Biomatrix on the Basis of Polypropylene Mesh and Fetal Fibroblasts

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A technology for obtaining a biomatrix on the basis of polypropylene mesh, used in clinical surgery, and cultured fetal fibroblasts is proposed. The method was developed on experimental animal cells. It includes culturing of rat fetal fibroblasts on fibers of a polypropylene mesh, treated with wheat germ lectin. This technology can be used for obtaining a biomatrix with fibroblasts of different origin. This biomatrix can be used in experimental and clinical surgery for dressing and stimulation of effective healing of mechanical injuries to the connective tissue.

Key Words: *matrix; fibroblast; polypropylene mesh; lectin*

Special wound-healing dressing is used for the treatment of burns, trophic ulcers, abrasions, and other stubborn unhealing wounds; polypropylene meshes are used in hernial surgery. The former are used to dress the wound surface, the latter close the hernial opening. They serve as matrices for *de novo* regenerating connective tissue. However, this treatment is often associated with complications: implanted mesh is rejected in 6% cases, seromas develop in 15-20% cases.

It was found that human auto- and allogenic fibroblasts transplanted into a connective tissue wound or burn wound retained their viability at the site of transplantation and promoted wound healing without formation of coarse cicatrices, stimulating the regenerative processes and accelerating epithelialization. Collagen gels are used as the supporting matrix or substratum for fibroblasts [2,4]. However, the resultant biomatrices can be used only in surface wounds. Biomatrices on the basis of solid synthetic substrates easily installed at the site of the defect and adhering to the connective tissue, which are used in clinical practice, can be used in deep injuries to the connective tissue, in hernias of the anterior abdominal wall.

We developed a technology for obtaining a biomatrix on the basis of polypropylene mesh and cultured experimental animal fetal fibroblasts.

MATERIALS AND METHODS

Lintex polypropylene mesh with 75×75 μ cells was used in the study.

Lectins (agglutinins) from pea seeds (*Pisum sativum* agglutinin; PSA), wheat germ (WGA), peanut seeds (*Arachis hypogaea* agglutinin; AHA), potato tubers (*Solanum tuberosum* agglutinin; STA), black elder bark (*Sambicus nigra* agglutinin; SHA), and edible snail (*Helix pomatia* agglutinin; HPA), manufactured by Diagnosticum Company, were used in the study.

Primary fetal cells and subcultures of rat and mouse fetal fibroblasts were obtained by the standard method [1]. The cells were cultured in Eagle's MEM with 2 mM L-glutamine, 10% FCS, and 0.05% lactalbumin hydrolysate.

Lectin receptors were detected by the rosette formation test on mouse fetal fibroblasts grown on

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slides on days 2 and 4 of subculturing. The test was carried out with rabbit erythrocyte suspension (0.5%) in phosphate-buffered saline (PBS), pH 7.2. We previously found that mouse fibroblasts did not form rosettes with rabbit erythrocytes, in other words, had no surface receptors for them. Rabbit erythrocytes are routinely used for detecting lectins in plant extracts by the hemagglutination test [3]. Hemagglutination test of the studied lectins with rabbit erythrocytes showed high agglutination titers (1:1024 to 1:254144). In order to detect lectin receptors, fibroblast monolayer was successively treated with 0.1% lectin solution and 0.5% rabbit erythrocyte suspension in PBS, after which the preparations were made, stained after Romanowskii—Giemsa, and rosette-forming cells were counted.

Rabbit erythrocytes were also used as markers of lectin binding to the mesh fibers. Similar fragments of the mesh were plunged in 0.05 or 0.1% lectin solution at ambient temperature and incubated for 0.5 to 24 h at ambient temperature, then into 3% rabbit erythrocyte suspension in saline and incubated for 1.5 h at 37°C. After incubation with erythrocytes, the meshes were washed from free cells in saline. Erythrocytes bound to lectin-coated mesh fibers were lyzed in water and the optical density of the hydrolysate was measured at λ=405 nm.

In order to prepare the biomatrix (rat fetal fibroblast culture on the mesh), fragments of the mesh were placed on the bottom of penicillin flasks or Petri dishes and plunged in suspension of fresh or subcultured rat fetal cells at a concentration of 2×10^6/ml. The meshes were at first plunged in a minimum volume of cell suspension (1 or 4 ml in penicillin flasks or Petri dishes, respectively) providing their coverage with cells and best conditions for cell precipitation on mesh fibers and adhesion to them, because minimum volume of cell suspension prevents floating of light mesh to the surface. After 24-h incubation at 37°C, the culture medium was replaced with a fresh portion (3 or 10 ml, respectively) and culturing was continued for 7-9 days until the formation of a fibroblast monolayer on the mesh fibers and bridges from these cells between the fibers. In one series of experiments, the mesh fragments were made heavier with injection needles, and culturing was carried out in the complete volume of the medium from the beginning. Culture medium was replaced every 3 days. The resultant adhesion and growth of fibroblasts on polypropylene mesh fibers was monitored microscopically. The mesh for microscopy was dried after the end of culturing and stained after Romanowskii—Giemsa. The mesh washed from free stain was treated in two portions of 96° ethanol and in one portion of xylene for degreasing and dehydration, after which it was placed on a slide with a droplet of cedar balm and covered with a coverslip. Elongated and spheroid stained fibroblasts on mesh fibers and bridges formed by these cells between the fibers were seen under a microscope.

RESULTS

Lectin binding to mouse fibroblasts. Our task implied the search for substances increasing fibroblast adhesion to synthetic inert substrates. Human and animal fibroblasts, like many other cells and cell structures, have receptors to various lectins on their surface [3]. We detected these receptors on mouse fetal fibroblasts by the rosette formation test. The number of rosette-forming cells indicated that receptors for wheat germ lectin (WGA) were present on the surface of all cultured mouse fetal fibroblasts, except vacuolated degrading cells. Many cells had PSA receptors (86.3±3.4 and 52±4% on days 2 and 4 of culturing, respectively; Fig. 1) and a lesser number of cells had receptors to SNA (18±10 and 10.5±6.4%), AHA (13.5±5.0 and 11%), and HPA (10 and 16±6%). Receptors for AHA were detected only on spheroid cells (cells in mitosis) poorly fixed to the substrate, which was in line with published data on masking and impossibility of

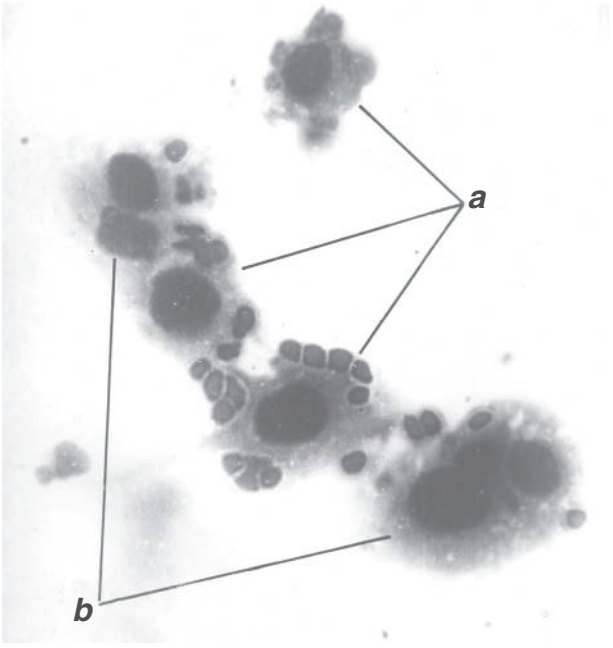

Fig. 1. Mouse fetal fibroblasts after treatment with PSA and rabbit erythrocytes (×900). *a*) fibroblasts with lectin receptors (rosette-forming cells); *b*) fibroblasts without PSA receptors. Here and in Figs. 2-4: Romanowskii—Giemsa staining.

Fig. 2. Fiber of a WGA-treated polypropylene mesh with agglutinated rat fibroblasts after 5-day culturing (×200).

Fig. 3. Fibers of WGA-treated polypropylene mesh with a fibroblast monolayer and a bridge of cells between the fibers after 5-day culturing (×200).

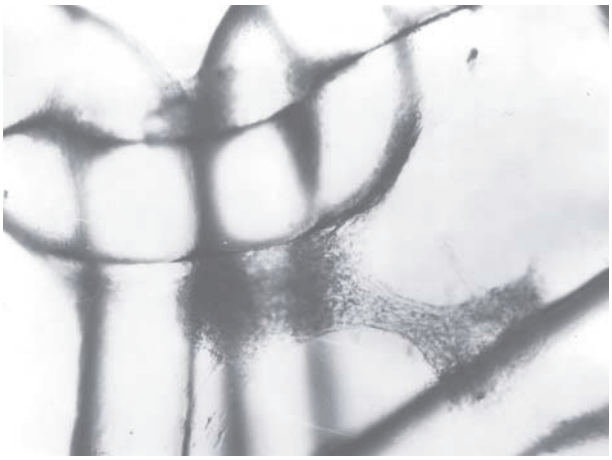

Fig. 4. Multilayer bridge from fibroblasts between WGA-treated polypropylene mesh fibers on day 7 of culturing (×100).

detecting these receptors on flat cells [3]. No STA receptors were detected on fibroblasts. Hence, WGA was chosen for the treatment of polypropylene mesh.

Binding of WGA to mesh fibers. In order to select optimal concentration of WGA solution for the treatment of mesh fibers, we tested 0.05 and 0.1% solutions. Lectin concentrations below 0.05% were not tested, because it was shown that they led to a lesser adsorption of fibroblasts on nylon fibers [5].

Optical density of hydrolysate of erythrocytes fixed on the mesh after its treatment with WGA solution was maximum and virtually the same after the mesh incubation with 0.1% WGA solution over 1 h (d_{405}=0.459) and with 0.05% WGA solution over 3 h (d_{405}=0.461). Increasing the time of incubation with 0.1% lectin solution to 3, 21, and 24 h led to reduction of the optical density of hydrolysate to d_{405}=0.395-0.256. Increasing the time of incubation with 0.05% lectin solution to 21 and 24 h reduced the optical density of hydrolysate to d_{405}= 0.302-0.210. After 30-min incubation, the optical density of the hydrolysate was 0.15-0.20 at both lectin concentrations. Hence, the duration of the mesh incubation with WGA is essential for its binding to the mesh fibers. The maximum and virtually equal amounts of this lectin bind to the mesh during incubation with its 0.1% solution for 1 h and with 0.05% solution for 3 h, and hence, we selected the second variant of the mesh treatment with WGA for our method of biomatrix obtaining as a more economic and providing the same result.

Cell culturing on intact and WGA-coated mesh fibers. Few fibroblasts bound to the mesh fibers after culturing (3-9 days) of these cells with intact mesh. By contrast, on WGA-coated mesh, the fibroblasts formed a monolayer and cell bridges between the fibers; in other words, they bind well to fibers and effectively proliferate on them, presumably, due to mitogenic characteristics of WGA.

A dynamic study showed some spheroid and flat fibroblasts on WGA-coated mesh on day 3 of culturing, but no formation of large cells conglomerations was noted. On days 5-7 of culturing, numerous fibroblasts were seen on the surface of almost all fibers (Fig. 2), and cell bridges appeared (Figs. 3, 4). On day 9, multilayer cell bridges between the fibers formed in many places.

Hence, due to the proposed technology we obtained a biomatrix based on polypropylene mesh and cultured fibroblasts due to their adhesion to WGA-coated fibers of polypropylene mesh and cell multiplication during culturing, with not only formation of a monolayer on the fibers, but also of multilayer bridges between the fibers. This technology can be used for obtaining biomatrices from fibroblasts of different origin.

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