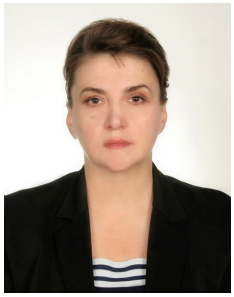
Scaffold constructs for cartilage tissue recovery



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Introduction

For an avoidance of migration and destruction of the isolated cells introduced into an organism, and also for building conditions of development vascular system to these cells various carriers, or the matrixes which are carrying out functions of a skeleton - stromas are used. At a choice of the most suitable substrate the objective assessment of a degree of their invading, and also vitality of cells is necessary for cells and their proliferative potential.
For today there are no accessible automatic systems of recognition, inventory and definition of a state of cells on carriers. At the same time the popular survey microscopy without computer analysis is a subjective and doubtful method. Earlier, in our work it was shown that polysaccarides-calcium phosphate microgranules (PS/CF) incorporated in Ti-alloys allow obtain new porous materials as implants [1]. However, depending on the conditions of operations are needed not only non-resorbable implants on the base of Ti alloys, but also resorbable porous implants.
In this work new computer method of an assessment of quality and quantity of cells on unit of a surface of matrixes is presented [2]. The computer method of an assessment of the three-dimensional morphological characteristic of cells in a matrix, and also assessments of structure of a matrix (the characteristic of the form of pores, their orientations, a degree of uniformity of allocation on all to its depth by means of program a package is used. Virtual three-dimensional models of matrixes and the cells located in them are received.
The objective of this preliminary *in vitro* biological study is to apply an objective method of an assessment of quality and quantity cells on unit of a surface of bio-matrixes and in unit of volume of a three-dimensional bio-matrix, to estimate the effect of the microstructure and surface physico-chemical properties of a porous scaffold construction obtained by dispersion of PS/CF in the collagen matrix (Col) and gelatin (GEL). The complexes are evaluated as tissue-ingineering scaffolds. The complexes can be formed into granules, films or produced as scaffolds. Their surface can be further modified by Sodium Hyaluronate. The weight ratio of PS/COL is 1:2. Sodium hyaluronate is added in 3% wt.
The stromal cells the culture of fibroblast-like cells and linear cells the gepatoma cells Gep-G2 were used in biological tests. A study was performed to evaluate the bone cell response to porous blocks scaffolds (PS/CF Col) and compare it to PS/CF microgranules. Biological studies were performed to estimate cell viability, proliferative activity.
Results indicate that both PS/CF as PS/CF Col blocks are resorbable bioactive and biocompatible material for bone tissue recovery.

Materials and methods

In experiences for cultivation of cells used granules on the basis of polysaccharides (PS) and calcium phosphates (CF). The spherical biogranules with size in the range 1000 - 2000μm, containing nanodispersed HAP incorporated in alginate (ALG) matrix (*«Biomed», Russia*) were used.
Porous matrixes were obtained by dispersion of HAP-ALG microgranules with size in the range 50 - 100μm in collagen (COL) and gelatin (GEL) solutions.

Cells culture

As source of stromal cells the culture of fibroblast-like cells isolated from green mice bone marrow (B10.GFP - the mice are obtained by crossing C57BL/10SnY and C57BL/6-TgN (ACTbEGFP) 1 mice line (Osb-Jackson Laboratory, England). The culture medium: DMEM + 10% FCS + 10mM HEPES + 100ME/1250ml Insulin + 6 ng/ml FGFb + 50 mkg/ml gentamycin sulfate. As linear cells the gepatoma cells Gep-G2 are used. The culture medium: DMEM + 10% FCS + 10mM HEPES + 50 μg/mL gentamycin sulfate.

The investigated material with added cultural medium without cells was placed preliminary in 24-well plates for 24 h. Fresh cultural medium containing the suspension of cells at $1x10^6$ cells per well was added at next day. Control cells were seeded directly into plastic wells on the same plate. The cultivation was carried out in CO_2-incubator in an atmosphere of 5% CO_2 for 3 days at 37°C.

At analysis of invading of matrixes, after end of cultivation fixed in 10% wt. neutral formalin, washed in flow flowing medium (8-12 h), them filled in gelatin (3% wt.) and produced serial cryostat sections. Preparations imbued a hematoxylin and the eosine, the received results were photodocumented.

Cultivation on matrixes was spent under the control of optical microscopy. After achievement of 75-80 % of invading on control to plastic, regarding a stuff spent definition of vitality of cells (method Mosmann and Monks, on activity respirations, without dissolution of crystals and definitions of absorbency of solution), and imbued the second part of a stuff a hematoxylin and eosine for visualization of cells.

Results and Discussion

The all matrixes investigated showed different stability for all period of cultivation. HAP-ALG granules were destroyed after 3 days of cultivation. This fact creates difficulties to estimate cell viability and proliferative activity in matrix by optical microscopy.

Nevertheless, the fact is established that the culture of fibroblast-like cells of bone marrow of green mice B10.GFP and cells of gepatoma Gep-G2 remain the ability to adhesion properties. At cultivation the cells fall into bottom of well plate where maintain their usually form. Therefore, the investigated scaffolds don't possess cytotoxicity action on the culture of cells. The cells cultured for 3 days on investigated bio-matrixes exhibited different levels of proliferation when compared to that cultured on standart TC plactic.

HAP-ALG granules which are widely used as resorbable osteoplastic material for bone remodeling were destroyed at these conditions of cultivation (Fig.1 a,b).

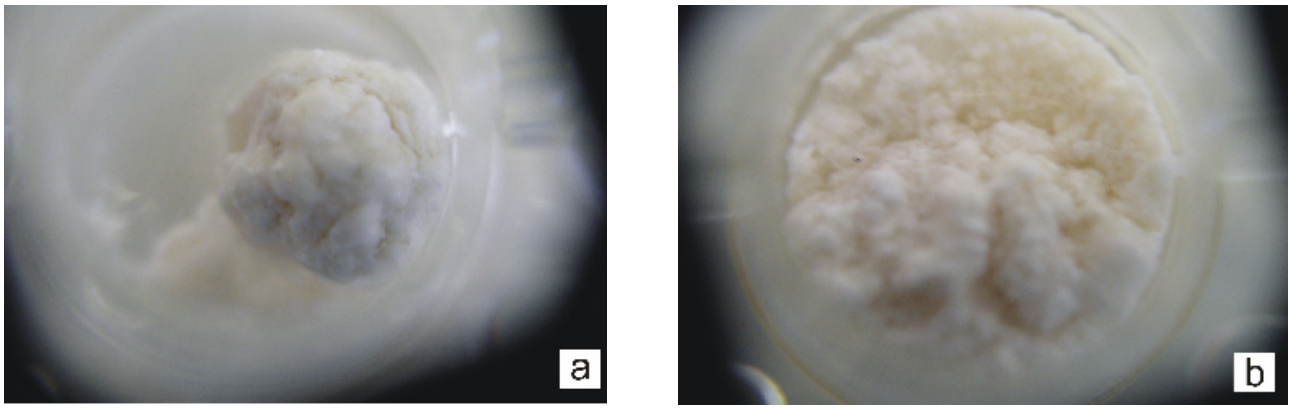

Fig. 1. Optical micrographs of HAP-ALG granules cultured for 3 days: (a) 1 day after cultivation; (b) 3 days after cultivation

Despite it, the cells observed in the precipitate of destroyed matrix on the bottom of well plate showed the absence of citotocitity and ability to proliferation (Fig. 2 a,b).

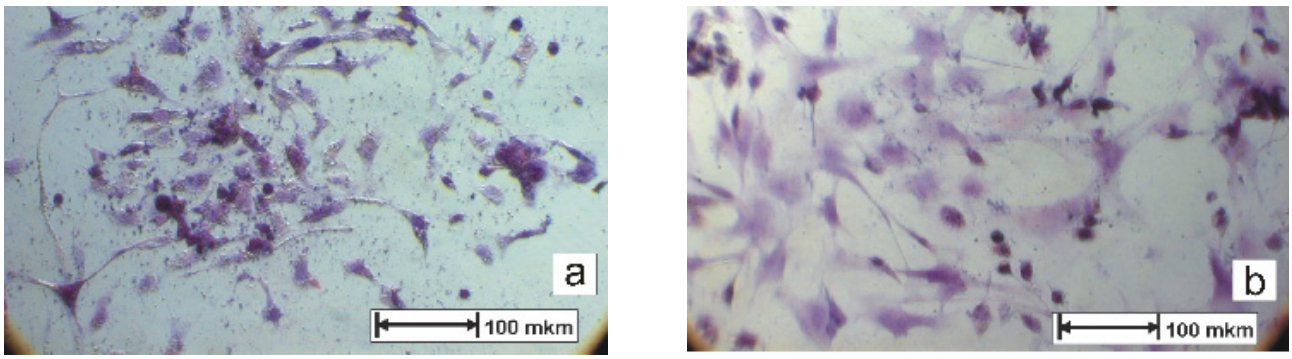

Fig. 2. Optical micrographs of cells proliferation cultured for 3 days on the surface of HAP-ALG granules:
(a) – gepatoma cells GEP-G2; (b) fibroblast-like cells from bone marrow B10.GFP

Porous matrixes were obtained in system HAP-ALG-Gelatin and HAP-ALG-Collagen (Fig.3 a,b). Both materials have open porous structure. The diameter of porous is equal 100-300 μm. In Fig.3 (b) clearly observed cross-linked bands. It may be proposed that due to this fact the matrix obtained in presence of collagen is the most stable matrix at cultivation. In opposite, gelatin containing matrix is the very gentle material swelling at cultivation period.

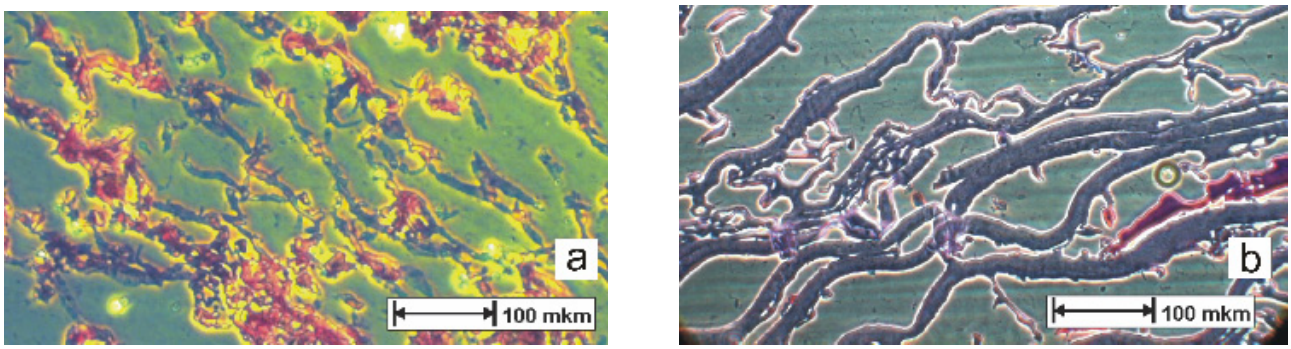

Fig. 3. Optical micrographs of porous matrixes: (a) – HAP-ALG-GEL; (b) HAP-ALG-COL

Unfortunately, in matrixes obtained pH value decreased slowly from 7.2 to 6.5 during all period of observation and this fact need next work to improve the properties of matrixes. Also negatively act on cells viability the additional cross-linking of matrixes by CaCl2.
In Fig.4 (a,b) the proliferation of cells on the bottom of well plate in HAP-ALG-COL without (a) and with (b) CaCl2 cross-linking (a) is presented. The morphology of cells is similar to control cells cultivated on plastic.

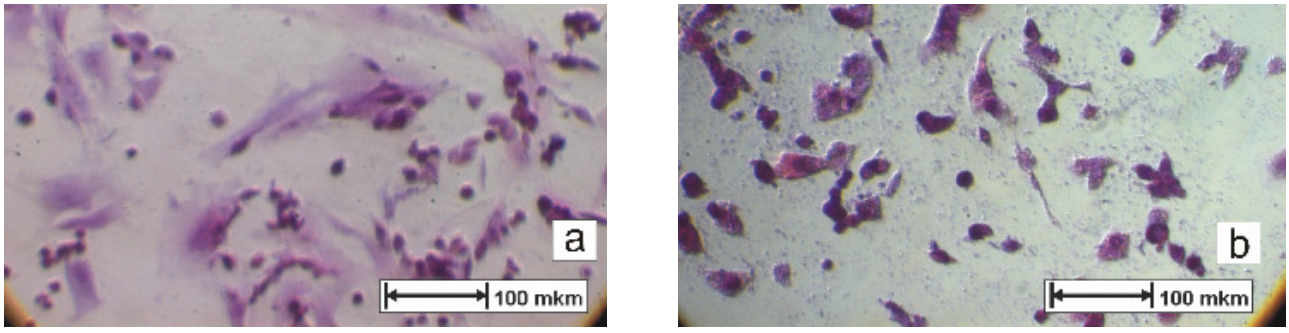

Fig. 4. Optical micrographs: (a) proliferation of fibroblast-like cells from bone marrow B10.GFP cultured for 3 days in the bottom of plate (HAP-ALG-COL matrix without CaCl2 cross-linking); (b) gepatoma cells GEP-G2 cultured for 3 days in the bottom of plate (HAP-ALG-COL matrix using CaCl2 cross-linking)

Biological tests shown that PS-CF-COL and PS-CF -GEL scaffolds being the smoothest material with microporous structure (100-300 μm) and in opposite PS/CF blocks are stable in culture medium during all period of cultivation.
It is important fact, the modification of HAP-ALG –Collagen by Sodium hyaluronate allows to increase considerably the proliferation activity of cells. In Fig.5 are presented results: (a) the structure of HAP-ALG-Collagen complex modified by Sodium hyaluronate in presence of cells attached to matrix and (b) proliferation activity of cells on the bottom of well plate.

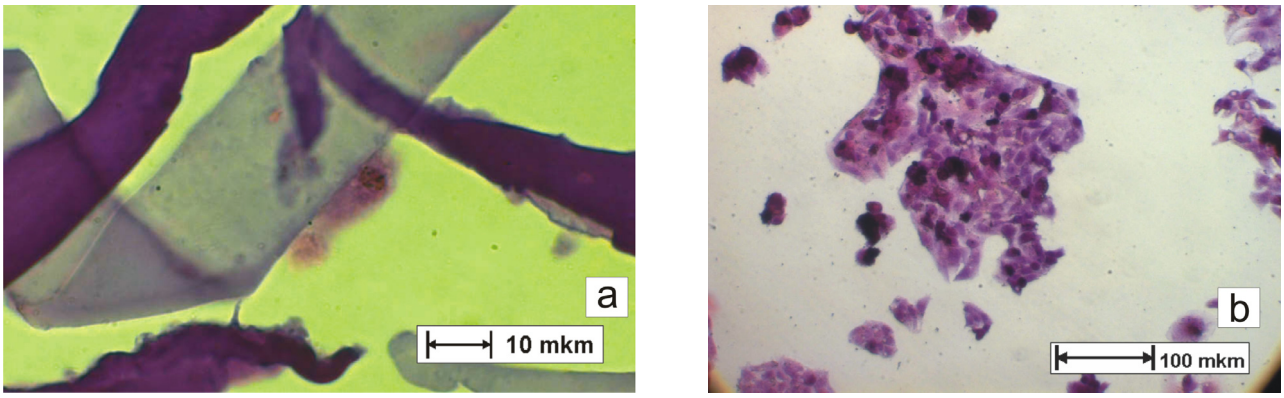

Fig. 5. Optical micrographs of cells proliferation cultured for 3 days on the surface of HAP-ALG-COL matrix modified by Sodium hyaluronate :
(a) – gepatoma cells GEP-G2 on the surface of matrix; (b) proliferation activity of gepatoma cells GEP-G2 on the bottom of well plate

The results from cytocompatibility tests demonstrate that Sodium hyaluronate enhances cell attachment and proliferation on HAP-ALG-COL matrixes. The in vitro evaluation of the cultivated matrixes indicates that the complex HAP-ALG-Collagen complex modified by Sodium hyaluronate is the most appropriate for 3-D culture, manifested by better cell growth.

Conclusions

- Use of the offered procedures allows to estimate a state cells on matrixes therefore optimization of selection matrixes for invading on them of cells becomes possible.
- A cultivation of cells with the materials being tested showed proliferation activity and the absence of the toxic effect.
- In further will be continue the work on computer visualization cells in bio-matrixes.

References

1. A.E. Sytschev, et al. (2005) *Porous biomaterials based on titanium nickelide and hydroxyapatite*, II France-Russia Seminar “New Achievements in Materials Science”, 10-12 November, Moscow, Russia, pp. 32-33.

2. D.N. Livak, et al. (2007) “*The computer methods of qualitative and quantitative analysis of viable adhesion cells in surface unit of thin - films and unit of volume of 3-D matrices*”, Russian and International scientific conference "Stem cells and the perspectives of their use in health protection", Moscow, 30-31 May, p.111.