TRANSLATED FROM RUSSIAN

PROTOCOL
RESEARCH OF ANTIBACTERIAL PROPERTIES OF THE "ARGIFORM" MATERIAL

1. The research of the antibacterial properties of the material was carried out in accordance with the normative document "Guidance on experimental (pre-clinical) study of new pharmaceutical substances", Moscow, 2000. Practical policies.
A comparative experiment was conducted on research of antibacterial properties of "ARGIFORM", a water-containing biopolymer material with silver ions, as well as a research of "FORMACRYL", a polyacrylamide material of a similar chemical composition but not containing silver ions.

Samples:
"ARGIFORM": The submitted samples are in a sterile form. "ARGIFORM" is filled in disposable injection plastic syringes, which are tipped and vacuum-sealed in individual blister packaging. Each set consisting of a blister packaging with a syringe and an injection cannula which are packed into a separate branded carton box. The marking, description and the trademark are printed on the boxes.
"FORMACRYL": The submitted samples are in a sterile form. Disposable injection plastic syringes filled with the material "ARGIFORM" are tipped and vacuum-sealed in individual blister packaging.

2. Methods

2.1. Bacterial strains exploited

1. Staphylococcus aureus ATCC 25923.
2. Staphylococcus aureus ATCC 43300 MRSA.
3. Escherichia coli ATCC 25922.
4. Pseudomonas aeruginosa ATCC 27853.

2.3. Nutritional media

Plates with Mueller-Hinton agar (Acumedia Manufacturers, Inc., USA).

3. Description of research

2 cylindrical 8 mm wells were cut out in the agar and 0.5 ml of the materials under examination were injected from syringes. Additionally, 10 ml of a melted Mueller-Hinton agar was poured on the surface of the plates for gel binding in the wells. The plate was dried a little in a thermostat at a temperature of 40 °C for 20 min.
A bacterial suspension for bacterial lawn was prepared:
Eighteen-hour colonies on a Tryptichase soya agar were suspended in a normal

saline solution to an optical density of 0.5 MacFarland. Further the agar surface was inoculated with a cotton plug, which had been previously immersed in the derived suspension and then squeezed out. The agar plates were incubated at a temperature of 35 °C for 18 hours.

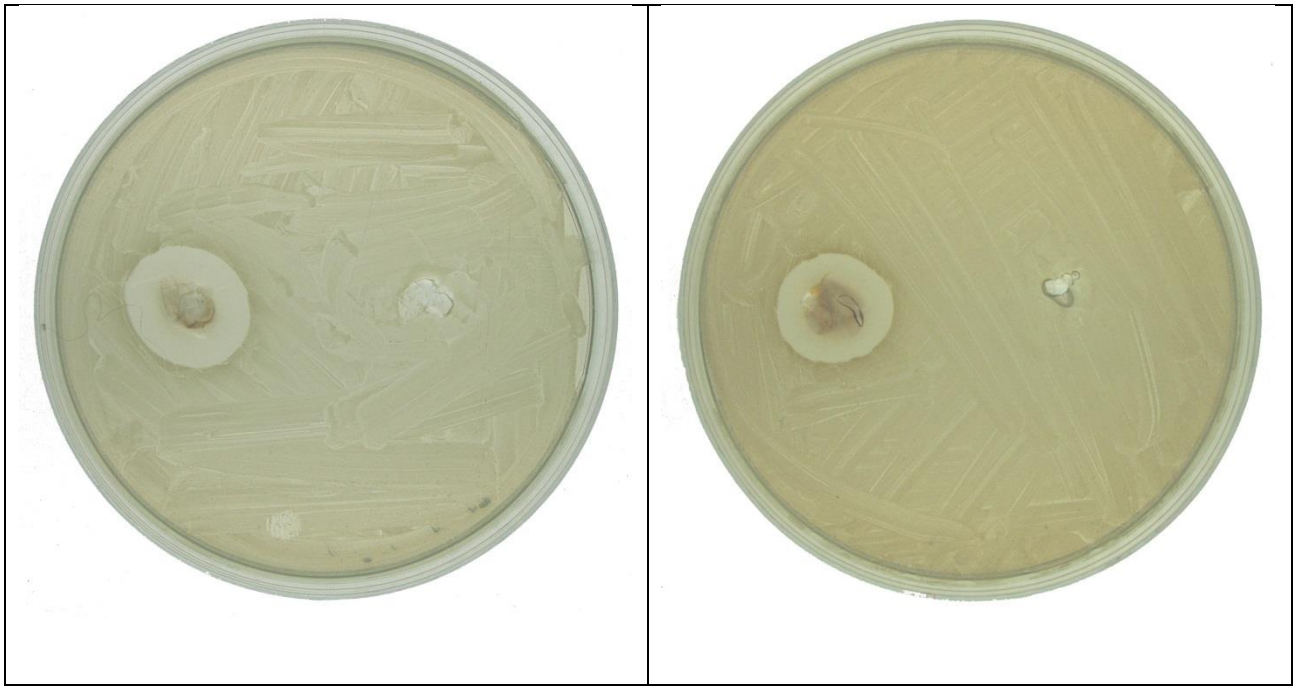
4. Results of the research

The bacterial growth in the well area with the material FORMACRYL (the right well at the photo #1) does not differ from the surrounding bacterial growth.
In the well area with the material "ARGIFORM" (the left well at the photo #1) there are areas of growth retardation in plates with each of the tested cultures.
Dimensions of areas of growth retardation around the well with the material "ARGIFORM" are shown in table #1.

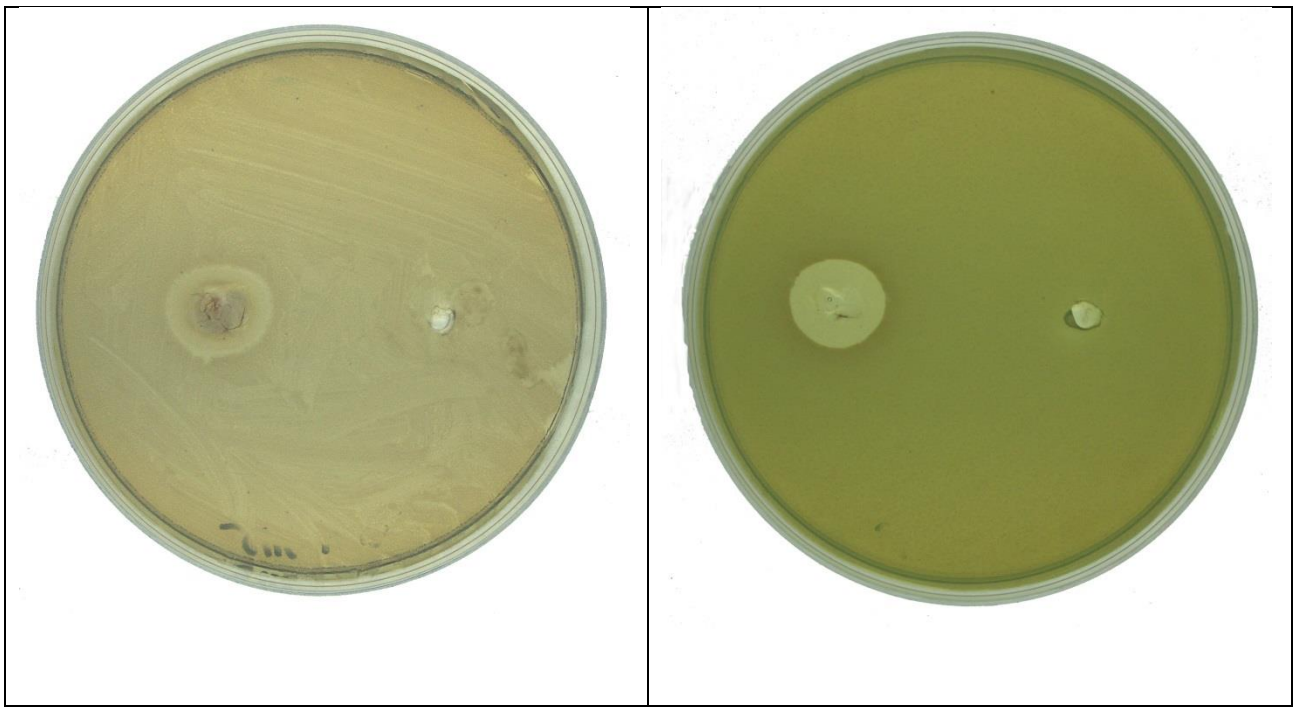
Table #1

Bacterial strains used in the test	S. aureus 25923	S. aureus 43300	E. coli 25922	P. aeruginosa 27853
Width of the area of growth inhibition, in mm	4.0	3.0	3.0	5.0

Photo #1



Staphylococcus aureus ATCC 25923 **Staphylococcus aureus ATCC 43300**



Escherichia coli ATCC 25922 **Pseudomonas aeruginosa 27853**

5. Conclusion on tests of antibacterial properties

Inoculations from the area of growth retardation disclosed no viable bacteria. These data demonstrate the antibacterial character of "ARGIFORM" material.
The results of the research demonstrate that "ARGIFORM" material, versus to "FORMACRYL" material (without silver ions), exerts an antibacterial effect on the used control bacterial strains.
"ARGIFORM" material has an bacteriostatic property.

Ions of Silver are bonded within polymer cross-linked structure to provide antibacterial protection of the implant. Silver contents reduce the possibility of colonization and proliferation of microorganisms within the hydrogel, including microorganisms already present in the patient.

Silver ions stay stabilized inside a combined molecule of polyacrylamide and N,N'- methylene-bis-acrylamide. Coming into contact with body tissue (after injection) silver ions start slowly dissolve in it and release into the surrounding tissues thus realizing their bacteriostatic effect.

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