TRANSLATED FROM RUSSIAN

RESEARCH OF CYTOTOXIC ACTION

1. The cytotoxic action of the material was researched in accordance with GOST R ISO 10993.5-99 «Medical goods. Study of biological action of medical goods. Part 5. Research of cytotoxicity: the 'in vitro' methods", on a cell line of a rat pheochromocytoma PC12 with the use of the MTT test.

In order to confirm that the bactericidal action of ARGIFORM material is subjected to the presence of silver ions in its composition only at definite time interval, comparative trials were carried out on cytotoxicity determination of 3 samples of the material.

2. Samples

2.1. ARGIFORM, a series produced water-containing biopolymer material with silver ions (hereinafter ARGIFORM), submitted 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 cannulae is put into a separate branded carton box. The marking, description and the trademark are printed on the boxes.

2.2. FORMACRYL CE 0123, a series produced polyacrylamide material without silver ions (hereafter 'PAAM'), submitted in a sterile form. Disposable injection plastic syringes filled with the material ARGIFORM are tipped and vacuum-sealed in individual blister packaging.

2.3. The material ARGIFORM 'washed of' silver ions by means of the the method described in p.3.2.3.1., which simulates 'washability of' silver ions of the human (hereinafter 'ARGIFORM washed off silver ions') is submitted in a sterile form. Disposable injection plastic syringes are filled with the material ARGIFORM are tipped and vacuum-sealed in individual blister packaging.

2.3.1. The methods of preparation of ARGIFORM material washed of silver ions

The content of silver ions in the tested material before the beginning of the experiment amounted to 38.8 mkg/g.
The washing of the silver ions from the material ARGIFORM designed for endoprosthesis replacement, was simulated by its infusion in distilled water at a temperature of (40 ± 1)°C. The correlation between the weight of the material and the volume of a model medium was calculated by the formula:

$$\frac{M \cdot K}{V},$$

where M is the maximum possible amount of the material used in clinical practice equal to 20, g;
K is the constant of aggravation which is equal to 10;
V is the human blood volume (5 l).

Intrusion of the silver ions into the extract which was changed after each determination was analyzed by means of AAS-30 spectrometer of atomic absorption, the sensitivity of the method is of 0.01 mkg/g.

Silver ions migration dynamics from the material is shown in table #1.

Table #1

Period of the exposition, days	0	1	2.5	7.5	10	17	23
Content of silver ions, mkg/g	38.8	0.17	0.15	<0.01	<0.01	<0.01	<0.01

From the present table it follows that after 7.5 days silver ions were not found, taking into the sensitivity of the method.

3. Experiment on cytotoxicity study

3 series of tests on cytotoxicity study were conducted.
1 series. Control materials tests.
2 series. Tests of samples of the polyacrylamide- based materials (PAAM and ARGIFORM) and their extracts.
3 series. Tests of gel samples . (PAAM and ARGIFORM 'washed off silver ions') and their extracts.

3.1. Experimental materials and methods

Cells lines

Rat pheochromocytoma cell line PC12 was used. The cells did not contain microplasm.

Preparation of the cells

The cells were cultured in the medium RPM I 1640 (PANECO) which contained 10% of embryonic calf serum and antibiotics to a state of a solid multi-layered confluent in 48-cup trays at a temperature of 37°C with a 5% content of CO_2.
Control materials

As a negative control brand BAER silicon material HV 3/422 was used.

As a positive control material phenol aqua solutions were used.

4. Methods of the experiment

MTT test was used for determination of the cytotoxic action of the samples.
For that a 5% solution of a MTT reagent was added to the cells and during 3 hours inoculated at a temperature of 37°C. The formed crystals (only in live cells) were dissolved together with the cells by adding a 25% natrium dodecylsulphate solution. The colored solution was photometred on the MultiScan "Plus P version 2.03" at 492 nm.

All the tests, including extractions, were carried out in sterile conditions in a FLOW laminar flow hood.
Gel extracts were got by inoculation of 1 g of ARGIFORM in 5 ml of a cultural growth medium RPMI 1640 with a pH of 7.4 at a temperature of 37°C for 24 hours.

The cytotoxicity was researched by 'per se' adding of 50 and 100 mg/cup of the material to cells in 3 parallel tests. Extracts from the material were researched by a full replacing of the growth

substance by the extracts.

5. Observation and results

Test results are shown in percentage to the control in which the cells contained only a cultural medium (control reagent).

5.1. Cytotoxicity test of control samples
The control materials cytotoxicity results are shown in table #2.

Table #2

#	Sample	Quantity	% to the control
1	Silicon	50 mg	98.5
2	Phenol water solution	0.015 ml	71.4
3	Phenol water solution	0,030 ml	52.4

5.2. Cytotoxicity test of samples of polyacrylamide-based materials (PAAM and ARGIFORM) and their extracts

The results of study of cytotoxicity of the materials and their extracts are shown in table #3.

Table #3

#	Sample	Quantity	% to the control
1	PAAM	50 mg	87.9
2	PAAM	100 mg	92.4
3	Argiform	50 mg	63.1
4	Argiform	100 mg	0
5	Extract of PAAM	100%	94.7
6	Extract of Argiform	100%	41.5

The comparative analysis of the samples of PAAM and ARGIFORM showed, that only a sample of ARGIFORM gel causes necrocytosis and only when it contains a certain quantity of silver ions. From the table it is obvious that the cytotoxic effect is observed only in a sample of ARGIFORM and only at mass action. Thus, the cytotoxicity is conditioned by the presence of silver ions in the material which ensure its antibacterial property.

5.3. Cytotoxicity tests of samples of PAAM gels, ARGIFORM ‘washed of the silver ions’ and their extracts

In the next series of experiments was compared the cytotoxicity of PAAM materials, ARGIFORM ‘washed of silver ions’ and their extracts.

As follows from the results of the experiment shown in table #4, the experimental subjects did not show any cytotoxic activity.

Table #4

#	Sample	Quantity	% to the control
1	PAAM	50 mg	104.8
2	PAAM	100 mg	109.4
3	ARGIFORM ‘washed off silver ions’	50 mg	106.3
4	ARGIFORM ‘washed off silver ions’	100 mg	98.1
5	PAAM Extract	100%	112.9
6	Extract from ARGIFORM ‘washed off silver ions’	100%	105

6. Conclusions

The ‘in vitro’ tests on the cell culture of a rat pheochromocytoma PC12 showed that the antibacterial material ARGIFORM does not exert any cytotoxic effect after freeing from silver ions. The experiment shows that the polyacrylamide material which is the base of the tested material does not possess cytotoxicity and that the suffocating influence of ARGIFORM material on cells is of a temporary character and its bactericidal properties are caused by presence of silver ions.

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