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BACTERICIDAL ACTIVITY OF NANO SILVER AGAINST PATHOGENIC MICROORGANISMS WHICH CAUSE PECULAR DISEASES OF GENITAL SECRETION TRACK

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Abstract. Nano silver was known as a bactericidal agent for almost all bacterial species and has been intensively investigated over the world to apply for disinfection in different fields of human activities and environmental protection. Specifically, genital exudate media contain numerous dangerous bacteria such as *Neisseria gonorrhoeae, Enterococcus faecalis, Staphylococcus aureus, Candida albicans* etc. which as a rule are drug-resistant with respect to antibiotics. This research presents the results of the application of nanosilver in replacing antibiotics in elimination of the above mentioned microorganisms by using low nanosilver concentrations of about 0.01 mg/cm^2 immobilized on non-woven fabric. The experimental data showed that nanosilver-impregnated fabric samples at a concentration 0.01 mg/cm^2 gave antibacterial rings of 10 mm, 9 mm and 8 mm, respectively, for *N. gonorrhoeae; C. albicans + E. coli* and *C. albicans + E. coli + S. aureus*.

Keywords: nanosilver, antibiotics, excretion track, non-woven fabric, drug resistant.

Classification numbers: 1,2; 1.3; 1.3.1.

1. INTRODUCTION

Nano-particles of silver (AgNPs) or nanosilvers have been intensively investigated over the world to apply for disinfection in different fields of human activities, medicine, and environmental protection [1-5]. Nanosilver is a broad spectrum antimicrobial agent with a long history because of its high microbicidal activity, short disinfection time [6]. Bactericidal activity of nanosilver containing materials can be applied in medicine for reduction of infections on the open wounds and burns treatment [7, 8]. It can be applied on textile fabrics for elimination of microorganisms [9], applied on bacterial cellulose membrane [10, 11], and applied for disinfection in water treatment [12]. In comparison to antibiotics, which are nowadays facing on

the drug resistance, nanosilver can destroy bacteria in via cell membrane destruction of microorganisms.

Inflammation of genital track is a primary cause of appearance of numerous disturbances affecting the human health, the working ability, the reproduction power and especially the fertility. If these disturbances were not discovered and treated timely it may result in heavy consequences such as inflammation of small pelvis, out-womb pregnancy, infertility, cervix cancer, increasing risk of HIV, HPV diseases etc. [13].

Recent research results [14-16] showed that inflammation of genital track is often encountered diseases in the world, especially in developing countries. According to WHO, every year about 330 ÷ 390 million women over the world have got the diseases transmitted through genital track [17, 18]. In United States every year about 10 million take vaginal examination, among which 28 % were revealed infected. Other countries reported approximately the same rate of genital track infections, oscillating between 25 ÷ 65 % of the examined people.

The bactericidal activity of silver nanoparticles against the pathogenic, MDR as well as multidrugsusceptible strains of bacteria was studied by many scientists [19-22], and it was proved that the silver nanoparticles are the powerful weapons against the MDR bacteria such as *Pseudomonas aeruginosa,* ampicillin-resistant *E. coli,* methicillin- resistant *S. aureus* (MRSA) and vancomycin- resistant *S. aureus (VRSA)* [2, 23, 24].

In Viet Nam, the women of fertility age have a rate of genital track inflammation much higher than that in the region and in the world. The research of Vietnam National Hospital of Dermato-Venereology indicated that at 5 provinces the proportion of women of age from 15 ÷ 49 years old infected with at least one of the genital track diseases was 70.56 % [1]. In recent years various bacteria and fungi causing genital track inflammation become more and more drug- resisted and more difficult to treat. The searching for new drugs capable of killing drug-resistant pathogenic microorganisms is of vital importance.

In this study, we attempted to kill 5 microbial pathogenic species frequently encountered in hospitals such as *E. col; S. aureus; E. faecalis; C. albicans; N. gonorrhorae* using AgNPs. The objectives of this research are: (1) to use nano silver to kill microbial pathogenic species frequently encountered in hospitals, (2) to compare the bactericidal capacity of AgNPs in various concentrations for 5 microbial pathogenic species.

2. EXPERIMENTAL

2.1. Chemicals and materials

AgNPs solutions used in the research were prepared according to the chemical wet method described in [25, 26] with physico-chemical characteristics described in [27-29] and AgNPs concentration 500 mg/L. Antibacterial activity was determined in various media such as conventional agar, blood agar, chocolate agar and Sabouraud agar for 5 specific bacterial types, including 5 species of each *E. coli; N. gonorrhoeae; E. faecalis; S. aureus* and *C. albicans*. All these pathogenic microorganisms were isolated from patient's specimen at the Vietnam National Hospital of Dermatology and Venereology. Determination of bactericidal activity of fabric impregnated with AgNPs was carried out using a cultivating medium prepared by dilution of the lyophilized medium procured from Merck. Skin irritation experiment was carried out on rabbits.

2.2. Determination of MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) of AgNPs

AgNPs solutions for 5 types of pathogenic microorganisms causing gynaecological diseases was carried out by using twice-dilution technique as demonstrated in Table 1.

Table 1. AgNPs concentration at different dilution levels.

Dilution	1	1/2	1/4	1/8	1/ 16	1/ 32	1/ 64	1/ 128	1/ 256	1/ 512	1/ 1024	1/ 2048	1/ 4096
[AgNPs] ppm	500	250	125	62. 5	31, 2	15. 6	7.8	3.9	1.9 5	0.9 7	0.49	0.24	0.12

0.2 mL of a AgNPs solution diluted respectively with exposition times of 15 min, 1 h, 2 h and 4 h, respectively. Then pour these suspensions onto agar disks and keep them in incubator for 24 h under respective conditions: for *E. coli; E. faecalis; S. aureus* at 37 ^{o}C; for *N. gonorrhoeae* humidity > 70 %, at 37 ^{o}C in the presence of 3 ÷ 10 % CO_2; for *C. albicans* at 25 ÷ 30 ^{o}C.

2.3. Determination of antibacterial capacity of AgNPs in respect of the pathogenic bacteria causing gynaecological diseases

A AgNPs solution of concentration 500 ppm immobilized on non-woven fabric with weight density 25 ± 2 g/m^2, pull strength MD: 6 ± 1.5 kg/50 mm and CD: 0.9 ± 0.2 kg/50 mm, elasticity MD: 30 ÷ 60 %, CD: 50 ÷ 100 %, thickness: 0.4 ± 0.15 mm, thermostability: ≤ 130 ^{o}C. AgNPs coating was conducted with a rate of the fabric motion 30 ÷ 70 m/min, coating rate of AgNPs solution 0.84 ÷ 3.36 L/min, drying temperature: 100 ÷ 120 ^{o}C. AgNPs density of the impregnated fabric was in a range of 0.005 ÷ 0.01 mg/cm^2.

2.4. Skin irritation effect of non-woven fabric immobilized with AgNPs

The experiment was carried out on rabbits. Pieces of non-woven fabric impregnated with nanosilver solution of concentration 0.01 mg/cm^2 were applied on the shaved patches of rabbit's skin, watched for 3 days and recorded irritation spots.

3. RESULTS AND DISCUSSION

3.1. Minimum nanosilver concentration for killing bacteria of the excretion track

Bactericidal activity of SNPs solutions of different concentration and exposition times in respect of the pathogenic microorganisms in the excretion track presented in Fig.1 showed that all types of the tested microorganisms were killed by nanosilver solutions of different concentration and contact time. It can be seen that *N. gonorrhoeae* was rather sensitive to AgNPs, even just at the moment of contact (0 min) 1.95 ppm of nanosilver already killed completely the bacterial species, while for *E. coli* and *E. faecalis* it needed 125 ppm.

Bactericidal activity of different AgNPs concentration solutions and exposed times in respect of the pathogenic microorganisms in the excretion track is presented in Fig.1. All types of the tested microorganisms were killed in different conditions. It can be seen that *N. gonorrhoeae* was rather sensitive to AgNPs, even just at the moment of contact (0 min) 1.95 ppm of nanosilver already killed completely the bacterial species, while for *E. coli* and *E. faecalis* it needed 125 ppm.

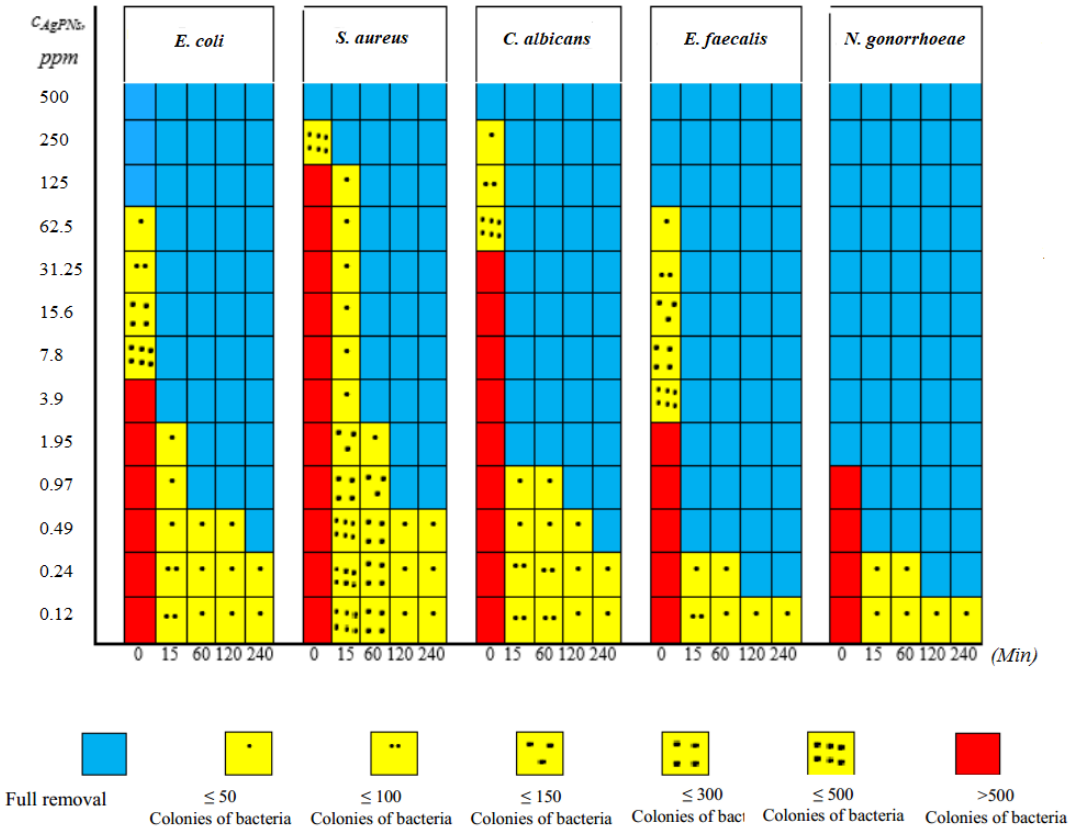

Figure 1. Bactericidal activity of AgNPs solutions of different concentrations and exposition time with respect to the exudative track microorganisms.

Gram positive *S. aureus* and sporous fungal species *C. albicans*, although both demonstrated a good capability of resistance against impact of AgNPs, also were killed at an higher nanosilver concentration (500 ppm). Exposition time also played a role in bacterial disinfection. With 60 min exposition, it required only 3.9 ppm of nanosilver solution to kill all the 5 pathogenic microorganisms. With 4 h exposition, it needed only 0.97 ppm to kill the most recalcitrant *S. aureus*, while for *E. coli* and *C. albicans* need 0.49 ppm.

Thus, minimum nanosilver concentration used for inhibition of bacteria in the exudative track was relatively low. Instant inhibition nanosilver concentration with respect to all the types of the studied microorganisms was 500 ppm, for *E. coli; E. faecalis* and *N. gonorrhoeae* 250 ppm. With prolonged contact time, minimum inhibition nanosilver concentration considerably decreased: 3.9 ppm for the 5 microorganisms with exposition time of 60 min, while after 2 h of contact the minimum concentration was 0.97 ppm. These results confirmed that nanosilver concentration of 500 ppm can be considered as a minimum concentration used to manufacture AgNPs impregnated fabrics intended for treatment of gynaecological diseases.

3.2. Capability of nanosilver-impregnated non-woven fabric to kill bacteria causing diseases of the exudative track

Figure 2 shows SEM images of a non-woven fabric without (a) and with immobilized (b) AgNPs of different concentration. One can see that AgNPs were evenly incorporated into fabric surface.

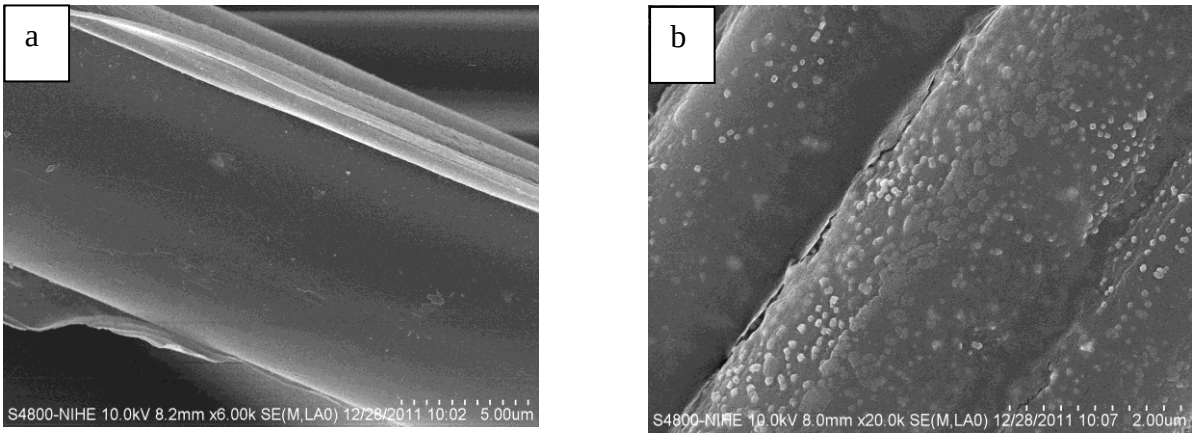

Figure 2. SEM image of non-woven fabric without (a) and with AgNPs (b) incorporated.

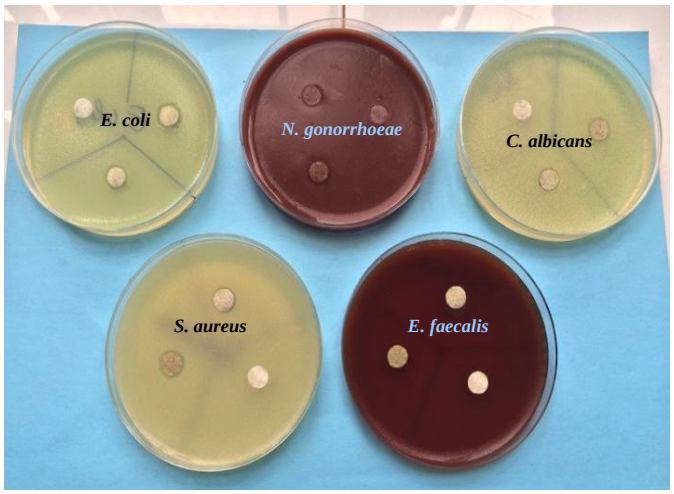

Figure 3. Antibacterial rings of 3 nanosilver-impregnated fabric pieces with respect to the bacteria in the excretion track.

Table 2. Antibacterial activity of AgNPs-impregnated non-woven fabric against the bacteria spp. in the exudative track depending on nanosilver content.

Sample	SNPs solution volume (mL) *	SNPs content, mg/cm^2	Bactericidal activity
1	0.1	0.005	Antibacterial rings are not clear
2	0.2	0.01	Antibacterial rings could be watched clearly
3	0.3	0.015	Antibacterial rings could be watched very clearly
4	0.4	0.02	Antibacterial rings are largest

*Surface of the pieces of nano-impregnated fabric was 10 cm^2

The experimental data illustrated in Table 2 and Fig.3 showed that antibacterial rings began to appear on nanosilver-impregnated non-woven fabric at a nanosilver content of 0.005 mg/cm^2 in respect of the studied microorganisms, including *E. coli; N. gonorrhoeae; E. faecalis; S. aureus* and *C. albicans*. By increasing nanosilver content the rings' diameters increased proportionally.

3.3. Assessment of bactericidal activity of AgNPs-impregnated non-woven fabric in respect of the bacteria causing ureteritis, diseases of genital track and vaginal discharge

Table 3 presents bactericidal efficiency of AgNPs-impregnated non-woven fabric towards microorganisms which cause gynaecological diseases in vaginal discharge environment, depending on nanosilver content. It can be seen that *C. albicans* sample mixed with different bacteria isolated from patient's specimen resulted in similar features presented in Table 2. In the presence of natural bacterial suspension the fabric samples impregnated with SNPs of concentration 0.01 mg/cm^2 exhibited simultaneously bactericidal power towards one to three microbial types. Only in the simultaneous presence of more than 4 types of microorganisms the shape inhibition rings did not appeared. However, in practice the simultaneous presence of 4 bacterial types in specimen is scarce due to the nutrient competition rule. Nano-impregnated fabric sample with concentration 0.005 mg/cm^2 clearly exhibited bactericidal power towards 3 types of bacteria causing gynaecological diseases.

Table 3. Bactericidal efficiency of AgNPs-impregnated non-woven fabric towards microorganisms which cause gynaecological diseases.

Fabric samples immobilized with SNPs	Inhibition ring diameter				
	C. albicans	*C. albicans* + *E. coli*	*C. albicans* + *E. coli* + *S. aureus*	*C. albicans* + *E. coli* + *S. aureus* + *E. faecalis*	*N. gonorrhoeae*
0.01 mg/cm^2	7 mm	9 mm	8 mm	Not clear	10 mm
0.005 mg/cm^2	0 mm	7 mm	7 mm	Not clear	9 mm
Blank	0 mm	0 mm	0 mm	0 mm	0 mm

The experimental data showed that nanosilver-impregnated fabric samples at a concentration 0.01 mg/cm^2 gave antibacterial rings of 10 mm, 9 mm and 8 mm, respectively, for *N. gonorrhoeae, C. albicans + E. coli* and *C. albicans + E. coli + S. aureus*. From these data one can choose a nanosilver concentration suitable for manufacturing antibacterial fabric. At concentration 0.01 mg/cm^2 AgNPs-impregnated non-woven fabric is able to kill all 5 microorganisms which cause exudative track diseases.

Assessment of skin irritation effect

Assessment of skin irritation caused by using of AgNPs-impregnated non-woven fabric was carried out in Central Institute for Drug Quality Control. The results presented in Table 4 showed that the prolonged exposition up to 72 h did not reveal any irritation on the skin of the tested rabbits, proving that nanosilver-containing non-woven fabric is safe for skin treatment.

Table 4. Skin irritation effect of nanosilver-containing fabric on rabbits.

Rabbit	Weight, kg	Exposition time						Total mark 24 ÷ 72 h	Responded mark/3
		24 h		48 h		72 h			
		B	P	B	P	B	P		
1	2.22	0	0	0	0	0	0	0	0
2	2.30	0	0	0	0	0	0	0	0
3	2.45	0	0	0	0	0	0	0	0
	Total skin irritation mark for 3 rabbits								0
	Average total skin irritation mark								0

4. CONCLUSION

Nanosilver shows up to be able to kill effectively bacteria of the secretion track such as *E. coli; N. gonorrhoeae; E. faecalis; S. aureus* and *C. albicans* with very low AgNPs concentration depending on exposition time. *N. gonorrhoeae* was rather sensitive to AgNPs, even just at the moment of contact (0 min) 1.95 ppm of nanosilver already killed completely the bacterial species, while for *E. coli* and *E. faecalis* it needed 125 ppm.

AgNPs-impregnated non-woven fabric with a concentration of 0.01 mg/cm^2 is able to kill at least 3 bacterial types inhabited in the secretion track. It gave antibacterial rings of 10 mm, 9 mm and 8 mm, respectively, for *N. gonorrhoeae; C. albicans + E. coli* and *C. albicans + E. coli + S. aureus.*

Nanosilver-containing non-woven fabric did not irritate skin therefore is suitable for manufacturing individual hygienic products.

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