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FIBROBLAST PROLIFERATION AND ADHESION *IN VITRO* ON POLYLACTIDE FILMS

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Abstract: Fibroblasts are a versatile biological model for the *in vitro* study of dynamic molecular regulatory processes underlying cell growth and proliferation, metabolism and transduction of intracellular signals. **The aim of the research** was to evaluate the proliferative activity and adhesive capacity of cultured *in vitro* fibroblasts on polylactide films of different thicknesses depending on the presence of the adhesive factor.

Materials and methods. Laboratory samples of polylactide films were made of L-PLA 4043D NatureWorks (USA) by extrusion and films with a thickness of 100. 125 and 150 μn with a smooth surface were obtained. The subject of the study was cultured fibroblasts (2nd passage) isolated from loose fibrous connective tissue of the peritoneum of a practically healthy person with an appendectomy with pre-signed informed consent. Small pieces of fabric under sterile conditions were ground into pieces from 1 mm to 2 mm and 2-3 pieces each were placed in petri dishes. Cell culture was carried out using DMEM/F-12 (1:1) (1X), FBS 20%, anti-anti (1x100), sodium pyruvate (1x100). Incubated at 37 C in an atmosphere enriched with 5% CO2 for 5 days.

Results. Thus, the addition of adhesive factor AF comprising gelatin to the nutrient medium activates the proliferation of cultured cells almost 1.98 times (p = 0.000) than without it; the largest number of attached fibroblasts is observed on the 3rd day with substrates of 100 μn and 150 μn in wells with AF, while in substrates with a thickness of 125 μn - on the 1st day and the 3rd day of cultivation. Based on supravitally stained samples, it was found that the adhesion of fibroblasts, depending on the thickness of the polylactide film, showed statistically significant differences (p = 0.022) between films with a thickness of 125 microns (37.67 ± 7.63) and 100 microns (20.75 ± 8.51), as well as an upward trend (p = 0.068) between 125 μN (37.67 ± 7.63) and 150 μN (25.67 ± 10.67). Only at a polylactide thickness of 125 microns is the number of fibroblasts on the surface of the film higher than in the well, and at other thicknesses, the number of cells in the well is greater than on the polylactide.

Keywords: proliferation, adhesion, polylactide, *in vitro*, fibroblasts.

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Introduction. Fibroblasts represent a universal biological model for studying in vitro dynamic molecular regulatory processes underlying cell growth and proliferation, metabolism and transduction of intracellular signals [4].

Most tissue engineering substitutes for living skin are created by culturing skin cells under laboratory conditions and combining them with a substrate. Application of skin equivalents accelerates wound healing, reduces pain syndrome, inflammation, as well as prevents scarring, contracture or pigment defects [13].

Polylactic acid, its copolymers and composites are part of the modern class of biodegradable materials and are widely used for the manufacture of various implants [1]. Polymer matrices made of polylactic acid are biocompatible products possessing bioactive properties in relation to regenerative processes and reactions of blood system during subcutaneous implantation in laboratory rats [3]. Biodegradable polymers such as polylactide and poly (e-caprolactone) are widely used in biomedicine as polymer scaffolds to promote tissue and cell growth during bone regeneration, as well as for drug delivery when drugs are mixed with a polymer. matrix and are gradually released as the biopolymer decomposes in the human body [10, 11]. However, due to its hydrophobicity, the interaction of cells with this material is far from optimal [9]. Biofunctionalization of the surface of many biodegradable polymers is one of the strategies used to improve the biological activity of such materials [6]. The high molecular weight polylactide is a colorless, glossy, rigid thermoplastic polymer which can be semi-crystalline and completely amorphous depending on the purity of the polymer backbone. Both lactic acid and polylactide exhibit optical activity, that is, they exist as two L- and D- stereoisomers. Lactic acid is very hygroscopic, so lactides are used instead [2].

The development of scaffolds for use in tissue engineering requires careful selection of properties such as mechanical characteristics, porosity and biodegradation. Scaffold surface properties are an important criterion because they affect cell adhesion, proliferation, and differentiation. It is known that the topography of the surface affects the cellular response, but the mechanisms governing this remain unclear [7].

The aim of the research was to assess the proliferative activity and adhesive capacity of cultured *in vitro* fibroblasts on polylactide films of different thicknesses depending on the presence of adhesive factor.

Materials and methods. Laboratory samples of polylactide films were made of L-PLA 4043D NatureWorks (USA) based on the UNTL "Polymer Nanocomposite Technologies" NEFU named after M.K. Ammosov by extrusion at the

following parameters: melt temperature - 175-180 °C; supply area - 170 °C; sealing zone (plasticisation zone) - 174 °C; injection zone (dosing zone) - 175 °C; die (extrusion head) - 180 °C. As a result, films with a thickness of 100, 125 and 150 nm with a smooth surface were obtained. These films can be easily cut with scissors, which corresponds to the requirement for the matrices.

An in vitro experimental study was carried out in the research laboratory "Cellular Technologies and Regenerative Medicine" of the Medical Institute of NEFU named after M.K. Ammosov. The subject of the study was cultured fibroblasts (2nd passage) isolated from loose fibrous connective tissue of the peritoneum of a practically healthy person with an appendectomy with pre-signed informed consent. Small pieces of fabric under sterile conditions were ground into pieces from 1 mm to 2 mm and 2-3 pieces each were placed in petri dishes. Cell culture was carried out using DMEM/F-12 (1:1) (1X), FBS 20%, anti-anti (1x100), sodium pyruvate (1x100). Incubated at 37 C in an atmosphere enriched with 5% CO2 for 5 days.

After achieving monolayer growth in polystyrene petri dishes (diameter 60x15 mm) on day 2 of culture, a passage was made into bottoms with a bottom size of 75 cm2. Two days later, by trypsinization, the cell suspension (1.0-1.3x106/ml) after 2 times washing with PBS and centrifuging was transferred to a bottom of 15 µl in 24-well standard culture plates with an area of 2 cm2. For everyone their three thickness of polylactide (100, 125 and 150 мкн) about 12 holes from which 6 holes were processed within 5 min. by an adhesive factor - AF (Attachment Factor 1X, Cascade Biologist TM) containing gelatin as an attachment factor were estimated. The polylactide supports cut along the diameter of the wells (1.75 cm) were placed on the bottom of the wells. The culture plates were incubated at 37 C in an atmosphere enriched with 5% CO2 for 5 days.

Daily cell counting was carried out from inverted microscope images (LOMO, Russia) with an increase of 60 times (eyepiece x15, lens x 4) and visualization of 5 fields of vision on each well. A total of 180 wells were analyzed, of which 90 wells with an adhesive factor (30 wells for thicknesses of 100 µn, 125 µn, 150 µn) and 90 wells (30 wells for thicknesses of 100 µn, 125 µn, 150 µn) - without it. The number of rounded (not attached) and elongated (attached) fibroblasts was calculated, as well as their sum - the total number of cells (Fig. 1).

Statistical analysis performed with IBM SPSS Statistics version 19. To compare related groups (in dynamics), the Wilcoxon test was used; to compare independent samples, the nonparametric Mann-Whitney and Kruskal-Wallace tests were used. The critical value of the significance level (p) was taken equal to 0.05.

Results and Discussion. Comparative analysis of the total number of cells (round and elongated cells) in the test wells depending on AF treatment revealed statistically significant differences (p = 0.000) on all follow-up days (1st to 4th days). In 90 wells with adhesive factor, the average value of the total number of cells turned out to be statistically significantly higher (on average 1.98 times) than without it (Fig. 2).

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It should be noted that on polylactide films, fibroblasts attach unevenly on the surface, significantly more cells are observed closer to the edge of the films than in the center. [7] observed an increase in cellular response with an in-

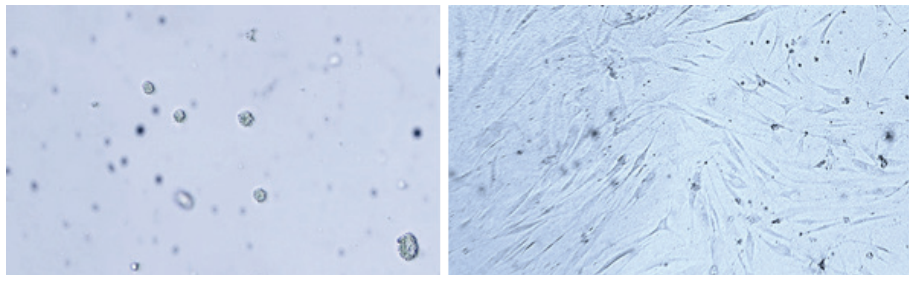

Fig. 1. Rounded unattached fibroblasts on the day of planting (left) and on the 4th day of cultivation (right) - spindle-shaped attached cells

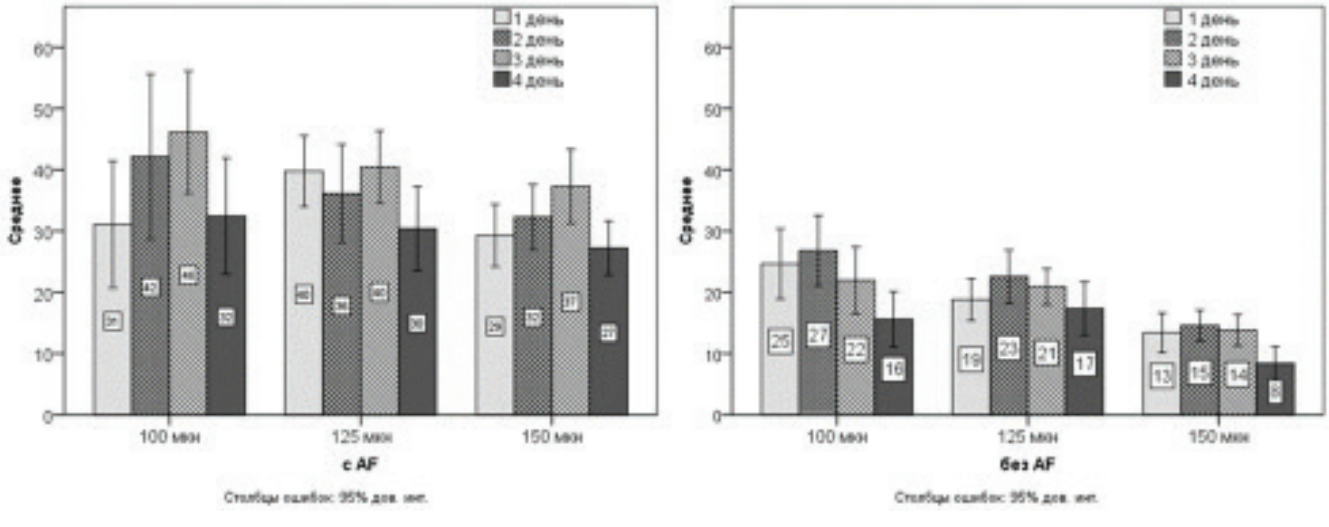

Fig. 2. The total number of cells in wells with AF and without AF in dynamics.

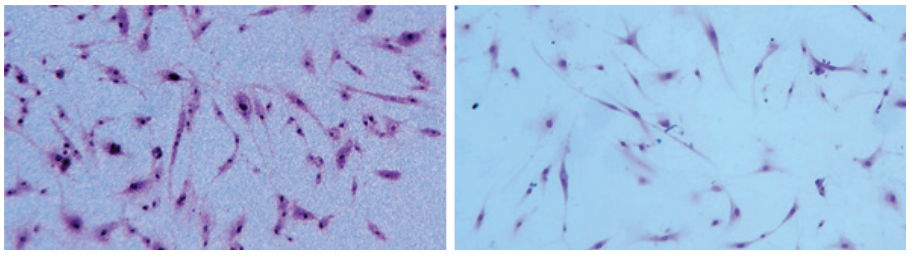

Fig. 3. Stained fibroblasts on day 4 of culture: (left - cells on the surface of the polylactide 125 μn thick, right - on the bottom of the well)

crease in edge density. This may possibly be due to preferred protein adsorption or conformational changes occurring on surface irregularities. Protein adsorption or conformational changes preferably occur on surface irregularities. This has been proposed as a mechanism of the so-called contact alignment phenomenon experienced by cells seeded on parallel ridges, where it has been found that focal adhesions are predominantly formed on the edges and lateral walls of such features [12].

Conclusion. Thus, the following conclusions can be drawn from the results obtained:

- addition in nutrient medium of an adhesive factor of AF including gelatin in the structure activates proliferation of the cultivated cages almost by 1.98 times (p =0.000), than without her;

- the largest number of attached fibroblasts is observed on the 3rd day with substrates 100 μn and 150 μn in wells with AF, while in substrates with thickness 125 μn - on the 1st day and the 3rd day of cultivation;

- according to supravitally colored samples, it was found that the adhesion of fibroblasts depending on the thickness of the polylactide film showed statistically significant differences (p = 0,022) between films with a thickness of 125 μn (37.67 ± 7.63) and 100 μn (20.75 ± 8.51), as well as a tendency to increase (p = 0,068) between 125 mkn (37.67 ± 7.63) and 150 μn (25.67 ± 10.67). Only with a polylactide thickness of 125 μn the number of fibroblasts on the film surface is higher than in the well, and with other thicknesses the number of cells in the well is higher than on the polylactide;

- on polylactide films, fibroblasts attach unevenly on the surface, significantly more cells are observed closer to the edge of the films than in the center.

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