Conference paper

Effect of Implantation and Construction Dental Materials on Fibroblast Cell Culture

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Abstract

An experimental study of biocompatibility of the basic prosthetic materials in the cell culture of human fibroblasts was performed. It revealed a negative effect on the morphology of the cells of chromium-cobalt alloy, the material for overdentures based on polymethyl methacrylate, the light-curing composite.

1 Introduction

The human body is increasingly subjected to psychological and environmental impacts contributing to the distortion of the reactivity of the immune and endocrine systems. Against this background, the implants and overdenture materials may cause atypical allergic and toxic-chemical reactions. In this regard, the comparative studies of biocompatibility of dental materials with the use of sophisticated methods of evaluation of the materials toxicity in cell cultures, in particular, human fibroblasts (HEF-T), are relevant. The relevance of these studies is also due to the attention to construction dental materials with the effect of shape recovery, such as titanium nickelide [1, 2, 3].

2 Experimental

Normal cells of human embryonic fibroblasts (HEF-T) were selected from the Collection of Tissue Cultures of the D.I. Ivanovsky Research Institute of Virology. The IGLA medium (produced by the M.P. Chumakov Institute of Poliomyelitis and Viral Encephalitis) was used for the cultivation.

To study the biocompatibility and effect on the growth activity of HEF-T cells of dental materials the MTT method was used. MTT is the colorimetric test which is used as a biological standard and is recommended in this capacity to assess the cytotoxic effect of different foreign substances on cells. The test is based on the direct correction of the number of viable cells and the intensity of metabolism of the MTT specific reagent to water-soluble dark-colored formazan under the influence of mitochondrial succinate dehydrogenase (dead cells and cells with reduced viability do not have this ability). Subsequent photometry of DMSO-dissolved formazan enables to compare accurately the change in optical density of the solution relative to control with the change in the number of viable cells, and to evaluate in cytotoxic studies the specific cell death induced by a certain cytotoxic agent. The pre-washed and autoclaved samples of the test materials were placed in the wells of the Costar 24 Well Plate (USA). The suspension of cells in the inoculum dose 13x105 cells/ml in the IGLA medium supplemented with 10 % fetal calf serum (SPA PanEco, Moscow) was added to each well of the plate. The plates with samples and cells were incubated in a CO2 thermostat at 37C for 48 hours. After incubation, the culture medium was removed and the MTT assay was carried out [1, 4]. After incubation of the cells, the culture medium was aspirated from the wells, 1 ml of the medium was added with 200 ml of MTT (3[4.5-dimethyl-thiazol-2-yl]-2.5-diphenyltetrazolium, Sigma) at an initial concentration of 5 mg/ml and incubated for 4 hours. Then the medium with MTT was removed and 1 ml of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals formed. The cell precipitation was resuspended for 5 min by pipetting. Cell viability was assessed by the solution color intensity, measuring the optical density at the wavelength of 545 nm at the Immunochem 2100 photometer (USA).

The grown cells, their size and volume were calculated in the form of a histogram using a manual automated cell counter - Scepter Millipore (Germany), which enables to obtain data about cell population, concentration, and distribution by size and volume. The fibroblasts were incubated for 96 hours. Then, the monolayer of cells grown on the bottom of the wells was studied visually through the Olympus SKH41 light microscope (Japan), and taken by the mixture of 0.02% Versene-Chimopsine and diluted in 1 ml of the IGLA medium for counting with the Scepter Millipore cell counter.

To study the cell morphology, the nature of their attachment to the substrate, staining with Acridine Orange was used. In the interaction with pure nucleic acids

the Acridine Orange forms complexes which are green fluorescent when bound to DNA and red fluorescent when bound to RNA. After incubation for 96 hours, the culture medium was removed, the wells were washed twice with 1 ml of PBS (phosphate-buffered saline) and 1 ml of 95% alcohol was added. After 15 minutes, the alcohol was removed, and the wells were dried. Then 1 ml of 0.01 % Acridine Orange was added for 10 min, the wells were washed twice with 1 ml of PBS. The coverslips with stained cells were removed and studied through the OptonAxioskop fluorescent microscope (Germany).

The following materials of the well-known manufacturing companies were studied: titanium Grade 4; titanium-niobium-zirconium alloy; titanium-niobium-tantalum alloy; titanium nickelide; chromium-cobalt alloy; zirconium dioxide for frames of non-removable dentures; zirconium dioxide for dental implants; ceramic press system; light-curing composite; material for overdentures based on polymethyl methacrylate; material for overdentures based on nylon.

3 Results and discussion

Based on the data obtained in the experiments on biocompatibility, the effect on growth activity of HEF-T using the MTT method, no samples (except for light-curing composite) after 24 hours of incubation provided any toxic effect on the cells, as the values did not exceed 20.0 % of the difference with the control sample parameters (Table 1). However, note a marked difference with the control characteristic not only for light-curing composite (– 28.3 %), but also for the material for overdentures based on polymethyl methacrylate (– 18.1 %).

Table 1. Determination of the Effect on Growth Activity and Viability of HEF Samples Cells Using MTT Assay

Sample	OD 545 nm	Difference with the control
Grade 4 titanium	0.727±0.087	+ 4.8 %
titanium nickelide	0.657±0.07	– 5.1 %
titanium-niobium-zirconium alloy	0.679±0.016	– 1.9 %

titanium-niobium-tantalum alloy	0.619±0.011	– 10.6 %
chromium-cobalt alloy	0.767±0.095	+ 9.8 %
zirconium dioxide for frames of non-removable dentures	0.741±0.047	+ 6.6 %
ceramic press system	0.620±0.053	– 10.4 %
zirconium dioxide for dental implants	0.749±0.09	+ 7.6 %
light-curing composite	0.496±0.098	– 28.3 %
material for overdentures based on nylon	0.650±0.027	– 6.1 %
material for overdentures based on polymethyl methacrylate	0.567±0.061	– 18.1 %
Cell monitoring	0.692±0.071	

In the study of the cells morphology after 96 hours of incubation, most of the materials did not inhibit the cell growth activity, and the fibroblasts themselves did not differ from the control. At the same time, the process of degeneration of HEF cells was revealed, which expressed in cell rounding, their shortening and detachment from the plastic on the bottom of the wells in the presence of the chromium-cobalt alloy, the material for overdentures based on polymethyl methacrylate and the light-cured composite (Tab. 2, Fig. 1). Thus, the concentration of cells in the presence of these materials, compared with the control, was 62.2 %, 72.0 %, 64.3 %, respectively.

Table 2. Determining the Mean Size and Volume of Cells by Scepter Millipore Automated Cell Counter

Sample and its position	Readings of the Scepter Millipore cell counter			
	Mean cell	Mean cell diameter	Concentration cells/ml	Sample/control cells ratio, %

	volume pL	μm		
Cell monitoring	3.38	18.63	6.78x104	
Grade 4 titanium	2.86	17.62	9.21x104	+ 135.8
titanium nickelide	2.65	17.18	7.64x104	+ 112.7
titanium-niobium-zirconium alloy	2.44	16.71	9.64x104	+ 142.2
titanium-niobium-tantalum alloy	1.45	14.06	6.96x104	+ 102.7
chromium-cobalt alloy	2.46	16.76	4.22x104	− 62.2
zirconium dioxide for frames of non-removable dentures	2.95	17.80	6.34x104	− 93.5
ceramic press system	2.18	16.09	5.99x104	− 88.4
zirconium dioxide for dental implants	2.7	17.27	6.11x104	− 90.1
light-curing composite	2.58	17.02	4.36x104	− 64.3
material for overdentures based on nylon	3.02	17.93	5.43x104	− 80.1
material for overdentures based on polymethyl methacrylate	2.8	17.52	4.88x104	− 72.0

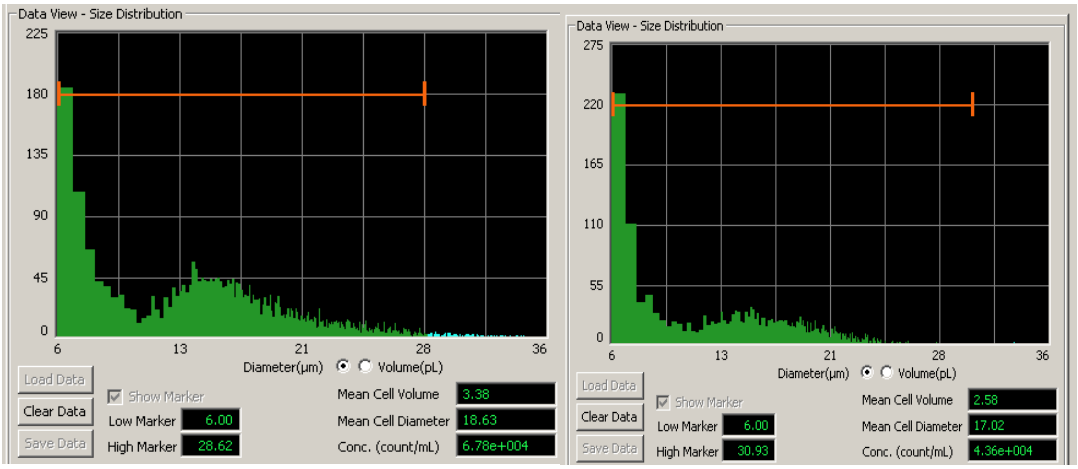
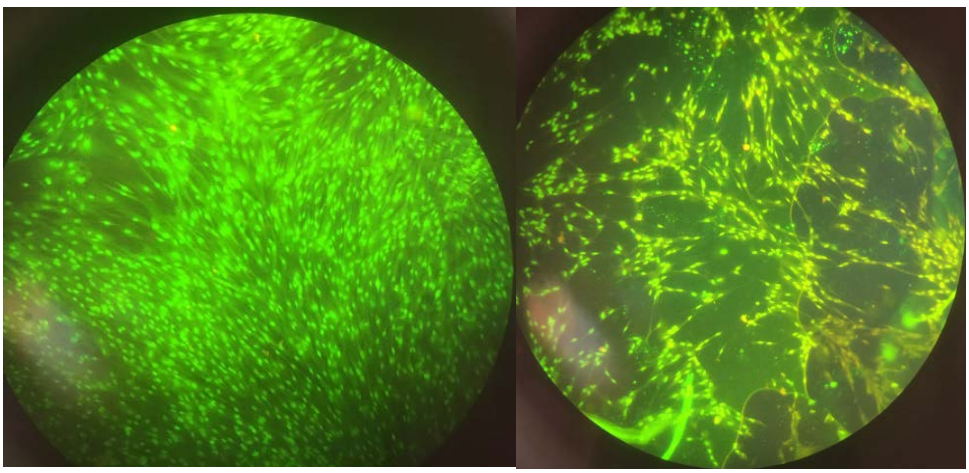


Fig. 1.Histogram of Readings of Scepter Millipore (a – control, b – in the presence of light-cured composite)

After Acridine Orange staining of the cell culture, the control cells fluoresced under UV light with yellow color, the maximum at 550 nm, the cell nucleus emitted bright fluorescence and the cytoplasm also radiated light. The incubation of the cell culture in the presence of chromium-cobalt alloy, the material for overdentures based on polymethyl methacrylate and the light-curing composite emitted fluorescent light of lower intensity (Fig. 2).



a b

Fig. 2. HEF-T Cell Culture after 96 Hours of Incubation (Acridine Orange staining: a — control, b — in the presence of light-curing composite)

4 Symmary

The possibilities of contemporary methods for the study of biocompatibility of dental materials, in particular in human fibroblast cell culture, enable to differentiate materials by the extent of their impact on cells. This experimental study revealed a certain negative impact on human fibroblasts of such materials as chromium-cobalt alloy, the material for overdentures based on polymethyl methacrylate and the light-curing composite. At the same time, titanium alloys, including titanium nickelide with a shape recovery effect, have good biocompatibility.

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