Effect of nanoparticles in growth of test - bacteria

Effect of nanoparticles in growth of test - bacteria

SV Stolyar[1,2], **LA Chekanova**[3], **RN Yaroslavtsev**[3], **VP Ladygina**[1] **and LS Tirranen**[1,4,5]

[1] Federal Research Center "Krasnoyarsk Science Center" of the Siberian Branch of the Russian Academy of Sciences; Krasnoyarsk, Akademgorodok, 50, Russia
[2] Siberian Federal University, Krasnoyarsk, Svobodnyi pr., 79, Russia
[3] Kirensky Institute of Physics, Krasnoyarsk, Akademgorodok, 50/38, Russia
[4] Institute of Biophysics of the Siberian Branch of the Russian Academy of Sciences, Krasnoyarsk, Akademgorodok, 50/12, Russia

E-mail: tiran@ibp.ru

Abstract. Confident effect of five magnetic composite nanoparticles (FeP@Ag, FeP@Pd, CoP, NiP, Fe_2O_3@АГ) on growth of test bacteria colonies (Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus) in five replicates each is considered. Reliable inhibitors of colonies of all five test bacteria were nanoparticles FeP@Ag. CoP nanoparticles are reliable inhibitors of growth of 4 test bacteria (except for test bacteria Escherichia coli). NiP nanoparticles are reliable inhibitors of growth of 2 test bacteria: Escherichia coli and Klebsiella pneumoniae. Bacteria Escherichia coli were most sensitive to the effect of magnetic nanoparticles; and bacteria Pseudomonas aeruginosa and Staphylococcus aureus were most resistant to the effect of magnetic nanoparticles. The prospects of the method are in the possibility of multiple reuse of the magnetic particles with antimicrobial properties for bacterial decontamination of the studied sources of water and removal of magnetic nanoparticles from the treated liquids by electromagnet. The method can find use in water treatment facilities for household, Industrial and medical wastes.

1. Introduction

On 29 January 2018 World Health Organization (WHO) published data of epidemiological surveillance service on high resistance level to many antibiotic classes of the bacterial strain series [1, 2]. This endangers efficiency of antibiotics [2]. Nanosize materials are considered new antimicrobial agents [3, 4].

The aim of the study is to find bactericidal, bacteriostatic and stimulatory action of various magnetic composite nanoparticles deposited on agar nutrient medium in Petri dishes on growth of five test- bacteria in five replicates each.

2. Materials and methods

The objects under study were magnetic composite nanoparticles FeP@Ag, FeP@Pd, CoP, NiP, Fe_2O_3@AG and five test - bacteria: Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus growing on fish-peptone agar (FPA).

All powder samples have been produced by chemical sedimentation method. FeP powders (d ~ 50 nm, C(P) ~ 5 %), NiP (d ~ 250 nm, C(P) ~ 5 %) and CoP powders (d ~ 300 nm, C(P) ~ 5 %) have been produced from solution of the following composition: salt of respective metal (sulfate), sodium

citrate, sodium hypophosphite, sodium hydroxide. Ag powder was produced from solution of the following composition: silver nitrate, sodium hypophosphite, sodium hydroxide. Pd powder was produced from solution of the following composition: palladium chloride, sodium hypophosphite, sodium hydroxide. The powder of Fe_2O_3 nanoparticles coated with polysaccharide arabinogalactan (Fe_2O_3@AG) has been produced solution of the following composition: ferrous sulphate, arabinogalactan, sodium hydroxide.

The powders were resuspended in a sterile flask with 100 ml of settled tap water, treated for 4 minutes in «Volna» apparatus (ultrasonic technological apparatus UATA-04/22-OM (УЗТА-04/22-ОМ) made by «Ultrasonic Technology Center» – www.u-Sonic.ru). Then the flask with water suspension was sterilized under 1 atm.

The surface of FPA nutrient agar medium pre-dispensed in sterilized Parti dishes was added 0.1 ml of produced water suspension of the powder and anointed with sterile spreader.
Suspension prepared from day-old test bacteria according to the Tarasevich optical turbidity standard by 10 units which was dispensed by Pasteur pipette into the replicator base wells (figure1).

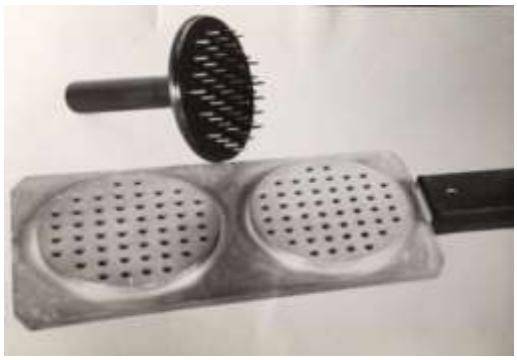

Figure 1. Replicator: handlewith pins (top) and base with wells (bottom).

The replicator handle was used to make replicates up to 25 test bacteria simultaneously on the surface of FPA agar nutrient medium with particles under study in Petri dishes. The dishes with inoculated test bacteria were incubated in a thermostat at 37 °C. The growth of test bacteria depended on the action of nanoparticles.

Response of test- bacteria to the action of nanoparticles was evaluated after three days of their joint growth by the difference in the size of test-bacteria colonies in experiment and controls, when the bacterial colonies grown in control had pronounced pigmentation [6]. Controls were dishes with test-bacteria not exposed to the action of nanoparticles. On figure 2 show sequence of applying bacterial suspensions into the replicator base wells.

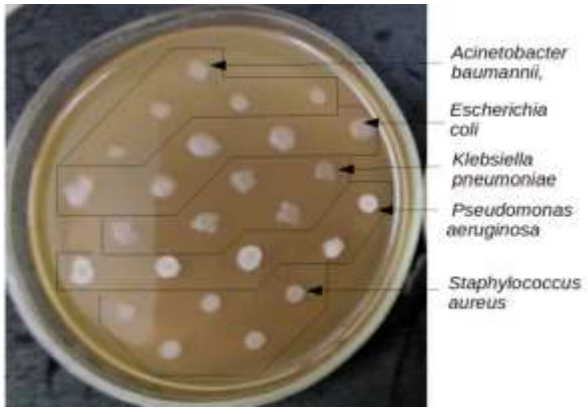

Figure 2. Sequence of applying bacterial suspensions into the replicator base wells.

The data were statistically processed according to the method of G.F. Lakin [7]. Account was made of arithmetical mean of the diameter of bacterial colonies under study, standard error of the mean. The criterion of evaluation was standard value of normalized deviate (t_{st}) for 95-99.9% -th level of significance.

The effect of magnetic composite nanoparticles on test bacteria was evaluated as positive (stimulating) or negative (inhibiting) when the size of test bacterial colonies in the experiment reliably increased or decreased as compared to control. If the size of colonies in the experiment did not differ reliably from control the action of magnetic composite nanoparticles was considered uncertain [6,7].

3. Results and discussion

Figure 3 shows results of experiment of effect of FeP@Ag magnetic composite nanoparticles of core-shell type, on the growth of five test bacterial colonies (each test- bacterium in five replicates) after 18 hours of incubation in thermostat.

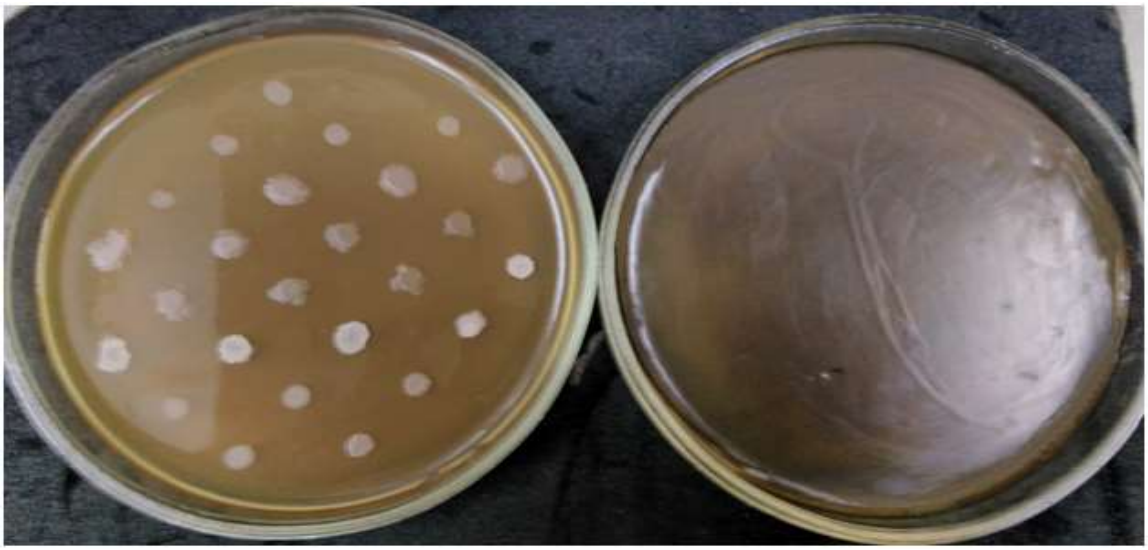

Figure 3. Antibacterial effect of FeP@Ag nanoparticles of core-shell type, on growth of five test bacterial colonies (each test bacterium in five replicates) after 18 hours of incubationin thermostat.
Note: Control – on the left: growth of colonies of 5 test-bacteria on the medium without nanoparticles. Experiment – on the right. Lack of growth of colonies of test - bacteria on nutrient medium with FeP@Ag particles.

In control on the nutrient medium without nanoparticles all colonies of test bacteria have grown up. In experiment no growth of all colonies of 5 test bacteria on the medium with FeP@Ag magnetic composite nanoparticles occurred.

Table 1 presents experimental data on - bactericidal effect of FeP@Ag particles on the growth of colonies of five test bacteria in experiment and in control.

Table 1. Effect of FeP@Ag particles on the growth of colonies of bacteria in five replicates.

Bacteria	Control $\acute{M} \pm \acute{m}$ (mm)	Experiment $\acute{M} \pm \acute{m}$ (mm)	t_{st}	$p \geq$, effect
Acinetobacter baumannii	7.60 ± 0.17	0	20.32	$p \geq 0.001$- bactericidal
Escherichia coli	7.36 ± 0.09	0	21.22	$p \geq 0.001$- bactericidal
Klebsiella pneumoniae	8.60 ± 0.19	0	31.57	$p \geq 0.001$- bactericidal
Pseudomonas aeruginosa	11.5 ± 0.27	0	19.21	$p \geq 0.001$- bactericidal
Staphylococcus aureus	5.20 ± 0.25	0	21.22	$p \geq 0.001$- bactericidal

Thus, the experiment showed bactericidal effect of FeP@Ag magnetic composite nanoparticles on growth of colonies of five test- bacteria in five replicates of each test bacterium.

Table 2 and 3 show results of antibacterial effect of FeP@Pd and NiP nanoparticles on growth of colonies of five test- bacteria (each test- bacterium in five replicates).

Table 2. Effect of FeP@Pd nanoparticles on growth of colonies of test- bacteria.

Bacteria	Control $\acute{M} \pm \acute{m}$ (mm)	Experiment $\acute{M} \pm \acute{m}$ (mm)	t_{st}	$p \geq$ effect
Acinetobacter baumannii	6.38 ± 0.07	7.72±0.436	3.04	$p \geq 0.05$ - stimulating effect
Escherichia coli	10.36 ± 0.26	7.48±0.146	9.55	$p \geq 0.001$- bacteriostatic effect
Klebsiella pneumoniae	5.56 ± 0.103	7.40±0.187	8.62	$p \geq 0.01$ - stimulating effect
Pseudomonas aeruginosa	8.98 ± 0.132	9.80±0.200	3.42	$p \geq 0.05$ - stimulating effect
Staphylococcus aureus	6.16 ± 0.144	6.50±0.267	1.54	uncertain effect

The data presented in table 2 show that reliable stimulating effect was found in 3 test - bacteria (Acinetobacter baumannii, Klebsiella pneumoniae and Pseudomonas aeruginosa). Reliable inhibiting effect affected Escherichia coli. FeP@Pd nanoparticles had no reliable effect on growth of Staphylococcus aureus colonies.

Table 3. Effect of NiP nanoparticles on growth of five colonies of test bacteria.

Bacteria	Control $\acute{M} \pm \acute{m}$ (mm)	Experiment $\acute{M} \pm \acute{m}$ (mm)	t_{st}	$p \geq$, effect
Acinetobacter baumannii	8,1 ± 0,368	6,74±0,452	2,34	uncertain effect
Escherichia coli	8,24±0,112	6,84±0,236	5,36	$p \geq 0,01$ bacteriostatic
Klebsiella pneumoniae	9,30±0,123	7,70±0,123	9,23	$p \geq 0,001$ bacteriostatic
Pseudomonas aeruginosa	4,84±0,160	4,98±0,132	0,67	uncertain effect
Staphylococcus aureus	5,08±0,036	5,02±0,330	0,33	uncertain effect

The data presented in table 3 are indicative of antimicrobial effect of NiP. Growth of colonies of test bacteria Escherichia coli and Klebsiella pneumoniae was reliably shown to be inhibited ($p \geq 0.01$ and $p \geq 0.001$, respectively) while the growth of colonies of test bacteria Acinetobacter baumannii, Pseudomonas aeruginosa and Staphylococcus aureus was uncertain.

Tables 4 and 5 present data on antimicrobial effect of CoP nanoparticles and Fe_2O_3 nanoparticles coated with polysaccharide arabinogalactan (Fe_2O_3@AG) on the growth of the same 5 colonies of test bacteria, each in five replicates.

Table 4. Effect of CoP on growth of 5 colonies of test-bacteria, each in 5 replicates.

Bacteria	Control $\acute{M} \pm \acute{m}$ (mm)	Experiment $\acute{M} \pm \acute{m}$ (mm)	t_{st}	$p \geq$, effect
Acinetobacter baumannii	10.1± 0.332	1.80±0.559	2.77	$p \geq 0,001$ - bacteriostatic
Escherichia coli	9.00 ±0.274	9.50±0.223	1.42	uncertain effect
Klebsiella pneumoniae	10.0± 0.418	8.70 ±0.11	2.91	$p \geq 0,05$ - bacteriostatic
Pseudomonas aeruginosa	7.00± 0.158	4.52±0.143	11.6	$p \geq 0,001$ - bacteriostatic
Staphylococcus aureus	7.24± 0.201	5.90 ±0.10	5.96	$p \geq 0,01$ - bacteriostatic

CoP nanoparticles have been found to reliably inhibit growth of colonies of test bacteria Acinetobacter baumannii and Pseudomonas aeruginosa ($p \geq 0.001$); Staphylococcus aureus ($p \geq 0,01$); Klebsiella pneumoniae ($p \geq 0,05$). Uncertain was the action of Escherichia coli only.

Table 5. Antimicrobial effect of Fe_2O_3 nanoparticles coated with polysaccharide arabinogalactan (Fe_2O_3@Ag), on growth of colonies of test-bacteria.

Bacteria	Control $\acute{M} \pm \acute{m}$ (mm)	Experiment $\acute{M} \pm \acute{m}$ (mm)	t_{st}	$p \geq$, effect
Acinetobacter baumannii	9.35±0.165	9.80±0.06	1.67	uncertain effect
Escherichia coli	9.60 ±0.292	11.0±0.158	4.22	p≥0.05 – stimulating effect
Klebsiella pneumoniae	10.1 ±0.400	11.0±0.158	2.09	uncertain effect
Pseudomonas aeruginosa	7.30 ±0.268	9.80±0.123	8.47	p≥0.01 – stimulating effect
Staphylococcus aureus	5.60 ±0.187	7.00±0.158	5.96	p≥0.01 – stimulating effect

4. Conclusion

So, FeP@Ag nanoparticles have been found to have bactericidal effect on all test-bacteria, each bacterium in five replicates, used in the experiment. Bactericidal properties of Ag nanoparticles are reported by other authors, too [8, 9].

Besides, CoP nanoparticles have been found to reliably inhibit growth of colonies of test-bacteria Acinetobacter baumannii and Pseudomonas aeruginosa (p≥0.001); Staphylococcus aureus (p≥0.01); Klebsiella pneumoniae 0.05. Action of Escherichia coli only was uncertainly stimulating.

The action of Fe_2O_3 nanoparticles coated with polysaccharide arabinogalactan uncertainly stimulated growth of test- bacteria Acinetobacter baumannii and Klebsiella pneumoniae and reliably stimulated growth of test-bacteria Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus.

It should be noted that number 1 to inhibit the growth of colonies of all five test bacteria were FeP@Ag nanoparticles; number 2– CoP nanoparticles – reliable inhibitors of growth of 4 test bacteria (except for bacteria Escherichia coli); number 3 – NiP nanoparticles – reliable inhibitors of growth of 2 test bacteria: Escherichia coli and Klebsiella pneumoniae.

Escherichia coli was most responsive to the effect of nanoparticles, most resistant were Pseudomonas aeruginosa and Staphylococcus aureus.

One of the reasons that the nanoparticles are more efficient than the classical antibacterial agents is their high ratio of surface to the volume – this gives rise to new mechanical, chemical, electrical, optical, magnetic and other properties different from their volumetric properties [3].

The silver nanoparticles as other metal-containing particles are specified by unique properties associated with high ratio of their surface to the volume defining their high efficiency (8, 9). Immense specific surface of the nanoparticles makes the “evaporation” processes more intensive to increase the concentration of metal ions. Further on the electrostatic interaction on the cell membrane makes the bacterial cell absorb the metal ions. Penetration of heavy metal ions inside the cell can trigger a cycle of chemical reactions in which the metal will act as a catalyst. The possible processes enumerated can trigger apoptosis processes of bacterial cell.

Effect of heavy metals on cytoplasmic membrane cause substantial changes. First of all, they are associated with impairment of their functions leading to the loss by the cells of amino acids, nucleotides< inhibition of transport processes and ultimately to the death of the bacterial cell [10].

The prospects of magnetic nanoparticles possessing antimicrobial properties is in their multiple use. Magnetic properties of nanoparticles at room temperature makes possible to easily remove the nanoparticles from the treated liquids by electromagnet after bacterial decontamination. Magentic nanoparticles with antimicrobial properties can find application at water treatment facilities of any liquid wastes (household, industrial, medical).

References

[1] Ustinov A V *New WHO report on antibiotic resistance* Available from: https://www.umj.com.ua/article/120095/novyj-doklad-voz-po-antibiotiko-rezistentnosti

[2] Baker-Austin C, Wright M S, Stepanauskas R and McArthur JV 2006 Co-selection of antibiotic and metal resistance *Trends Microbiol.* **14(4)** 176-82
[3] Martens E, Demain AL 2017 The antibiotic resistance crisis, with a focus on the United States *J. Antibiot.* **70** 520–6
[4] Huh Ae, Jung Kwon, Young Jik 2011 "Nanoantibiotics": A new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era *J. of controlled release* **156** (2) 128-45
[5] George M 2005 Whitesides, Nanoscience, Nanotechnology, and Chemistry *Small* **1** 172-9
[6] Tirranen LS, Torotenkova VN and Skazka TB 2011 Patent № RU 2410437 A method to determine bacteriostatic, bactericidal and stimulating action of antibiotic on microorganisms **3**
[7] Lakin GF 1990 *Biometry* (Moscow: Vysshaya shkola)
[8] Panacek A, Kvitek L, Prucek R e*t al*. 2006 Silver Colloid Nanoparticles: Synthesis, Characterzation and Their Antibacterial Activit. *J.of Physical Chemistry* **119** (33) 16248-53
[9] Rai M, Yadav A, Gade A, 2009 Silver nanoparticles as a new generation of antimicrobials *Biotechnol*. *Adv*. **27** 76–83
[10] Wang L, Hu C, Shao L 2017 The Antimicrobial Activity of Nanoparticles: Present Situation and Prospects for the Future. *International J. of Nanomedicine* **12** 1227–49