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Cellular Response after Stimulation of the Gelatin-Alginate Matrixes

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Summary: In the study the evaluation of the influence of gelatin-alginate matrixes on cells of Jurkat, A549 and Hurvec lines in *in vitro* tests was presented. Cellular cultures in the presence of the evaluated matrixes did not change their morphotic features and their count and proliferation. Gelatin-alginate matrixes in the form of sponges do not have anty cytotoxicy.

Keywords: agglutination; biocompatibility; cellular viability; cytotoxicy; gelatin-alginate matrixes; neoplastic cells; proliferation

Introduction

Polymer materials are used in medicine as dressings and hemostatic materials. The values of local application of therapeutic substance in dressings of bioresorbable polymers, such as gelatin and alginates have been known for a long time. It was observed that gelatin in preparations for parenteral application caused slower drug release. On the basis of the above described polymers the technology of producing porous gelatin-alginate matrix was worked out as a carrier of therapeutic substance which, such as the applied polymers should show biological indifference, that is, should not show cytotoxic action.[1–3] Tests of gelatin-alginate matrixes in contact with neoplastic cells will allow to determine mutual reactions between matrix and the cells. Quickly proliferating human neoplastic cells and correct cells of vessel endothelium were tested. Neoplastic cells are cells with potentially unstable genotype and phenotype and contact with foreign material could still more influence their morphological changes, proliferation, viability and as the result they could certainly cause quicker and vast damages in the organism. Comparatively, tests were performed on correct cells of blood vessel endothelium able for proliferation, the tissue exposed to a relatively high concentrations of substances transported through blood circulation after their introduction to the organism.

The purpose of the tests was evaluation of morphological changes, viability and ability to division of the chosen cellular cultures after their temporal contact with gelatin-alginate matrixes in testing in vitro.[11,12]

Materials

Four kinds of gelatin-alginate matrixes in the form of sponge prepared at Department of Drug Form Technology of Medical University of Wroclaw were used in the study. In order to obtain the form of sponge a liofilization of foam originated from foaming of mixture of sterile solution of gelatin (20%), natrium alginate (2% or 4%) and glycerol (3% or 5%) selected in an appropriate ratio was performed (Table 1). The obtained cylindrical mould was sliced in order to evaluate biological in vitro of the

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Table 1.
Content of the gelatin-alginate matrices.

Matrix	Gelatin	Natrium alginate	Glicerol
3A	8cz 20%	2cz 2%	3%
5A	8cz 20%	2cz 2%	5%
3B	8cz 20%	2cz 4%	3%
5B	8cz 20%	2cz 4%	5%

sponge. Biological material consisted of quickly proliferating human neoplastic cells of the lymphoblastic T lymphoma cells Jurkat grow in the suspension of medium, whereas epithelial lung carcinoma cells-A549 adhering to the bed in the culture, show the superficial growth on substrate and slowly proliferating human umbilical vein endothelial wells – Huvec, adhering to the bed in the culture. For Jurkat line medium RPMI-1640 (R8758) of *firm Sigma* with addition of 10% FBS (Fetal Bovine Serum) of *firm Cambrax* was used, for line A549 medium MEM (Minimum Essential Medium Eagle M2279) of *firm Sigma* with supplementation of 0.292 g/l L-glutamin and 10% FBS of *firm Cambrax* was used. For line HUVEC medium Ham's F12K was used supplemented with ECGS 0.03 mg/ml, heparin 0.1 mg/ml, 5% FBS, glutamin 2 mM. Penicillin 100 °U/ml streptomycin 100 μg/ml was used to protect against bacteria. The tests of sponges in contact with the above mentioned cellular lines were performed in The Laboratory of Cellular Cultures in The Department of Biochemistry in Medical University of Wroclaw.

Method

Conditions of Culture

Cellular cultures were performed in culture bottles with culture liquid for a given cellular line in an incubator at temp. 37 °C, at atmosphere 5% CO_2 for 24 hours addend. Cells were taken for the experiment when the culture of cells was in II logarithmic growth phase. In the purpose of detachment of cells of A549 and Huvec lines from medium the standard procedure-trypsinization with use of 0.25% Trypsin-EDTA solution (T4049) of firm Sigma.

Studies of Toxic Action with the Direct Contact Method

Culture plates with 24 round flat-bottomed pits of firm Nunclon Company (Denmark). Volume of a pit 1 ml, surface 1.9 cm^2. 1 ml of cells suspension in the correct medium was deposited in culture pits. The evaluated sponges were put on cells of Jurkat line 24 hours after the culture establishing. On cells of A549 and Huvec lines after attaching to the bed (after 2 days for A549 line and 7 days for Huvec line). Analogically prepared cultures to which sponges were not added constituted the control. The plates were put in an incubator at temp. 37 °C, at atmosphere 5% CO_2, relatively for 24 h and 48 h.

Evaluation of Toxic Action

Morphological Tests of Cells

The quantitative changes in cellular cultures were evaluated after 24 h and 48 h and viability tests were performed from MTT after previous morphological evaluation. The cells shape, adherence and detachment from the bed, agglutination, cellular lysis, division, proliferation, colony creating, ability to reproduction and cellular viability were evaluated.[4,5]

Studies of Cellular Viability

Cellular viability was determined with test of MTT. The test was performed with use of the set TOX-1, firm Sigma, in which mitochondrial dehydrogenases, of only living cells with metabolic active mitochondria, cause transformation of yellow stain MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) into non-soluble in water blue-violet formozans, which after dissolution in mixture 10% TritonX-100/0,1N HCl/izopropanol is distinguishable spectrophotometricly at $\lambda = 595$ nm. Absorbency was determined in the tested sample and control. Absorbency from the control sample was compared with absorption of control, where the same amount of cells as in the tested samples, was incubated without the evaluated materials. The percentage of living cells was calculated in relation to control. Absorption values K (respectively K_{Jurkat}, K_{A549}, K_{Huvec}) was accepted as 100%.

Results

Macroscopic Observations

In the all established cultures, after 18 hours the sponges were completely dissolved. The cultural medium was clear.

Microscopic Observations

The sponges added to the cellular cultures were sterile, which is proved by sterility of the culture. The cultures were left in the incubator respectively for 24 h or 48 h. After calculating the cells essential quantitative differences were not observed in relation to controls (quantitative differences did not exceed +, − 3% in any experiment). The cells reproduced and the cells shapes in the all tested cultural lines did not change. The cells of A549 and Huvec lines adhered to the bed and had correct morphological features. The cells of Jurkat line were single, they did not create adherent clusters. The amount of cells A549, Huwec, Jurkat in the cultures was comparable with the control. The amount of cells A549 of was in the range from 1.72×10^6 to 1.76×10^6 for of cells Huves 0.52×10^6 to 0.55×10^6 and cells Jurkat 2.20×10^6 to 2.20×10^6 Agglutination, vacuolisation, or lysis of cellular membranes were not observed. In none of the evaluated cultures, in which the influence of sponges was tested, dead cells were not observed in amounts other than for the control cultures, single dead cells. In all cultures numerous cells were observed in divisions. The degree of toxicity with use of morphological evaluation was assessed as 0. Single intracytoplasmatic granules, cellular lysis not observed. The cells were observed of line A549 to 7 days, Huwec to 14 days and Jurkat to 4 days after dissolving of sponges in culture (Figure 1–3).

Viability of Cells

Sponge elements after dissolving in cultural media did not have essential influence on cellular viability marked with test of MTT and evaluated by comparison with proper controls (Table 2). The obtained values for

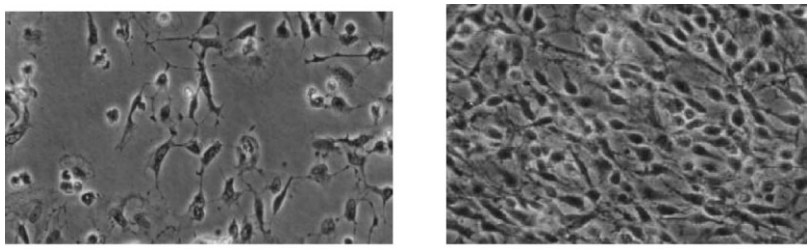

Figure 1.
Cells culture of line A549 (A) and after to put in of matrixes – 7 days (B).

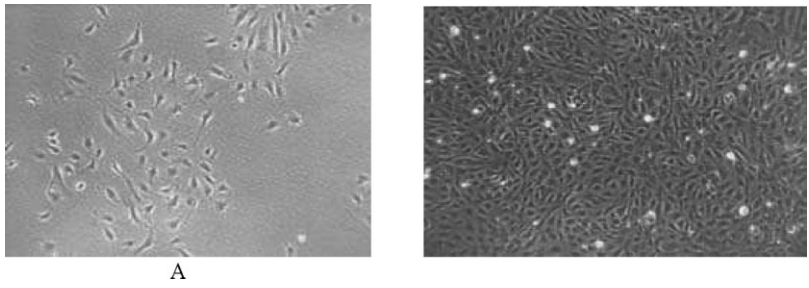

Figure 2.
Cells culture of line Huwec (A) and after put in of matrixes – 12 days.

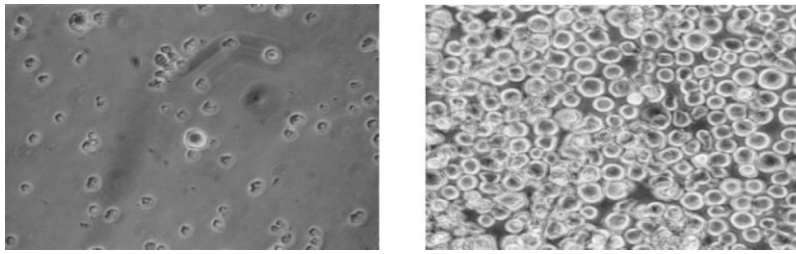

Figure 3.
Cells culture of line Jurkat (A) and after to put in of matrixes – 4 days.

all sponges were comparable. Viability of cells was over 90%. There were no essential differences in viability of cells between particular preparations of sponges. The evaluated gelatin-alginate sponges did not show cytotoxic action on the chosen cellular lines. The test on cellular viability showed lack of essential influence of the presence of gelatin-alginate matrixes on proliferation and viability of cells.

Discussion

Application in medicine of biodegradable polymers with large biological indifference such as: gelatin, alginate allows to produce an implanted carrier, which after some time will undergo resorption in the organism. Application of porous structure as well as specific properties of the used polymers will allow to obtain elongated in time release of therapeutic substance in precisely determined place. Testing of response of blood cells and cellular cultures with gelatin-alginate matrixes, is to select the matrix with optimal biological properties for implantation studies. Hemolytic action and morphotic changes in the picture of granulocytes, erythrocytes, blood platelets were not observed in tests of matrixes in contact with blood. Activation of endogenous coagulation system (shortening of time of blood recalcification and elongation of APTT, without influence on exogenous coagulation system was observed in tests of hemostatic action. Those changes are the result of contact of the material with blood. The measure values are in the range of the referential values.[6–8]

Evaluation of the influence of gelatin-alginate matrixes with the sponge structure on cellular lines was made after their temporal contact with quickly proliferating human carcinoma cells: of lymphoblasic line lymphoma T cellular – Jurkat, epithelial line of lung carcinoma - A549 and slowly proliferating correct cells of vessel endothelium of line Human Umbilical Vein Endothelial Cells - Huvec.

In the all established cellular cultures, the sponges were completely dissolved and the cultural medium was clear after 18 hours. After counting the cells, essential quantitative differences in relation with controls as well as among the evaluated kinds of sponges were not observed. The cells reproduced and had correct morphological features characterised for a given line. Agglutination, vacuolization, detach-

Table 2.
Viability of cells 24 hours and 48 hours after contact with the gelatin-alginate matrixes.

Matrix	A549 [%]		Huvec [%]		Jurkat [%]	
	24 h	48 h	24 h	48 h	24 h	48 h
3A	98	102	102	97	102	102
5A	99	103	98	98	102	103
3B	99	98	98	97	105	99
5B	97	98	97	97	100	98

ment from the bed or lysis of cellular membranes were not observed. Dead cells in numbers different than in the proper controls were not observed in any of the evaluated cultures. Cellular cultures in the presence of the evaluated matrixes did not change their morphotic features and their count and proliferation ability was on the level of control.

The elements of gelatin-alginate matrixes after dissolving in cultural media did not influence essentially the viability of cells. Cellular viability was over 90%. Essential viability in dependence on the kind of sponges were not observed. The obtained results prove that a properly prepared gelatin-alginate matrix does not show cytotoxic features. It is particularly significant in use of that material as a carrier of drug, cells. Thanks to its biological indifference, it will not affect negatively the morphotic picture of the correct and neoplastic cells after implantation into the organism, which has large diagnostic significance and creates possibilities of application of gelatin-alginate matrixes in medicine. From the evaluated matrixes with optimal biological, hemolytic, hemostatic and cytotoxic features, gelatin-alginate matrix containing 4% of natrium alginate and 3% of glycerol was characterised.

Conclusion

Gelatin-alginate matrixes in the form of sponges do not have anty cytotoxicy effects and do not cause morphotic changes of the evaluated cellular cultures. Gelatin-alginate matrixes in the form of sponges do not decrease viability and proliferation ability of the cells of the evaluated cultures.

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