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ISOLATION OF PRIMARY SKIN FIBROBLASTS FROM SKIN BIOPSY

Abstract

During the last decade, methods involving the use of cell therapy are being actively introduced in clinical practice in different fields of medicine. Patient-derived cells can be used for study of certain diseases associated with change in cellular phenotypes. Also they can be used for study of molecular mechanisms of a disease and subsequent drug screening approaches. Fibroblasts are of special interest for regenerative medicine. The global experience in treatment of skin lesions have demonstrated that fibroblasts applied to wounded area promote faster healing and epithelialization. Today, autologous and allogeneic fibroblasts are more often used in treatment of different skin traumas and defects along with various surgical materials. In this study we describe the method of isolation, maintaining and storage of human primary skin fibroblasts. The described method is fast (takes 30-40 min), simple and reproducible.

Keywords: primary skin fibroblasts, skin therapy.

INTRODUCTION

Cell technologies are actively developing in a modern world and for the last decade have reached a significant success especially in treatment of severe states that demand tissue regeneration. Cell therapy can be used in treatment of trophic ulcers of different etiology, large scale burns, including chemical and radiation burns, symptomatic treatment of hereditary diseases, tendon reconstruction and cosmetic surgery.

Because of the high potential in reparation processes, fibroblasts based cell therapy stands out from other cell based therapies. Isolation and culturing of skin fibroblasts is very promising, efficient and cost-effective technique in modern regenerative medicine. Because of lack of the immune response and impossibility of transmission of the infection, the use of autologous skin fibroblasts is more preferable.

Fibroblast are the heterogeneous population of cells, which include cells on different stages of differentiation, starting from multipotent mesenchymal stem cells and ending with fully differentiated fibroblasts [1]. The main function of fibroblasts is synthesis and organization of the extracellular matrix [9], by means of synthesis and destruction of collagen and elastin proteins [2]. Fibroblasts have a huge biosynthetic potential. A single mature fibroblast cell can produce up to 3.5 million collagen macromolecules per day [4]. Fibroblasts also produce different growth factors, cytokines, glycosaminoglycans, proelastin, nidogen, laminin and other important components of the extracellular matrix [1]. Fibroblasts are still capable to produce ECM components even after the transplantation [5], therefore creating appropriate environment for functioning and proliferation of other cells.

Today both autologous and allogeneic fibroblasts are used for healing of skin defects. However, the therapy with the use of autologous cells demonstrate longer clinical effect with no inflammatory response and risks of infection [7]. Allogeneic fibroblasts can be used in emergency situations. They provide fast closure and healing of a wound, however their lifespan is limited [8].

There are different methods that are successfully used for the isolation of fibroblasts from skin biopsy these methods usually include mechanical or enzymatic treatment of biopsy material. There are also methods that combine both treatments [3].

The goal of this study was to approbate the method of skin fibroblasts isolation, culturing and their long-term storage in the conditions of liquid nitrogen.

MATERIALS AND METHODS

Fibroblasts were isolated from skin samples obtained with the use of 4 mm disposable biopsy punch. Biopsy site was locally anesthetized by 2% lidocaine solution. For establishing of stable culture of skin fibroblasts we have used the method that have been described elsewhere [10]. Briefly, after the biopsy the sample was immediately placed into transportation medium containing DMEM supplemented with 10 % FBS and 1× antibiotic-antimycotic solution. In the laboratory skin biopsy was placed into a Petri dish containing small amount of the transportation medium, and then was divided with a scalpel into 12-18 smaller pieces of the same size. 700 microliters of culture medium containing high glucose DMEM containing glutamine and pyruvate supplemented with penicillin streptomycin mixture and 10% FBS (Gibco, Life Technologies), were added into each well of gelatinized 6-well plate. Two or three skin pieces were placed in the distance of 5 mm from each other in each well. Subsequently the plate was placed in CO_2 incubator at 37°C and 5% CO_2. The culture medium was replaced as needed, usually every 4-5 days during the first 15 days of incubation.

The first keratinocytes emerged at day 5-7 of incubation, while fibroblasts started to appear at day 14-19. Cells were passaged after the formation of monolayer (day 25-30). All subsequent passages were performed every 3-4 days. The culture medium was changed every 2-3 days. The morphology of cells was monitored with the use of Leica inverted microscope. 0.25% trypsin solution (Gibco, Life Technologies) was routinely used for cell detachment. After reaching the confluent state, the cell culture was regularly split in a ratio of 1:3. Cell freezing have been performed in Nunc cryovials in a freezing medium supplemented with 10% DMSO. Cells prepared for freezing were placed into insulated polystyrene box and put in -86°Bozo I.Ya. Deev R.V., Pinaev G.P. «Fibroblast» - specializirovannaya kletka ili funkcional'noe sostoyanie kletok mezenhimnogo proiskhozhdeniya? ["Fibroblast" - a specialized cell or the functional state of mesenchymal origin cells?] Citologiya [Cytology]. 2010, №2, V.52, P. 99-109.

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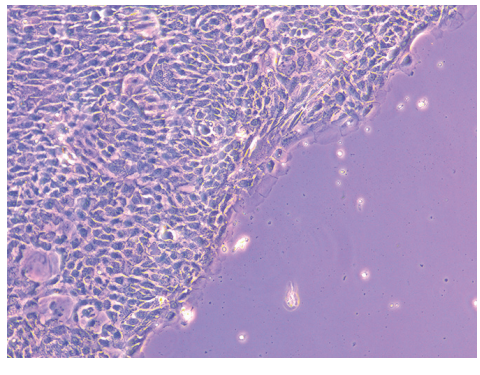

Figure 1. Keratinocytes outgrowth in primary culture of human skin fibroblasts at day 7 of culturing. Phase contrast, 100×.

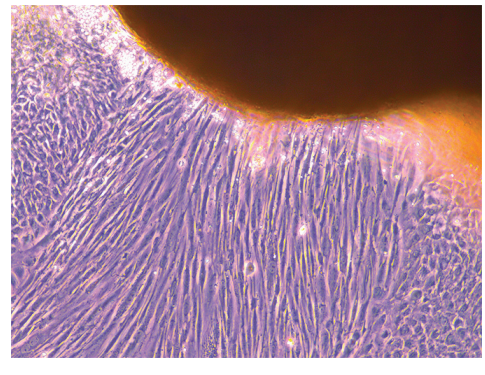

Figure 2. Heterogeneous population of keratinocytes and fibroblasts in primary culture at day 19 of culturing. The edge of skin biopsy can be observed. Phase contrast, 100×.

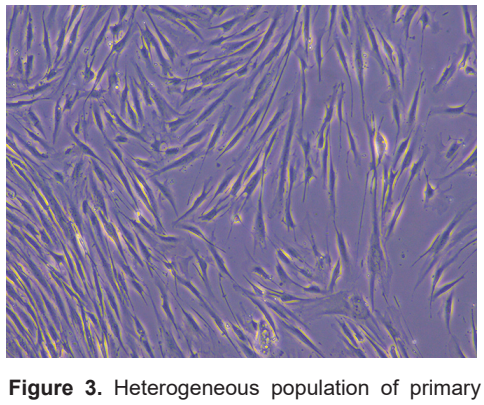

Figure 3. Heterogeneous population of primary human skin fibroblasts at day 25 of culturing. Phase contrast, 100×.

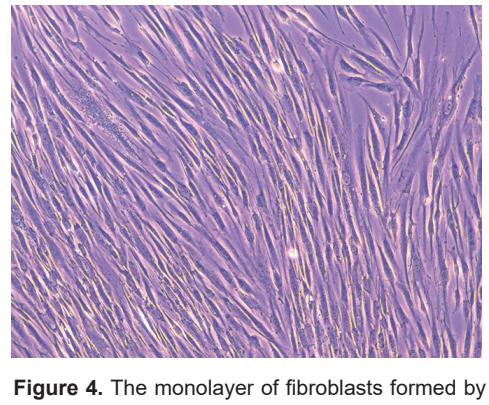

Figure 4. The monolayer of fibroblasts formed by day 28.

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INFLUENCE OFANTIOXIDANT THERAPY ON THE COURSE OF ADHESIONS IN THE ABDOMINAL CAVITY UNDER THE EXPERIMENTAL CONDITIONS

ABSTRACT

In the present work results of influence of antioxidants (emoxypin and mexydol) on formation of adhesions in an abdominal cavity at experimental animals in comparative aspect after modelling of adhesive illness are presented. It is established that in the groups received specified preparations authentic decrease of formation of adhesions is marked with optimization of antioxidant system. The quantity of remezotelizated cells on deserosaled sites of peritoneum in comparison with control group increases.

Keywords: adhesive process, antioxidants, laboratory animals.

In spite of progress of modern medicine, peritoneal adhesions remain one of the unsolved and actual problems of the abdominal surgery at present time. The frequency of the disease, the difficulty of timely recognition, lack of clear criteria of treatment policy and unfavorable outcomes make the problem of adhesions syndrome relevant [3, 4, 7]. Over the past few decades varieties of preventive measures from the formation of adhesions in the abdominal cavity were offered. However, until now there are no fairly effective means for prevention of adhesions in the postoperative period in modern medicine [5, 6, 8].

In recent times, studiers refer to abnormality in pro - and antioxidant balance of the body in the study of the reasons of development of various diseases, including inflammatory conditions [2].

The **aim** of the research is to determine the potency of drug influence having antioxidant properties: emoxypine and mexidol in comparison, used for prevention and development of adhesion in the abdominal cavity of the laboratory animals after the modeling of the peritoneal adhesions experimentally.

MATERIALS AND STUDY METHODS

The research study was carried out on 60 white non-inbred mature rats with initial body weight 230-250 gms, kept under standard vivarium conditions.

The rats were divided into 4 groups of 15 rats in each group for determination the potency of drug influence having antioxidant and antihypoxic properties (emoxypine and Mexidol) at course of adhesion in the abdominal cavity after modeling the process of peritoneal adhesions.

Group 1 – the intact group (n = 15). The laboratory animals of this group were not subjected to adhesions and no drugs were administered to them. The lipid – peroxidation and antioxidant defense measurements of the rats of this group were investigated for comparative assessment with the laboratory animals of other groups.

The other rats were included into the second, third and fourth groups. They experienced the modeling of the peritoneal adhesions. The midline laparotomy was performed under ether anesthesia in the conditions of experimental operating room. Then the removal of the serous membrane of the visceral peritoneum of the terminal segment of the small intestine situated 2 cm above the ileocecal angle, was performed with the help of gauze turunda up to the slight hyperemia and the capillary hemorrhage occurred over the whole circle of the intestine within 2 cm round. Further, the laparotomy wound was sutured with the sterile non-absorbable capronic ligatures. All the laboratory animals in pre- and postoperative period were in the vivarium in equal conditions. The laboratory animals were devitalized by etherization's overdosage in 14 days after modeling of the peritoneal adhesions. After mortification the blood for research was taken by decapitation and the visual estimate of the adhesive process in the abdominal cavity was carried out.

Group 2 – the control group (n = 15) was used for comparative assessment of the postoperative therapy effectiveness with groups 3, 4. The laboratory animals were subjected to the modeling of the peritoneal adhesions of the peritoneum. After that during 7 days of the postoperative period the laboratory animals were administered abdominally as a single dose 2 ml of sterile physiological saline (NaCl 0, 89%).

In the third group (n = 15) the rats, after being modeled, were administered Mexidol (60mg/kg) abdominally as a single dose during 7 days of postoperative period.

The fourth group (n = 15) was composed by the rats, modeled to peritoneal adhesions. They were administered emoxypine (60mg/kg) abdominally as a single dose during 7 days of the postoperative period.

In 14 days the laboratory animals after