Original Article

Assessment of the effect of biocompatibility of fibroblasts and scaffolds on the cell cycle in vitro

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ABSTRACT

In regenerative medicine, to create tissue equivalents of the skin, great importance is paid to the study of the biocompatibility of scaffolds and skin cell culture. In the current scientific work, the dynamics of fibroblast cell cycle parameters in the in vitro system were evaluated by flow cytofluorometry in two experimental models. The first model is to evaluate the biocompatibility of fibroblasts with scaffolds under 2D and 3D cultivation conditions. The second model is an assessment of the biology of fibroblasts after exposure to UV radiation. The fibroblasts necessary for the study were isolated and cultured using mechanical dissociation of the tissue. Cell lines of the third passage were used in the experiment. To study the effect of ultraviolet irradiation on the parameters of the fibroblast cell cycle in vitro culture, cell lines were irradiated with a UV lamp. To analyze the biocompatibility of the fibroblast cell line with scaffolds, fibroblasts were seeded on two types of carriers: "G-DERM" and "Transwell". It was found that for two weeks of cultivation, high cell biocompatibility was observed in the G-DERM scaffold under 2D cultivation conditions. UV irradiation causes the cell cycle to stop as a compensatory adaptive process in response to the action of damaging radiation. Temporary exposure and dose-dependent exposure to UV radiation for 30 seconds do not have a pronounced lethal effect on cell cultures.

Keywords: UV radiation, Skin cells, Fibroblasts, Scaffold technologies, Cell cycle

Introduction

Physiological, biochemical, and molecular genetic reactions occurring in cells in response to the action of various damaging factors are complex, sometimes unpredictable, and heterogeneous processes. Due to the lack of uniform universal markers, a common set of signs has been established, among which the most informative is the analysis of the cell cycle [1-4]. In scientific studies over the years, it has been noted that ultraviolet (UV) irradiation and different cultivation conditions in the in vitro system affect the morpho-functional parameters of cells [5-7]. In particular, the direct mutagenic effect of UV radiation on the DNA structure, the occurrence of mutations of tumor suppressor genes, and an increase in ROS production were revealed [8, 9]. At the same time, UV radiation has an activating effect on the synthesis of growth factors, proliferation, etc. by skin cells [10]. It is known that the disease of melanoma of the skin is directly dependent not only on the duration but also on the intensity of UV radiation. Thus, even short-term but high-intensity radiation causes a powerful carcinogenic effect [11-13].

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Along with external influences, the biology of cells in vitro is influenced by different cultivation conditions, which include the biocompatibility of cells with scaffolds. Currently, there is a large amount of fundamental research on the biocompatibility of scaffolds with skin cells in the aspect of creating equivalents of damaged tissues and organs for regenerative medicine [14-16].

However, the search for optimal solutions for practical application is still underway. One of the indicators of biocompatibility is the analysis of the cell cycle, its dynamics, and mechanisms of regulation. In particular, it is known that G2-M cells are the most vulnerable in the S-phase and during proliferation [17, 18].

Fibroblasts are a heterogeneous population of skin cells. This is determined by the topographic localization in the body and its location in the fibroblastic differon [19], which in turn determines the complexity of interpreting the dynamics of the cell cycle indicators. Thus, mitotically active and postmitotic fibroblasts are isolated in the dermis of human skin. Even fibroblasts of the same anatomical site, but of different layers (papillary and reticular) have differences in cellular morphology, proliferative potential, extracellular matrix production, production, and response to growth factors and cytokines [20]. The existing differences in the dynamics of the fibroblast cell cycle are necessary to maintain tissue homeostasis [21-23]. The heterogeneity of fibroblasts is based on the variability of genome expression under the influence of microenvironment factors [23]. The immunophenotypic profile of cultured skin fibroblasts normally corresponds to the profile of mesenchymal cells. They express vimentin, CD44, CD49b, CD54, CD90, CD105, but do not express CD34, CD45, CD133, CD117, HLA-DR, nestin [24]. Thus, modern research methods in the field of cellular and molecular biology make it possible to get closer to understanding the subtle mechanisms of cellular homeostasis, ways of regulating the cell cycle, in this regard, the analysis of the cell cycle remains one of the main criteria for assessing the reactivity of cells to external conditions. The relevance of studying the biocompatibility of scaffolds and skin cell culture, as well as the effect of UV radiation, is determined by the significance of the practical application of the created tissue equivalents of the skin in regenerative medicine.

In this regard, the purpose of this study is to evaluate the dynamics of fibroblast cell cycle parameters in the in vitro system using flow cytofluorometry in two experimental models. The first experimental model is to evaluate the biocompatibility of fibroblasts with scaffolds under 2D and 3D cultivation conditions. The second experimental model is an assessment of the biology of fibroblasts after exposure to UV radiation.

Materials and Methods

The material for the experiment was skin samples from the upper eyelid region of women aged 36 to 58 years, which were obtained as a result of plastic surgery (blepharoplasty). Fibroblast cells were isolated using mechanical tissue dissociation and cultured in T25 vials (TTP, Switzerland) in RPMI-1640 medium with the addition of 10% embryonic calf serum. The formation of a confluent monolayer was visually controlled using an inverted Eclipse TS100 microscope (Nikon, Japan). When the surface coverage of the vial was reached by 40%, the cells were transplanted. The cells were removed with 0.25% trypsin solution with the addition of ethylenediaminetetraacetic acid. Cell lines of the third passage were used in the experiment.

To study the effect of UV irradiation on the parameters of the fibroblast cell cycle in vitro culture, cell lines were irradiated with a 36 W Vitronic UV lamp (Oase, Germany) with a wavelength of 253.7 nm for 30 seconds. To do this, cells in the amount of $1x10^4$ were seeded into a Petri dish and irradiation was performed when the surface coverage of the cup was at least 50%. The analysis of the fibroblast population in the control group without irradiation and the experimental group with irradiation was carried out 24 hours after exposure.

To analyze the biocompatibility of the fibroblast cell line with scaffolds, fibroblasts were seeded on two types of carriers - "G-DERM" (JI-Group, Russia), which is a biopolymer based on a hyaluronic acid hydrocolloid and an adhesive peptide complex; "Transwell" (Corning, the Netherlands) - membrane inserts made of tetrafluoroethylene with collagen coating. Fibroblasts were applied to scaffolds in the amount of $1x10^4$ cells/ml. For this part of the work, two skin equivalent models were formed, depending on the scaffold used: CulTw – cell culture on Transwell, 3D cultivation, and CulGD - cell culture on G–Derm, 2D cultivation. The fibroblast population was analyzed for 15 days of cultivation.

Flow cytofluorometry: - the immunophenotype of fibroblasts was determined by the expression of the CD90 label (Beckman Coulter, USA), with an assessment of the proportion of fibroblasts as cells of mesenchymal origin; - determination of the proportion of fibroblasts by phases of the cell cycle - G0-G1, S, G2-M.

A blue laser (λ=488 nm) was used for studies on a cytofluorimeter. Data collection and processing were carried out using the Flow Max software (Partec, Germany) in terms of forward, lateral scattering, and fluorescence intensity over four channels (3 channels per blue laser and 1 channel per purple). The number of viable fibroblasts was calculated using trypan blue staining (BioRad, USA) using an automatic RWD C100 cell counter (RWD, USA).

Statistical data processing for the correlation indicator was carried out using the Student coefficient at the significance level of differences $p<0.05$ in Microsoft Excel 2010.

Results and Discussion

An analysis of the dynamics of cell cycle parameters in two experimental groups revealed distinctive features that are determined by the adaptive mechanisms of fibroblast cell lines. At the same time, in the first case, adaptation to the scaffold is triggered, and in the second case, it is the result of compensatory adaptation to the effect of UV radiation.

The fibroblast cell line, which was applied to the surface of scaffolds, showed the CD90 positive cell immunophenotype as a marker of mesenchymal stromal cells, in the amount of 75.7% in the CulTW group and 88.7% in the CulGD group **(Figure 1)**. Accordingly, the cultured cells were represented by a young generation of fibroblastic differon cells with a high proliferative potential for the formation of adhesion to the surface of scaffolds.

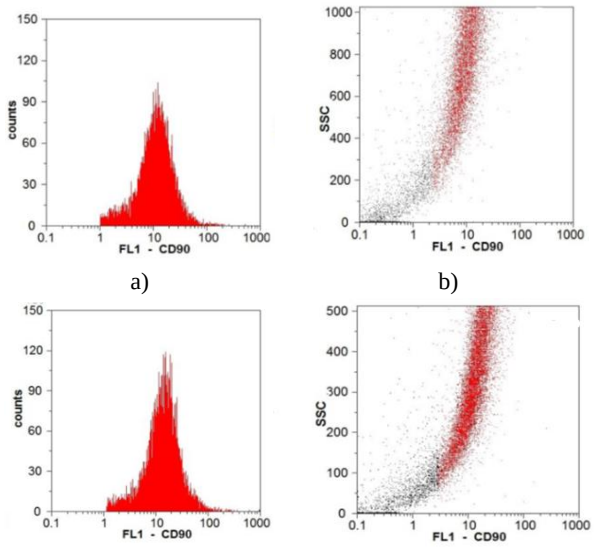


Figure 1. Immunophenotype of the CD90 positive dermal fibroblast line in the experimental groups CulTw (a, b) and CulGD (c,d): a, c) histograms of the number of CD90 positive fibroblasts; b, d) dot graph (red indicates CD90 positive fibroblasts among the total number of cells)

Flow cytometry revealed some differences in the distribution of fibroblasts by phases of the cell cycle, depending on the chemical and physical properties and spatial organization of scaffold structures (weaving structures). So, in particular, in the stage of proliferative rest (G0-G1 stage), the number of fibroblasts differs slightly, regardless of the type of scaffold – in the CulTw group this indicator was 47%, and in the CulGD group - 43%. Simultaneously, the number of fibroblasts in the polyploidization stage (S-stage), as an indicator of an increase in the amount of DNA, is higher in the CulTw group compared to the CulGD group – 27% and 12%, respectively. Whereas in the CulGD group, compared with the CulTw group, the number of fibroblasts at the stage of mitotic activity (G2/M) was higher - 45% and 27%, respectively. Thus, the chemical and physical properties and spatial organization of the structures of the G-Derm scaffold are more optimal for the proliferative activity of fibroblasts in comparison with the Transwell scaffold. It is possible to notice a higher rate of coating of the scaffold surface **(Figure 2)**. It should be noted that the data obtained correspond to two weeks of fibroblast cultivation on the surface of scaffolds, whereas later cultivation dates have not yet been analyzed, and perhaps 3D cell cultivation on Transwell in the delayed period will be more optimal compared to 2D cultivation on G-Derm.

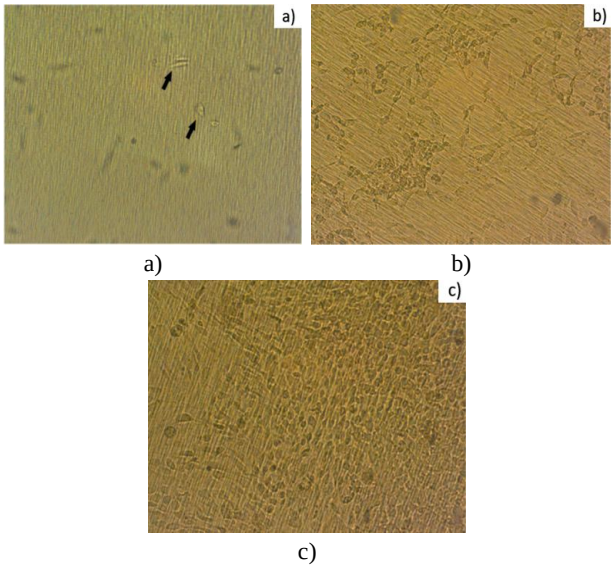
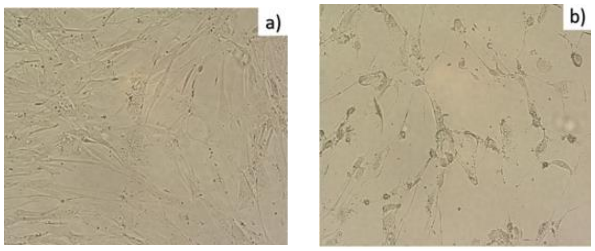


Figure 2. Dermal fibroblasts on the Transwell scaffold: a) on the second day; b) on the 13th day; c) on the 15th day of cultivation. Note: vital drug

Analysis of the fibroblast cell cycle in the second experimental group after UV irradiation for 30 seconds revealed an increase in the number of cells in the polyploidization stage (S-stage), compared with the control culture - 11% and 9%, respectively. The reverse dynamics is observed in the proliferation stage (G2-M period) - the number of mitotically dividing fibroblasts decreases (50% and 32%, respectively). Also, after UV irradiation, the number of cells in the proliferative resting stage (G0-G1 stage) increases - 41% and 7%, respectively.

A slight increase in the number of cells in the S-stage is most likely due not to the preparation of cells for division, but to the launch of a checkpoint system that reflects the implementation of repair processes rather than DNA replication [25]. A decrease in the number of cells in the division stage and an increase in the proliferative dormancy stage is a compensatory adaptive reaction with the cell cycle stopping as a protective mechanism against damage by UV rays [26]. The effect of external factors on the cell is accompanied by the activation of various intracellular and intercellular, as well as systemic adaptive reactions and processes that are aimed at eliminating or limiting damage and its consequences on cell structure and function [27, 28].

The revealed changes in the cell cycle of fibroblasts of the control group without irradiation and with irradiation for 30 seconds did not affect the total number of cells when analyzed after 24 hours. The choice of a 30-second time exposure was made after a series of experiments with time exposures of 60 and 300 seconds. An increase in exposure time caused a dose-dependent statistically significant decrease in the total number of cells compared with the control group without irradiation - from 2.32 x 105 to 1.5 x 10^5 ($r = - 0.89$, $p = 0.01$). An increase in the irradiation time of fibroblasts to 300 seconds led to pronounced morphological changes, which were expressed by an increase in the granularity of the cytoplasm and thinning of the processes up to their complete absence **(Figure 3)**.



a) b)

Figure 3. Dermal fibroblasts: a) without irradiation, b) 24 hours after irradiation for 300 seconds. Note: vital culture; light microscopy

It can be noted that in cell cultures there was a significant increase in the number of dead cells. The revealed irreversible changes in cells were most likely caused by a violation of the functions of mitochondria, a violation of the structure and function of the nuclear apparatus [29]. It can be concluded that the vulnerability of fibroblasts to this type of exposure is, firstly, dose-dependent, and secondly, delayed reactivity of cells after exposure is not excluded [30].

Conclusion

Thus, preliminary results on the analysis of the fibroblast cell cycle under in vitro conditions under exposure to ultraviolet light and cultivation on various scaffolds revealed that for two weeks of cultivation, high biocompatibility of cells is observed in the G-DERM scaffold under 2D cultivation conditions. It is known that the spatial arrangement of cells and biocompatibility with scaffold according to the principle of histomorphological correspondence, in compliance with the principles of spatial and functional similarity, is largely determined by the physical and chemical composition and spatial weaving of scaffold structures. UV irradiation causes the cell cycle to stop as a compensatory adaptive process in response to the action of damaging radiation. Temporary exposure and dose-dependent exposure to UV radiation for 30 seconds do not have a pronounced lethal effect on cell cultures. The data we have obtained on the cell cycle of fibroblasts in vitro under UV exposure and cultivation on scaffolds require further studies of cell death pathways, identification of cytotoxicity, and analysis of the preservation of proliferative activity in cells. The question of the degree of damage to fibroblasts and the threshold value of the influence of the analyzed external aggressors for the return of cells to their original state using reparative stress resistance mechanisms remains open.

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Conflict of interest: None

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Ethics statement: Skin samples from the upper eyelid region were obtained from women patients aged 36 to 58 years strongly after receiving agreement for participation in the experiment. All raw data are available upon request from the corresponding author.

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