NANOTECHNOLOGIES

Micro- and Nanostructural Characteristics of 3D Porous Carriers *Elasto*PHB®-3D

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Microstructure of the surface and micro- and nanostructure of the internal surface of 3D porous carrier *Elasto*PHB®-3D were studied by methods of electron microscopy and atomic force microscopy. Biological properties of *Elasto*PHB®-3D samples were evaluated using culture of L929 mouse fibroblasts. High porosity and pore size of biodegradable matrixes *Elasto*PHB®-3D and their good biofunctional properties as the substrate for cell culturing allow us to recommend *Elasto*PHB®-3D as a promising carrier for cell transplantation and creation of artificial organs.

Key Words: *scanning electron microscopy; atomic force microscopy; nanostructure; 3D-carriers*

Recent works in the field of transplantology and artificial organs proposed a principally novel approach to the restoration of the function of vital organs — cell and tissue engineering. The results of experimental and clinical studies suggest that cells transplanted without implanted carrier rapidly die because of the absence of proper conditions for their proliferation and differentiation [1,8-10].

Spatial organization of cells in organs and tissues is a critical parameter determining normal functioning of tissues and organs [11]. Therefore, an important role in the creation of hybrid systems is allocated to spatial distribution of transplanted cells and perspective of growing of the new tissue in a volume of 3D matrix. Porous carriers in the form of sponges and gels are very promising for clinical practice: for reconstitution of the cartilage and nervous tissues, for stimulation of integration and differentiation of retinal precursor cells, *etc*. [1,9].

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A new trend in biotechnology is construction of 3D porous matrixes from natural biodegradable polymers characterized by high biological safety and capable of regulating the time of implant bioresorption [1].

A technology for preparing a 3D porous matrix *Elasto*PHB®-3D on the basis of poly(hydroxybutyrate-co-hydroxyvalerate) co-polymer Bioplastotan®, a component of film carrier *Elasto*PHB®, was developed in Biomaterial Study Center, Institute of Transplantology and Artificial Organs [3,4]. The possibility of using *Elasto*PHB®-3D matrixes for cell culturing with their subsequent transplantation was demonstrated [2]. Apart from controllable biodegradation rate, important properties of implantable carriers are parameters of their surface and volume porous structure. At the same time, there are no data on the morphology of the internal pore surface of *Elasto*PHB®-3D.

Here we studied porous structure of *Elasto*PHB®-3D by methods of scanning electron microscopy (SEM) and atomic force microscopy (AFM).

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MATERIALS AND METHODS

Experiments were carried out on 3D carriers (sponges) *Elasto*PHB®-3D fabricated from polymer composition on the basis of Bioplastotan® co-polymer synthesized at Institute of Biophysics, Siberian Division of Russian Academy of Sciences [2]. *Elasto*PHB®-3D samples had cylindrical shape (10±2 mm in diameter, 1.2±0.5 thickness) and calculated porosity 95±2% (Fig. 1, *a*).

Surface microstructure of 3D carriers was studied by the method of SEM on a JSM 6360LA electron microscope (Jeol) at accelerating voltage of 5-10 kV and magnification ×50-300. Conducting coating necessary for electron microscopy was made by the method of ionic platinum dusting over 40 sec at constant voltage of 30 mA on a JFC-1600 Auto Fine Coater setup (Jeol).

Internal micro- and nanostructure of *Elasto*PHB®-3D samples was analyzed by high-resolution AFM (~10 nm) on a NTegra Tomo probe microscope (NT-MDT) coupled to a Leica UC6NT ultramicrotome. This integration makes it possible to reconstruct 3D image of the internal structure of the sample from AFM images of layer-by-layer ultrathin sections (<100 nm) [5].

For preparing ultrathin sections, *Elasto*PHB®-3D samples were embedded in epoxy resin. The images were registered using NSG10S non-contact silicon probe transmitters (NT-MDT) with a resonance frequency of 200-300 kHz and rigidity of about 10 N/m, typical tip curvature radius <10 nm. Measurements were performed by a semicontact method with initial probe oscillation amplitude of 10-20 nm. Working probe oscillation amplitude was chosen experimentally. Scanning was performed with horizontal frequency of 1.5 Hz and 256×256 points resolution.

Biological properties of *Elasto*PHB®-3D samples were evaluated using culture of L929 mouse fibroblasts. Sterile samples of the matrix were placed into 24-well polystyrene plates (Corning-Costar). The concentration of cells in the suspension for seeding was 13,000 cells/ml. The cells were cultured at 37°C in CO_2-incubator in a humid atmosphere containing 5±1% CO_2.

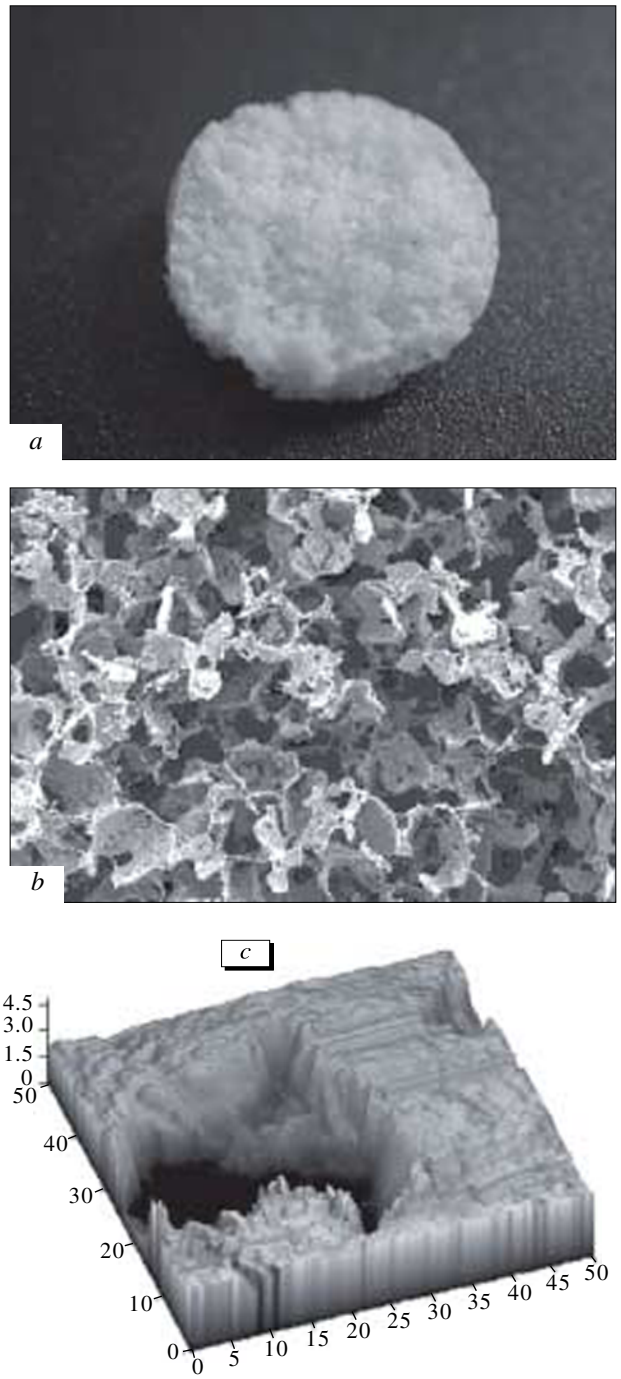

Fig. 1. 3D Porous carrier *Elasto*PHB®-3D. *a*) general appearance; *b*) cross-section microphotographs, ×50; *c*) AFM image of internal porous structure (50×50×3 μ).

RESULTS

The surface of *Elasto*PHB®-3D is characterized by a system of large pores (100-200 μ, Fig. 1, *b*), which several times surpasses the size of cells in organs and tissues (not more than 20-30 μ). Large pores intercommunicate via a system of small pores with a diameter of 3-50 μ. At present, matrixes with porosity of ≥80% and pore size of 100-500 μ are fabricated [7,9].

The mean roughness of the internal pore surface of *Elasto*PHB®-3D is ~150 (Fig. 1, *c*).

Biofunctionality of *Elasto*PHB®-3D sponges for cell culturing was proved on L929 mouse fibroblasts. On day 3 of culturing, solitary adherent cells were observed on matrix surface (Fig. 2). They

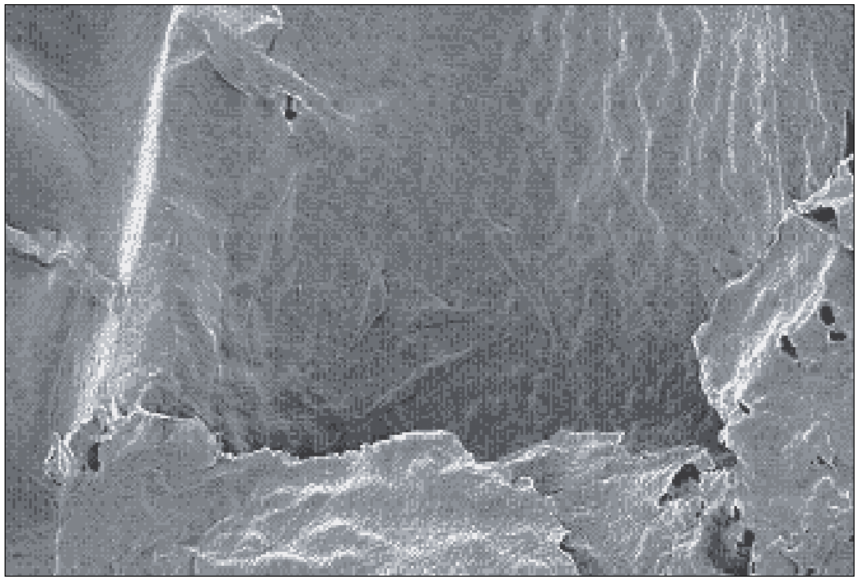

Fig. 2. *Elasto*PHB®-3D surface after 3-day culturing of L929 mouse fibroblasts, ×400.

primarily had spindle shape, which indicated their preserved viability.

High porosity and pore size of biodegradable matrixes *Elasto*PHB®-3D and their good biofunctional properties as the substrate for cell culturing allow to recommend *Elasto*PHB®-3D as a promising carrier for cell transplantation and creation of artificial organs.

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