples,  $10^3$  bacteria remain, but it is also excellent antimicrobial activity with over 96 % reduction of the initial bacterial count.

The reduction of *Bacillus subtilis* after 2 h of contact was over 96 % for all tested samples, while after 24 h of contact, the reduction was almost complete, over 99 %. The number of microorganisms decreased from  $10^4$  to  $10^2 \text{ CFU cm}^{-3}$ . All treated fabrics have effective antimicrobial activity after a short time of contact against the bacteria *B. subtilis*. The reduction of *E. faecalis* bacteria is slightly worse than *S. aureus*. After 2 h of contact, gauze, cotton wool, and compress showed antimicrobial activity with over 80 % reduction of bacteria. However, after 24 h of contact, all samples except for gauze reached a complete reduction of bacteria with over 99 %.

We can conclude that all samples treated with 30 ppm colloid showed excellent antimicrobial activity against all tested Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecalis*.

Finally, the antimicrobial activity against the pathogenic fungus *C. albicans* was tested, and the results are shown in Table 8.

According to their structure, fungi are different from bacteria. They are complex eukaryotic microorganisms with cells resembling animal cells. Tests have shown much weaker antimicrobial properties against this microorganism than against all other tested bacteria.



**Table 8.** Antimicrobial activity of tested samples treated by the micro-dispersion method with colloid concentration 30 ppm after 2 ( $C_0 = 300,000 \text{ CFU cm}^{-3}$ ) and 24 h ( $C_0 = 260,000 \text{ CFU cm}^{-3}$ ) of contact with *Candida albicans*

Sample	Time of contact with <i>Candida albicans</i> , h			
	2		24	
	C / CFU cm <sup>-3</sup>	R / %	C / CFU cm <sup>-3</sup>	R / %
Gauze	100,000	66.67	60,000	76.92
Sanitary pads	250,000	16.67	90,000	65.38
Cotton wool	190,000	36.67	90,000	65.38
Compress	160,000	46.67	9,100	96.50
Bandage	130,000	56.67	10,000	96.15

After 2 h of contact, the reduction is insignificant, while after 24 h of contact, the microbial reduction for compress and bandage is over 96 %, while for the other samples, it is between 65 and 77 %. We can say that there is low antimicrobial activity in the samples, and according to the results, there is no further reproduction of the fungus. Lower antimicrobial activity against *C. albicans* was observed for Ag attached to textile fibers [14] and inorganic supports such as hydroxyapatite [13] and magnetite [12], compared with the antibacterial performance of silver nanoparticles against *E. coli* [17,18]. However, this is also an indication that the samples treated with the 30 ppm colloidal solution would not be toxic to eukaryotic cells, so we can assume that they would not be toxic to human cells either. Consequently, these experiments will be done in the future.

#### 4. CONCLUSION

The processing methodology for the treatment of wound dressing materials was optimized regarding the antimicrobial properties of the obtained materials against *E. coli* as a reference culture. Based on considerations of the appearance of the material, the structure after processing, as well as the antimicrobial activity, the "deep coating" method was rejected, precisely because of the damage to the material structure. The second method was spraying a precisely determined volume ( $2 \text{ cm}^3 \text{ g}^{-1}$  of the material) at a silver concentration of 30 ppm in the colloid solution. All tested samples treated with this solution showed satisfactory and, in some cases, excellent antimicrobial properties against all tested strains of microorganisms. The antimicrobial efficiency of the tested materials against the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* was almost maximal after 2 h of contact (reduction of 95 and 99 %, respectively). After 24 h of the contact between the bacteria and the material, there was negligible growth of microorganisms, and we can consider that the efficiency is maximal.

In the case of Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecalis*, a longer time of contact is needed for the complete reduction of bacteria (over 99 %), except for *B. subtilis*. After 2 h of contact between samples and *S. aureus*, a satisfactory bacterial reduction was achieved, but after 24 h the bacterial reduction is maximal. This result is important taking into account the fact that *S. aureus* is considered one of the most common causes of skin infections. For *E. faecalis*, a longer contact time is needed to achieve maximum reduction. Antifungal activity testing against the fungus *C. albicans* gave moderate antifungal activity results.

The obtained results for all tested wound dressing products against tested microorganisms confirmed good antimicrobial activity, and we expect these products to reach the market soon.

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## Antimikrobna aktivnost medicinskih materijala obrađenih srebrom

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(Stručni rad)

Izvod

Cilj ovog rada je ispitivanje antimikrobne efikasnosti pamučnih materijala kao što su gaza, higijenski ulošci, vata, komprese i zavoji obrađeni komercijalnim koloidnim rastvorom srebra (kompanija Koloid doo). Ispitan je uticaj koncentracije koloidnog rastvora srebra kojim je obrađena tkanina na antimikrobnu aktivnost prema Gram-negativnim bakterijama *Escherichia coli* i *Pseudomonas aeruginosa*, Gram-pozitivnim bakterijama *Staphylococcus aureus*, *Bacillus subtilis* i *Enterococcus faecalis* i gljivici *Candida albicans*. Redukcija bakterija postignuta testiranim materijalima obrađenim rastvorom srebra koncentracije 30 ppm (15 do 20 µg Ag na 1 g materijala) prema Gram-negativnim bakterijama *E. coli* i *P. aeruginosa* je skoro maksimalna nakon dva sata kontakta, 95 i 99 %, redom. U slučaju Gram-pozitivnih bakterija *S. aureus*, *B. subtilis* i *E. faecalis*, potrebno je duže vreme za potpunu redukciju broja bakterija, osim za *B. subtilis*, gde je dovoljno dva sata kontakta za maksimalno smanjenje početnog broja bakterija. Antifungalna aktivnost prema gljivici *C. albicans* je umerena.

*Ključne reči:* optimizacija procesa; tekstilni materijali; pamuk; mikroorganizmi; koloidni ratvor srebra

