

Metal nanoparticles in DBS card materials modification

**A Metelkin¹, G Frolov¹, D Kuznetsov¹, E Kolesnikov¹, K Chuprunov¹,
S Kondakov^{1,2}, A Osipov^{1,2}, J Samsonova^{1,2}**

¹ National Research Technological University "MISIS", 2, Leninsky ave., Moscow, 119049, Russia

² M.V. Lomonosov Moscow State University, Department of Chemistry, 1-3 Lenin Hills, Moscow, 119991, Russia

E-mail: ksekse@mail.ru

Abstract. In the recent years the method of collecting and storing Dried Blood Spots (DBS) on special cellulose membrane (paper) has gained wide popularity. But possible damage of biosamples caused by microorganisms in case of their incomplete drying is a disadvantage of the method. It can be overcome by treating sample-collection membranes with colloidal solutions of metal nanoparticles, having antibacterial effect. The team studied antibacterial properties of nonwoven material samples with various coatings (alcohol sols of copper, aluminium, iron, titanium, silver and vanadium nanoparticles). Colloidal solutions of nanoparticles were obtained by means of electroerosion method with further low-temperature plasma condensation. Antibacterial activity of fiberglass and cellulose membrane samples with nanoparticle coatings was studied using *B. cereus* and plaque bacteria cultures. It was revealed that nanostructured coatings can suppress bacterial activity; in addition they can diffuse from the membrane surface into medium which leads to widening the areas of inhibiting testing cultures' growth. Thus, membrane materials treatment with alcohol-sols of metal nanoparticles can be seen as promising for conferring antibacterial properties to DBS carriers.

1. Introduction

In the recent years the method of collecting and storing Dried Blood Spots (DBS) on special cellulose membrane (paper) has gained wide popularity. But possible damage of biosamples caused by microorganisms in case of their incomplete drying is a disadvantage of the method. It can be overcome by treating the collecting membranes with metal nanoparticles having antibacterial properties. Modification of membranes surfaces with nanomaterials and polymers with antibacterial properties is among most promising methods for maintaining stability of physico-chemical parameters of membranes. Silver and zinc oxide nanoparticles range among the most active nanomaterials of this type. Silver and zinc oxide nanoparticles, as well as based on them antibacterial polymers gain wide popularity due to their prominent antibacterial properties [1]. Considerable effect is obtained because of high specific surface area of the particles providing good contact of the nanoparticles' surfaces with the environment. In addition to that, small particles sizes let them penetrate through cell membranes, thus increasing their antibacterial activity [2]. In particular, silver nanoparticles are widely used as an antibacterial agent in wound dressings, implant coatings, etc. [3]. Still, mechanism of their activity is not totally clear. Antibacterial effect is in proportion to the amount of silver ions (Ag⁺) released and to their interaction with a bacterial cell membrane [4]. High specific surface area of the particles surfaces enables greater number of atoms to interact with the environment so, antibacterial activity of silver



nanoparticles depends on their size [5]. Ions of silver interact with peptidoglycan cell wall and with cytoplasmic capsule [6]. Besides, ions of silver prevent DNA replication by reacting with sulfhydryl groups of bacteria proteins, especially with enzymes engaged in electron transport [7]. Thus, studies aimed at creating nanostructured coatings for DBS membranes providing antibacterial effect seem very promising.

2. Research target and methods

2.1. Research target.

In the present work properties of the following membranes were studied: cellulose membrane TFN («Munktell», Germany); glass fiber membrane Grade 8964 («Ahlstrom», USA) and glass fiber membrane GFSP 223000 («Millipore», USA), see the list in Table 1.

Table 1. List of materials and coatings under study

#	Material	Coating
1	Millipore CFSP 223000	Silver alcohol-sol
2	Millipore GFSP 203000 Glassfiber conjugate pad	Aluminium alcohol-sol
3	Mista PPDS-0300 (Grade 89640)	Tantalum alcohol-sol
4	Munktell TFN 179 g/cm ²	Titanium alcohol-sol
5	Grade 8950	Iron alcohol-sol
6	Grade 8964	Vanadium alcohol-sol
7	Grade 319	Copper alcohol-sol

2.2. Research methods.

Method for alcohol-sols of metals application onto porous material surfaces.

Colloidal solutions of silver, copper, tantalum, vanadium, titanium, iron and aluminium in ethyl alcohol were prepared using specially designed electro-pulse dispersion-condensation equipment. Porous materials were saturated with the obtained alcohol-sols after the following technique:

- after short shaking of colloidal solution a 5 ml aliquot was drawn with a single-use syringe;
- the solution was slowly and evenly applied onto the samples of nonwoven material, situated in a petri dish, up to complete saturation (when a small amount of free liquid appeared);
- the material was air dried due to alcohol evaporation at room temperature.

The samples were saturated twice following the same method.

To study the properties of membranes after application of biological fluids, membrane samples about 5 mm on a side were treated by blood serum by immersing them into biosample till full saturation, then the samples were air dried at room temperature.

Test-objects: *B. cereus* bacilli, spores of STI-1 vaccinal strain and tooth plaque bacterial culture.

Tooth plaque bacteria were obtained by isolation from teeth surfaces with an aseptic cotton wool ball and transferred to a test tube with Hottinger broth. The bacteria were cultivated in the broth for 18-20 hours at 35°C; bacteria concentration in the broth culture was 1×10^8 CFU/ ml.

In the experiments the team used Hottinger broth and solid medium (SM) for antibiotic sensitivity testing (AGV medium, produced by FSSIC 'Microgen', Ministry of Health and Social Development of the Russian Federation, Moscow).

Antibacterial properties of the samples were assessed by means of disk-diffusion method (zones of microbial growth inhibition determination) and by suspension method.

3. Results and discussion

Antibacterial activity of nonwoven materials (fiber felts of various composition, such as fiberglass and cellulose) with various coatings (Table 1) was studied by disk-diffusion method. Sample identification numbers were from 1 to 7, number 8 was a control sample. Tooth plaque bacteria and *B. cereus* bacilli were used as test cultures as the most resistant for disinfectology purposes. In the first experiment squares of the studied nonwoven materials as well as the control material square treated with silver-based nanodisperse system were placed in petri dishes on the surface of SM with test-culture inoculation (Figure 1).

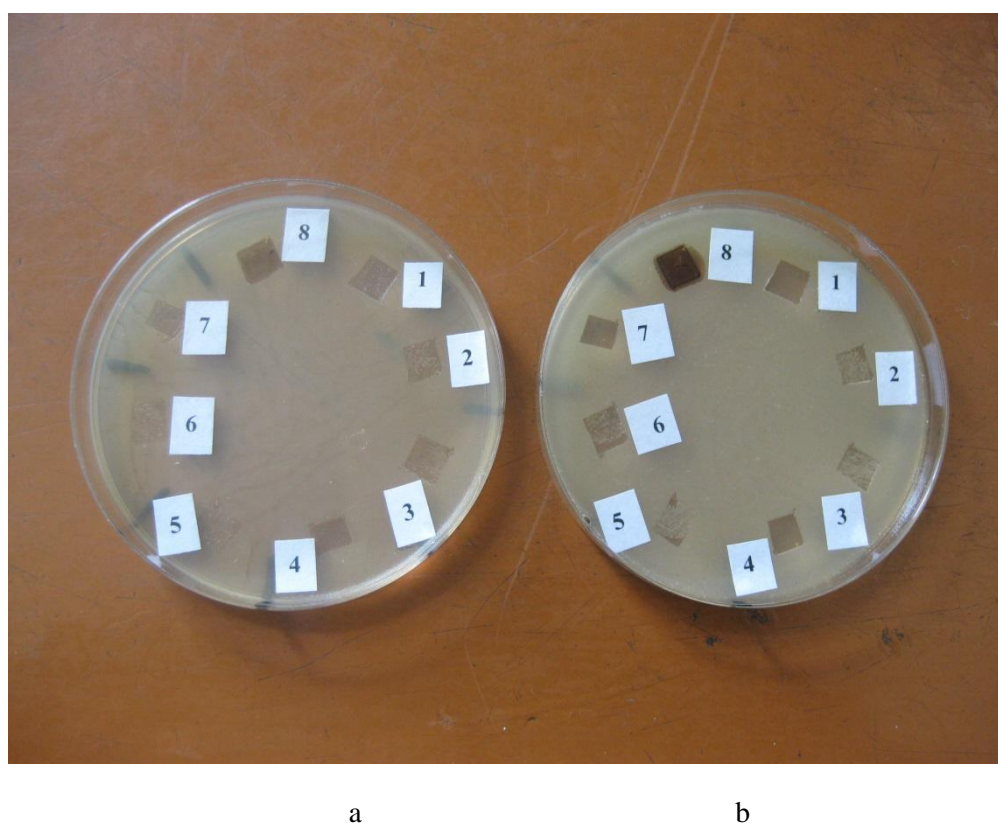


Figure 1. Antibacterial activity of nonwoven materials to tooth plaque bacteria (a) and *B. cereus* (b)

It can be observed from the Figure 1 that microbial growth inhibition zones were formed only under the squares. Growth inhibition of tooth plaque bacteria was especially pronounced under samples ## 1, 2, 3, 4, 7 while under samples ## 5 and 6 bacterial growth inhibition was very slight. On the *B. cereus* bacterial lawn growth inhibition was prominent under samples ## 1, 2, 4, 6, 7 and weak under samples ## 3, 5. Growth inhibition under the control sample # 8 is observed only under the sample itself.

In the second variant of the experiment squares of the studied nonwoven materials as well as the control material square # 8 treated with (ZnO+1.2% cationic surfactant) were placed in petri dishes on the surface of SM with test-culture inoculation of tooth plaque bacteria (Figure 2). The squares were previously wetted with distilled water.

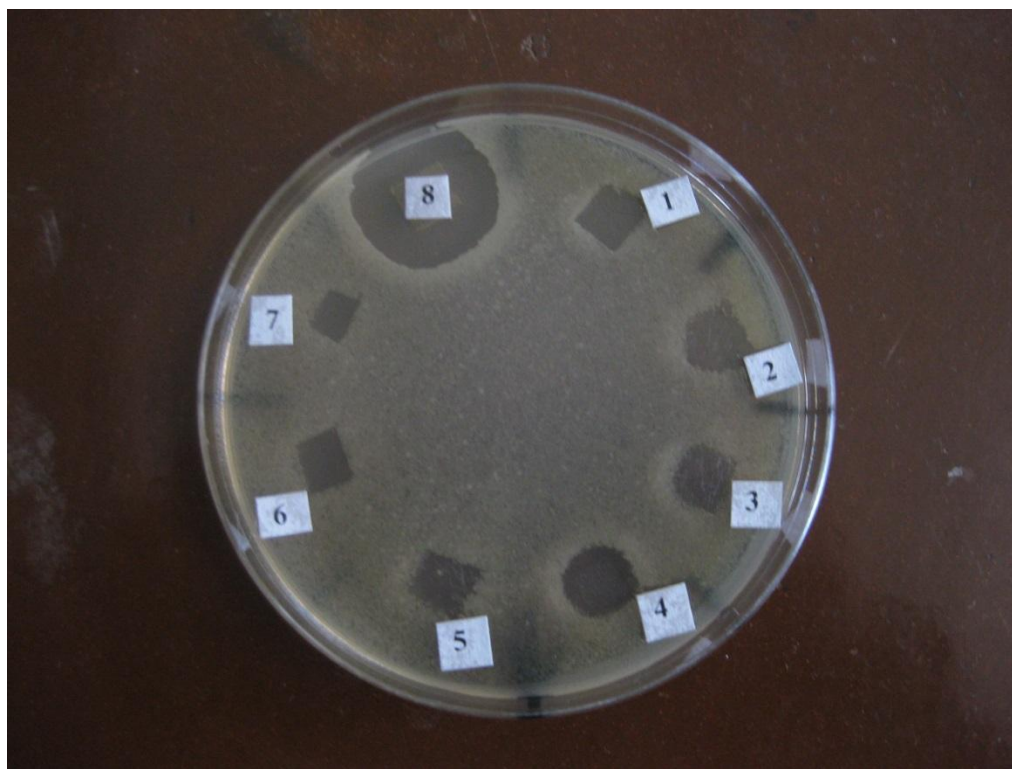


Figure 2. Antibacterial activity of pretreated nonwoven materials towards tooth plaque bacteria

Zones of tooth plaque bacteria test-culture growth inhibition were revealed, exceeding the outlines of the squares #2 (by 2 mm), #3 (by 1.5 mm), #4 (by 3 mm), #5 (by 1 mm) as well as a prominent zone of tooth plaque bacteria growth inhibition around the contours of control square #8.

Thus, pre-treatment of the studied materials displayed in some of them the ability of the coating to diffuse into agar and widen the zones of test-culture growth inhibition.

4. Conclusions

It was revealed that nanostructured coatings can inhibit activity of bacteria and they also can diffuse from membrane surfaces into agar thus widening test-culture growth inhibition areas. Treatment of membrane materials with alcohol sols of metal nanoparticles can be promising for adding antibacterial properties to DBS carriers.

Acknowledgments

The authors gratefully acknowledge the financial support of the Ministry of Education and Science of the Russian Federation within the Federal Target Program “Research and development on priority directions of scientific-technological complex of Russia in 2014-2020” (project No. RFMEFI57814X0010)

References

- [1] Hajipour M J, Fromm K M, Ashkarran A A, de Aberasturi D J, de Larramendi I R, Rojo T, Serpooshan V, Parak W J and Mahmoudi M 2012 Antibacterial properties of nanoparticles. *Trends in Biotechnol.* **30** (10) 499–511
- [2] Park H J, Park S, Roh J, Kim S, Choi K, Yi J, Kim Y and Yoon J 2013 Biofilm-inactivating activity of silver nanoparticles: a comparison with silver ions. *J. Industr. and Eng. Chem* **19** (2) 614–619

- [3] Sotiriou G A and Pratsinis S E 2010 Antibacterial Activity of Nanosilver Ions and Particles. *Env. Sc. & Technol.* **44** (14) 5649-5654
- [4] Peng J J Y, Botelho M G and Matinlinna J P 2012 Silver compounds used in dentistry for caries management: a review *J. of Dentistry.* **40** (7) 531–541
- [5] Radzig M A., Nadtochenko V A., Koksharova O A., Kiwi J, Lipasova V A and Khmel I A 2013 Antibacterial effects of silver nanoparticles on gram-negative bacteria: Influence on the growth and biofilms formation, mechanisms of action *Coll. and Surf. B-Biointerf.* **102** 300.
- [6] Chaloupka K, Malam Y and Seifalian A M. 2010 Nanosilver as a new generation of nanoparticle in biomedical applications. *Trends in Biotechnol.* **28** (11) 580-588
- [7] Seth D, Choudhury S R, Pradhan S, Gupta S, Palit D, Das S, Debnath N and Goswami A 2011 Nature-inspired Novel Drug Design Paradigm Using Nanosilver: Efficacy on Multi-Drug-Resistant Clinical Isolates of Tuberculosis. *Current Microbiol.* **62** (3) 715-726