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Biological Properties Of @ -3-Calcium Phosphate in The Aspect of Tissue Engineering.

Kharkova NV[1]*, Reshetov IV[1], Zelianin AS[1], Philippov VV, Sergeeva NS[3], Sviridova IK[4], and Komlev VS[5]

[1]I.M. Sechenov First State Medical University.
[2]Lomonosov Moscow State University.
[3]P Hertsen Moscow oncology research institute.
[4]Federal State Budgetary Institution National Medical Research Radiological Center of the Ministry of Health of the Russian Federation.
[5]Institute of Metallurgy and Material Science.

ABSTRACT

3- Calcium phosphate scaffold is a perfect structure for the bone tissue engineering as it has a chemical and crystal similarity to the bone mineral composition and it is a promising material for bone defects treatment. Some explorations in vitro with colonized cells and scaffold properties analysis for further experiment in vivo were performed during these studies. Experiments in vitro cytotoxicity acute assessment of given materials and dynamics of slew cells have been performed on cell line model of homologus pooled human antibody. The viability of HPHA in dynamic experiments was evaluated using the method To conduct the test in MTT-experiments in vitro at the end of cultivation HPHA on the conditioned plastic- HPHA-polistirene (control) and on the samples of bioceramics (experiment). It was revealed that since 3 days monitoring the HHA pool on sample materials of that laboratory group exceeded advanced control units. When analyzing the process of change of the population in conditions of cultivation of HHA on samples of calcium phosphate bioceramics TCP until 4 weeks has shown to reach maximum growth of the pool of experiment both HPHA and control occurs, usually in the first week of the experiment, however, in the experimental group the process of cell expansion flows significantly harder than in the control. The results suggest that the samples of TCP have satisfactory adhesive abilities concerning cells and not toxic to these cells. Our scaffolds are a promising implant material to be used in bone tissue engineering.

Keywords: scaffolds, tissue engineering, bone tissue, fibroblasts, in vitro, biocompatibility and cytotoxicity testing, tri-calcium phosphate, bone tissue, ceramics

Corresponding author

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INTRODUCTION

The use of bone grafts is very common in plastic surgery, orthopaedics and Oncology treatment for bone defects after trauma and after removal of malignant tumors. Bone graft is the gold standard among bone transplants, because it has a high degree of efficiency to repair bone structures. (1-4) However, the donor area has a limit on the amount of the material taken, and also has soreness in the postoperative period of the donor places and of course bone graft mortality cases take place.(1, 5, 6,17-20). Therefore, an alternate bone implant with bone properties for the reconstruction of bone defects must be created. Perfect skaffold is a 3D implant created using 3D printer, obtaining biocompatibility, biorezorbing properties, and possessing mechanical properties (1.7) Tri-calcium phosphate is a favourite in the range of other bone scaffold materials available, as a composite of this material is similar to the mineral composition of natural bone (1, 8-11). The biocompatibility and cytotoxicity testing, which you have to carry out before you introduce any material had been performed and this is described in this article (12-15)

Materials, methods and results of screening in vitro of two laboratory samples of batches of bioceramics

For this study α- 3 –TCP was used where size of pellets reached 300-600 MKM, with 54% porosity, pore size range: 1-5µkm , with specific surface: 0.51 m2 .

Before the start of the study the ceramic samples were washed thoroughly in 5-6 portions of distilled water to a volume of 130-150 ml and then sterilized them in a heatboard (150 C degrees , for 120 minutes). Screening of samples carried out in 24 '- holed cards (Costar, United States). Before the experiment in advance prepared sterile material (100 mg per hole) was placed into the tablets, afterwards the culture medium was introduced in a volume of 1 ml for a complete saturation of bioceramic materials with grown medium. Each sample board was introduced in triplets.

Experiments in vitro cytotoxicity acute assessment of given materials and dynamics of slew cells have been performed on cell line model of homologus pooled human antibody (HPHA, clone No. 1608) received from the collection of model cell cultures of medical-Genetic Science Center of RMSA (Russian Medical Science Academy), Moscow. The cell line had been treated in the complete grown (CGM) medium of the following composition: medium(Institute of poliomyelitis and viral encephalitis named after mr. Chumakov, RMSA, Moscow), 10% fetal calf serum , glutamine (600 mg/l), gentamicin (50 µg/ml). In the experiments cells in logarithmic growth phase (preconfluent monolayer) were used. To obtain the suspension single cells monolayer HPHA was farmed with 0.25% trypsin solution (Sigma, United States), then the resulting suspension cells were carefully washed by centrifugation twice more in a large volume of CGM , their calculation and an assessment of the viability was performed, staining the cell suspension of 0.04% trypan blue. Then in the boards with ceramic samples (experience) and empty boards (control) HHA were placed (180 thousand CL/cm2) in volume 1.5 ml of CGM and incubated: to identify acute cytotoxicity for 24 hours, to evaluate the matrix properties of bioceramics-4, 7, 11, 14, 18, 21 days regularly (twice a week) complete replacement of CGM. All operations with materials and HHA were carried out under sterile conditions, cultivation-in humid air containing 5% CO2 at 37 degrees C.

The viability of HPHA in dynamic experiments was evaluated using the method (T. Mossman, 1986), which is based on the ability of dehydrogenases of living cells to recover 3-(- 4.5-dimetiltiazolil-2)-2.5-difeniltetrazoly bromide (MTT,Sigma, United States) into pharmazone blue crystals , insoluble in water. As shown previously, the amount of formed blue cristals can characterize the proliferative activity (viability/quantity) of various types of human and animal cells. To conduct the test in MTT-experiments in vitro at the end of cultivation HPHA on the conditioned plastic- HPHA-polistirene (control) and on the samples of bioceramics (experiment) 1000 µl of culture medium was taken out of each hole and 125 µl of MTT solution at a concentration of 5 mg/ml was added. Via 3 hours incubation (5% CO2, 37degrees C) 250 µl of culture medium was poured out of each hole. The dissolution of formazane obtained was carried out by using isopropyl alcohol (150 µl on the hole). The sludges formed as a result product of protein precipitation in isopropanol, were released by 10 min. centrifugation of plates at 3000 rpm. Following that, 100 µl supernatant from each hole was transmitted into 96-well microtiter-shaped Tablet (OSTAR, United States) and the optical density of the solution was evaluated with the MSS spectrophotometer- 340 (Sweden) at 540 nm wave-length. As spectrophotometric testing (form) pure CGM samples and tests containing control samples in

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CGM (no cells) were used. To determine cytotoxicity of materials the pool of viable cells (PVC) , which survived after the incubation with these materials was calculated according to the formula:

PVC = {OD *(experiement): OD (contol)} x 100%
OD *-optical density of the phormazan solution.

While assessing matrix properties of bioceramic materials the changes of the pool of HHA (Δ) within a specified period was determined according to the formula:

Δ = {OD(certain time) –OD before}: OD before x 100%
OD (certain time)

-the optical density at a certain time
OD before - the optical density in the previous term

The positive calculated value of the pool showed growth of the HPHA population, negative value HPHA meant the death of a part of the population.

Aggregating of the results was conducted by Student*s method considering reliable differences at $p < 0.05$.

Evaluation Acute cytotoxicity and matrix properties of the surface of bioceramics @TCP samples of laboratory batch

This installment also contained 8 types of samples of the same composition, which is described in the previous section, but obtained by means of another method of deposition from aqueous solutions. It is shown that the samples of TCP of this lab group have no acute cytotoxicity against the culture of HHA. So, through 24 hours cultivation on this material quantity value of the optical density of the phormazan solution in the experimental group was not different from control values, and a pool of viable cells was within 70-89% of controls (table 1). The results suggest that the samples of TCP have satisfactory adhesive abilities concerning cells and not toxic to these cells (table 1).

Table 1: The optical density of the phormazan solution is (MTT-test, u/m.) and the size of the pool of viable cells (PVC %) through 24 hours cultivation β-TCP

№ sample*	Materials	Measured parameters	
		Optical density (min.)	Pool of surviving cells PVC
1	Polisteren (1*)	0.634_+0.031	100
2	Polisteren (control 2)*	0.454+- 0.001	100
3	TCP	0.348+-0.023	77.0

The the study of matrix properties of this lab party TCP samples revealed that the population of HPHA with long-term (up to 28 days) cultivation was constantly growing and statistically higher than reference values. This was reflected both in the high values of optical density of the phormazan solution in the experimental group in the dynamics of surveillance (table 2), and in excess of control indicators for the pool of viable cells (table 3).

Table 2: The value of the optical density of the phormazan solution (MTT-test, min.) in dynamics of cultivation on HPHA polisterene (control) and samples of bioceramics β-TCP (experiment).

№ Materials	Value of optical density phormazan solution . Dynamics experiment (day)							
	1	3	7	10	14	17	21	28
1. Polis-teren (contr.1)	0.634+---0.031	0.810 +- 0.004	1.072 +- 0.052	1.301 +- 0.033	1.362 +- 0.070	2.010 +- 0.000	1.993 +- 0.055	3.173 +- 0.007

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2. Polis-teren (contr.2)	0.455 +- 0.001	0.700 +- 0.016	0.878 +- 0.089	1.669 +- 0.089	2.061 +- 0.029	2.509 +- 0.077	3.186 +- 0.106	3.412 +- 0.00
3. TCP	0.348 +- 0.023*	0.740 +- 0.006	0.978 +- 0.013	1.942 +- 0.118	2.346 +- 0.024	2.973 +- 0.055	3.586 +- 0.122	3.931 +- 0.061

* statistically reliable difference with the control ($p < 0.05$)

So, if in the control groups when cultured on polisterene HPHA the value of optical density of the phormazan solution during 28 days observation rose from 0.634 to 3.173 (u/m). and with up to 0.455 3.412 min., in the experience of the sample with the TKF 0.348 to 3.931 (unit/m). That is statistically reliable received excess of optical density of the phormazan solution over reference values. The results indirectly show the numerical superiority of the pool of fibroblasts on test material compared to control (cultural plastic polistiren). Rating HHA pool changes (relative to the control in each experiment) and the speed of growth of the cellular population dynamics cultivation TCP confirmed this fact (table 3, table 4).

Table 3: The value of the pool of viable cells (% relative to the control) when cultivating fibroblasts by polisterene (control) and samples of bioceramics β-TCP (experiment).

№	Materials	The value of the pool of viable cells relative to control (% Dynamics experiment (day)							
		1	3	7	10	14	17	21	28
1	@TCP	76	106	111	116	114	118	113	115

It was revealed that since 3 days monitoring the HHA pool on sample materials of that laboratory group exceeded advanced control units.

When analyzing the process of change of the population in conditions of cultivation of HHA on samples of calcium phosphate bioceramics TCP until 4 weeks has shown to reach maximum growth of the pool of experiment both HPHA and control occurs, usually in the first week of the experiment, however, in the experimental group the process of cell expansion flows significantly harder than in the control. So,by the end of the first week of the cultivation of population growth for advanced materials samples HPHA amounted to 181% vs 69.1-93.0% in control I and II, respectively, and further when increased the density of cells on the surface, declined. In total, by the end of the experiment, a pool of HPHA in experimental groups increased by 275-375% in control-to 200-275% (table 4, Figure 1 a, b)

Table 4: Population growth rate (1%) HPHA when cultivating them on polistirene (control) and TCP sample synthesized by precipitation from solutions (experiment) in Dynamics experiment 28 vs 1 day.

№	Samples	Population growth HPHA(in %) in the dynamics of the Observation (day)			
		7vs 1	14vs7	21vs14	28vs21
1	Polistiren (1)	69.1	27.1	46.3	59.2
2	Polistiren (II)	93.0	134.7	54.6	7.1
3	TCP	181.0	139.9	52.9	9.6

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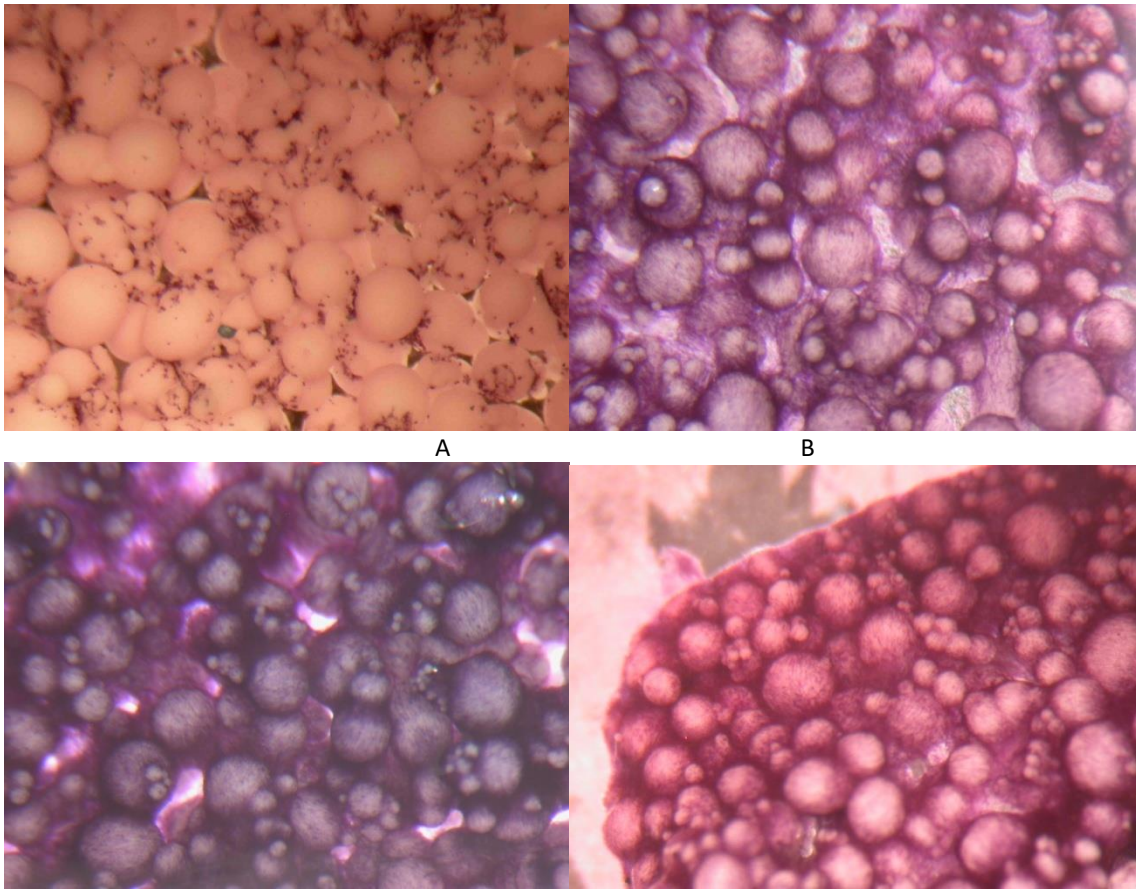

Figure1: Changes in the pool HPHA when cultured on B-phase ceramics HA/TCP (80/20)- a) 1 day, b) 6-7 days, c) 14 days, d) 21 days (MTT- test,x16)

CONCLUSION

The studied samples from the group 3-calcium phosphate ceramics in vitro are not toxic to the cells (HHA), i.e. citocompatible and have an ability of long-term support of the proliferation of HPHA in vitro, the proliferation of cells in the granules of bioceramics goes harder than that with polistirene, confirming their good adhesive properties.

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