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**«Перспективные материалы с иерархической структурой
для новых технологий и надежных конструкций»**

**VIII ВСЕРОССИЙСКАЯ НАУЧНО-ПРАКТИЧЕСКАЯ
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**BONE-LIKE MULTILEVEL CALCIUM PHOSPHATE COATING
MODULATES AN INTERACTION OF MESENCHYMAL STEM CELLS
AND TUMOR CELLS**

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Leukemia initiation and progression are connected with a disfunction of health cell microenvironment and human multipotent mesenchymal stromal cells (hMMSCs), in particular. Modern biomaterial biocompatibility research focuses the biomaterial hierarchic effects on MMSC behavior (that occur at the nano-, micro- and macroscales) because MMSCs are the fundamental units that produce/regenerate bone tissue. Scaffold-like micro-arc rough calcium phosphate (CP) coatings on titanium substrates have bone-like multilevel organization and influence both MMSCs (1) and tumor cell fate (2). Continuous monitored interaction of MMSCs and tumor cells is a promising tool for oncology, cellular and molecular biology, biotechnology and environmental research. The aim was to investigate a modulation of *in vitro* interaction of human MMSCs and leukemic T lymphoblasts (Jurkat T cells) caused by micro-arc CP coating with the help of Cell-IQ and RTCA advanced tools for continuous monitoring.

The method used was described closely by (3). Commercially pure titanium plates (10×10×1 mm³) were used as substrates for the deposition of CP coatings. Bilateral CP coatings were prepared in the anodal regime using a Micro-Arc 3.0 apparatus (ISPMS, Tomsk, Russia). An aqueous solution prepared from 20 wt % phosphoric acid, 6 wt % dissolved hydroxyapatite nanopowder (particles with a diameter of 40-100 nm), and 9 wt % dissolved calcium carbonate was used to obtain CP coating. Before biological testing, the samples were dry-heat sterilized.

One of each substrates with CP coating was placed in a well vertically at one edge of sterile 12-well plastic culture flat-bottom plates and attached to the wall of the well with a clip. In this position, the samples were not shifted when the plates were moved on the sample stage of the instrument and did not damage the forming cell layer. An average velocity of cell division (AVCD) of human adipose-derived MMSCs (hAMMSCs) contacted *in vitro* with allogenic Jurkat T cells was studied by means of Cell-IQ v2 MLF integrated phase-contrast microscopic platform for real-time surveillance imaging of living cells. Both 50 µL suspensions (5×10⁴ viable cells) of the CD73CD90CD105⁺hAMMSCs and Jurkat T cells were applied into the center of the well of 12-well plastic plates for 7 days at 100% humidity in a 5% CO₂ atmosphere at 37°C until the formation of a monolayer. A nutrient medium was once replaced. To determine cell invasion (chemotactic motility) through 8 µm pores the real-time cell analysis (RTCA DP Analyzer) with the CIM-plate was used.

Statistical processing of results was carried out using the SPSS 17.0 software package. The parameters (mean (X); standard deviation (SD)) were calculated. Because of non-normal distribution, non-parametric Mann-Whitney test was used to evaluate the significant differences between the samples.

SEM showed the microrelief of macroscale CP surface with irregularities. The peaks of the CP topography consisted of spherulites of up to 10–30 µm in diameter. Single or open interconnected pores (1–10 µm in diameter) were revealed. Atomic force microscopy (AFM) showed that CP relief was embedded with 500–1000 nm submicron particles. The particles assembled in globules that were 1–2 µm in diameter and 30 nm in height. The positioning of the globules provided porous (pores of 1–2 µm diameter) microspherulites (3–5 µm diameter, 300 nm in height).

An average velocity of cell division (AVCD) of fibroblast-like adherent hAMMSCs was 0.27–0.63 divisions/h. No micro-arc CP coating or Jurkat T cells influenced this process statistically (Table

Секция 4. Научные основы разработки материалов с многоуровневой иерархической структурой, в том числе для экстремальных условий эксплуатации

1). At the same time, CP coating diminished significantly the percent of dividing hAMMSCs contacted with leukemic Jurkat T cells (Table 1, 3D-coculturing).

RTCA system showed hAMMSC invasion towards tumor cells and not vice versa. For all this, cellular interaction led to increasing viability of Jurkat T cells and decreasing the number of viable hAMMSCs.

Table 1. Behavior of hAMMSCs contacted in vitro with Jurkat T cells in 2D- or 3D-cultures for 7 days, $X \pm SD$

Group number	Cell culture	Software processing of Cell-IQ video images	
		An average velocity of cell division (AVCD), divisions/h before monolayer formation	% of dividing hAMMSCs
2D - coculturing			
1	hAMMSCs (control)	0.53 ± 0.36 n=13	85 ± 27 n=6
2	hAMMSCs + Jurkat T cells	0.41 ± 0.24 n=21	103 ± 56 n=10
3D - coculturing			
3	hAMMSCs (control)	0.45 ± 0.38 n=11	68 ± 34 n=7
4	hAMMSCs + Jurkat T cells	0.42 ± 0.21 n=21	$14 \pm 8(4)^*$ n=6 P2=0.009; P3=0.01

Thus, Cell-IQ and RTCA are useful tools for *in vitro* real-time simulation and imaging of morphofunctional interplay between healthy cells and tumor cells. Tumor Jurkat T cells could control health hAMMSCs and promote stromal microenvironment for their survivability by means of secretable molecular products. Multilevel micro-arc CP coating forms bone-like inorganic structure that is capable to modulate *in vitro* interaction of human AMMSCs and leukemic T lymphoblasts. The results obtained may be useful for replacement surgery applications of orthopedic implants in cancer patients.

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