biosilver

FABRICS

A COMPARATIVE STUDY OF THE ANTIBACTERIAL ACTIVITY OF NON-WOVEN PP FABRICS SAMPLES, COATED BY BIOSILVER SOLUTIONS

APPENDIX 2

A comparative study of the antibacterial activity of non-woven PP fabrics samples, coated by BIOSILVER solutions

Test samples:
10 samples of non-woven PP fabrics, coated by BIOSILVER solution, including unmodified samples (control, № 6-10).

№1 - (spunbond 35 g / m^2, coated by «BIOSILVER solution»);
№2 - (spunbond 50 g / m^2, coated by «BIOSILVER solution»);
№3 - (spunbond 20 g / m^2, coated by «BIOSILVER solution»);
№4 - (needle-punched non-woven PP fabrics 150 g / m^2, coated by «BIOSILVER solution»);
№5 - (needle-punched non-woven PP fabrics 250 g / m^2, coated by «BIOSILVER solution»);

Control (fabrics noncoated by BIOSILVER):
№6 - (spunbond 35 g / m^2);
№7 - (spunbond 50 g / m^2);
№8 - (spunbond 20 g / m^2);
№9 - (needle-punched non-woven PP fabrics 150 g / m^2);
№10 - (needle-punched non-woven PP fabrics 250 g / m^2);

Test strains:
E. coli (gram-negative), strain B-1373, St. aureus (gram-positive), strain B-1266.

Materials and reagents:
- Saline solution 0,9% NaCl;
- Lysogeny broth (LB), – standardized growth medium (composition: tryptone - 10g / l, yeast extract - 5g / l, sodium chloride - 10g / l, 0.6% glucose, pH-7.2);
- Nutrient fish agar, NFA, Levine EMB Agar

Research Methods and Results
As a normative document, the Russians Guidelines for the laboratory assessment of the antimicrobial activity of textile materials were taken.

1. Disk diffusion method DDM

0.1 ml of a suspension of test strains with a concentration of 1-10 × 105 cells / ml were sown on the agar in Petri dishes. Under aseptic conditions, inoculated agar, wells were made and placed in each sample of experimental or control fabrics 1 × 1 cm in size. Non-woven material samples in the wells were moistened with saline: 150 µl was added to samples № 4, 5 to samples № 1,2,3 - 100 µl. The plated cups were kept in a thermostat at 37 ° C for 24 hours. The result was taken into account by the presence of culture lawn lysis zones around sample wells.

Results.

Table 1
Testing samples of non-woven PP fabrics samples, coated by BIOSILVER solutions for antibacterial activity by the Disk diffusion method DDM

Sample	Zone of growth inhibition in mm	
	S. aureus	E. coli
1 - 35 g / m^2, coated by "BIOSILVER solution"	15	15
2 - 50 g / m^2, coated by "BIOSILVER solution"	17	15
3 - 20 g / m^2, coated by "BIOSILVER solution"	12	11
4 - needle-punched 150 g / m^2, coated by "BIOSILVER solution"	18	17
5 - needle-punched 250 g / m^2, coated by "BIOSILVER solution	18	17
6 control - 35 g / m^2	0	0
7 control - 50 g / m^2	0	0
8 control - 20 g / m^2	0	0
9 control - needle-punched 150 g / m^2	0	0
10 control - needle-punched 250 g / m^2	0	0
Bacterial culture control	Typical uniform growth	Typical uniform growth

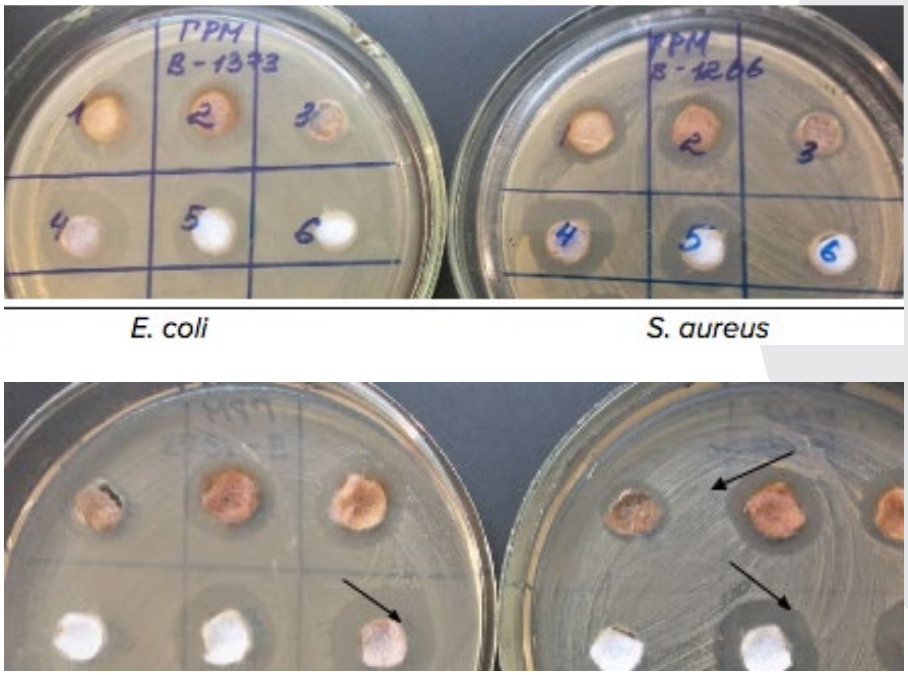

Fig. 1. Testing samples of non-woven PP fabrics samples, coated by BIOSILVER solution for antibacterial activity by the Disk diffusion method DDM (arrows indicate lysis zones, zone sizes in table 1). On the left is a Petri dish with E. Coli culture, on the right - with S. aureus culture.

The discussion of the results

As can be seen from the data presented, all samples of silver-coated non-woven fabrics exhibit an antimicrobial effect: the lysis zones were the largest for samples № 4, 5, then there was sample № 2, then № 1, the smallest lysis zone was for sample № 3. Control samples №6,7,8,9,10 (silver-free materials) no lysis zone.

Conclusion: this technique can be used in the Technical Specifications for non-woven PP fabrics, coated by BIOSILVER solutions as an indicator of authenticity by the presence of antimicrobial activity.

2. Joint incubation of non-woven PP fabrics samples with cultures of test strains in the liquid medium of LB.

Non-woven PP fabrics samples 2 × 2 cm in size were cut into 4 parts, placed in test tubes and sterilized in an autoclave for 15 minutes at a temperature of 115 ° C. Then, 5 ml of LB medium and 0.1 ml of a suspension of test strain cells with a concentration of 105 cells / ml were introduced into each tube. The test tubes were incubated at 37 ° C. After 10 minutes, 4 hours and 24 hours of joint incubation of cells and samples, culture fluid samples were taken, which were titrurated using the standard ten-fold dilution method, followed by plating on Petri dishes with timing medium. After 20 hours of incubation the number of grown colonies was calculated and the concentration of viable bacteria in CFU / ml of culture fluid was calculated.
Suspensions of test strains prepared in a similar manner, but without the addition of non-woven PP fabrics samples, were used as a culture control.
The results are presented in the tables below.

Table 2
The titer of the strain S. aureus in the culture fluid after joint incubation with samples № 1-6

№ Sample	The titer of culture fluid S. aureus, CFU / ml Incubation time		
	10mn	4h	24
1 - 35 g / m², coated by "BIOSILVER solution"	$1,7\times10^5$ ($1,0\times10^6$)	$1,4\times10^5$ ($4,8\times10^4$)	$2,2\times10^8$ ($2,8\times10^8$)
2 - 50 g / m², coated by "BIOSILVER solution"	$1,3\times10^5$ ($1,3\times10^6$)	$4,6\times10^3$ ($5,1\times10^4$)	$3,6\times10^7$ ($2,3\times10^7$)
3 - 20 g / m², coated by "BIOSILVER solution"	$1,9\times10^5$ ($1,1\times10^6$)	$2,9\times10^5$ ($3,2\times10^5$)	$4,6\times10^8$ ($3,4\times10^8$)
4 - needle-punched 150 g / m², coated by "BIOSILVER solution"	$1,1\times10^4$	$3,5\times10^3$	$3,1\times10^5$
5 - needle-punched 250 g / m², coated by "BIOSILVER solution	$1,1\times10^4$	$2,1\times10^3$	$7,8\times10^4$
6 control - 35 g / m²	$2,2\times10^5$	$1,1\times10^7$	$9,3\times10^9$
7 control - 50 g / m²	$2,2\times10^5$	$1,1\times10^7$	$9,3\times10^9$
8 control - 20 g / m²	$2,2\times10^5$	$1,1\times10^7$	$9,3\times10^9$
9 control - needle-punched 150 g / m²	$2,2\times10^5$	$1,1\times10^7$	$9,3\times10^9$
10 control - needle-punched 250 g / m²	$2,2\times10^5$	$1,1\times10^7$	$9,3\times10^9$
11 control of S. aureus strain	$2,2\times10^5$ ($1,6\times10^6$)	$1,2\times10^7$ ($6,2\times10^7$)	$1,1\times10^{10}$ ($3,4\times10^9$)

Note: for samples № 1, 2, 3, experiments were repeated. The results of the second experiment are shown in parentheses.

Table 3
The titer of E. coli strain in the culture fluid after joint incubation with samples No. 1-6

№ Sample	The titer of culture fluid E.coli, CFU / ml Incubation time		
	10mn	4h	24
1 - 35 g / m², coated by "BIOSILVER solution"	$1,8\times10^3$ ($4,1\times10^4$)	$2,3\times10^2$ (0)	$8,3\times10^3$ ($3,5\times10^{4)}$
2 - 50 g / m², coated by "BIOSILVER solution"	$1,3\times10^3$ ($3,7\times10^4$)	$1,1\times10^1$ (0)	$1,5\times10^3$ ($2,8\times10^{4)}$
3 - 20 g / m², coated by "BIOSILVER solution"	$1,4\times10^4$ ($5,2\times10^4$)	$3,0\times10^4$ ($4,8\times10^3$)	$1,7\times10^5$ ($4,2\times10^4$)
4 - needle-punched 150 g / m², coated by "BIOSILVER solution"	0	0	0
5 - needle-punched 250 g / m², coated by "BIOSILVER solution	30	$1,4\times10^7$	$1,1\times10^9$
6 control - 35 g / m²	$2,7\times10^5$	$1,4\times10^7$	$1,1\times10^9$
7 control - 50 g / m²	$2,7\times10^5$	$1,4\times10^7$	$1,1\times10^9$
8 control - 20 g / m²	$2,7\times10^5$	$1,4\times10^7$	$1,1\times10^9$
9 control - needle-punched 150 g / m²	$2,7\times10^5$	$1,4\times10^7$	$1,1\times10^9$
10 control - needle-punched 250 g / m²	$2,7\times10^5$	$1,1\times10^7$	$9,3\times10^9$
11 control of E.coli strain	$2,6\times10^5$ ($6,8\times10^5$)	$1,5\times10^7$ ($3,6\times10^8$)	$2,7\times10^9$ ($2,4\times10^{10}$)

Note: for samples No. 1, 2, 3, experiments were repeated. The results of the second experiment are shown in parentheses.

The discussion of the results

For all samples, a bacteriostatic effect was achieved (reduction of titers by several orders of magnitude compared with the control) and even bactericidal action (samples № 4 and 5 on E. coli).

It should also be noted that the components of the nutrient medium (tryptone, yeast extract) impede the desorption of silver nanoparticles from the tissue into the solution. Given the large microbial load (10^5 - 10^6 cells / ml), the presence of a sufficient amount of nutrient medium (LB medium + collagen hydrolyzate), the growth rate of bacteria is not comparable with real conditions. In practice, such large microbial loads, combined with growth media rich in growth factors, are unlikely to take place in reality.
Conclusion
The aim of the study was to develop a simple and affordable method for testing non-woven PP fabrics, coated by BIOSILVER solutions for antimicrobial activity. The method of co-cultivation of fabric samples with test strains in a nutrient medium is laborious, complex and requires refinement. The simplest, most convenient, affordable is the disk diffusion method DDM, which is recommended for inclusion in the Specifications for biosilversilver-coated fabric as a test - an indicator of authenticity by the presence of antimicrobial action.

Disk diffusion method DDM

Description of the Disk diffusion method DDM: 0.1 ml of a suspension of test strains with a concentration of 1-10 × 10^5 cells / ml are sown on the agar in Petri dishes. Under aseptic conditions, holes are made in seeded agar and samples of biosilver-modified and unmodified (original) fabric (control) are placed in

them in the form of pieces cut out approximately to the size of the hole. From 1 to 3 pieces of fabrics are placed in each well, depending on the thickness of the test tissue. Pieces are cut from places selected randomly. Samples in the wells are moistened with water for moisture capacity, that is, until free water appears. Petri dishes with seeding are placed in a thermostat at 37 ° C and incubated for 24 hours. The result is taken into account by the presence of culture lawn lysis zones around the sample wells. Around the wells with samples of the biosilver-coated non-woven fabrics, growth inhibition zones should be observed. In the control sample (initial unmodified tissue), the lysis zone should be absent. The experiment is carried out in two parallel repetitions. If a negative result is obtained in at least one sample, the experiment is repeated on twice the number of samples. The results of the repeated experiment are final.

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