ASSESSMENT OF THE EFFECT OF CONNECTIVE TISSUE CELLS ON THE REGENERATION OF THE ORAL MUCOSA

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Abstract. *This study presents a comparative experimental analysis of a tissue-engineered construct composed of silk degummed gauze and allofibroblasts for the treatment of mucosal defects in rats. The dynamics of animal weight and the relative weight of internal organs did not exhibit statistically significant deviations compared to control and reference values, indicating the safety of the cell therapy and tissue engineering methods employed for the experimental animals. The proposed tissue-engineered construct effectively supports the maintenance of proliferating dermal fibroblasts within the scaffold.*

Keywords: *tissue-engineered construct, allofibroblasts, silk degummed gauze, experimental animals.*

Introduction. Globally, extensive research is underway to explore methods for treating patients with soft tissue defects of the face. Currently, treatment primarily relies on various surgical techniques, including conventional approaches such as free tissue transfer, local tissue rearrangement, and others. However, in many instances, these methods fail to achieve the desired outcomes for oral cavity defect reconstruction, as defined by contemporary standards [3, 8]. Expanding the application of fibroblasts in cell-based therapies for oral soft tissue diseases, including those associated with aesthetic deformities and their correction, holds promise as a foundation for developing strategies to replace and/or regenerate damaged tissues and mucosal defects within the oral cavity [1, 4, 10]. These cells are readily cultured in vitro without compromising their functionality [7, 9]. Due to their pivotal role in maintaining tissue homeostasis, fibroblasts, uniquely among cell types, possess the capacity to effectively establish an environment conducive to the proliferation and migration of other cell populations [2, 5].

Material and methods of research. To evaluate the efficacy of a tissue-engineered construct composed of silk degummed gauze and autologous fibroblasts, in conjunction with injectable autologous fibroblasts, for the treatment of oral mucosal defects, an experimental morphological study of the tissues was conducted. Furthermore, to assess the toxicity of the materials, a histological examination of internal organs, including the liver, heart, lungs, stomach, and brain, was performed. The experiment utilized 30 healthy adult male Sprague-Dawley rats with an initial weight of 180-220 grams. The experimental animals were divided into 5 groups according to the treatment protocol. To assess wound surface regeneration, tissue samples were harvested on days 3 and 7. The distribution of rats into groups based on the applied treatment was as follows: Group I, silk gauze + fibroblasts; Group II, silk gauze + fibroblasts + supplemental fibroblast injections; Group III, silk gauze alone; Group IV, fibroblast injections alone; Group V, defect without treatment (control).

Dynamic observation was conducted over a period of 1, 3, 5, 7, and 14 days. The overall condition of the animals and clinical signs of potential intoxication were assessed, including: general animal condition, food and water consumption, body weight changes, behavioral characteristics, and

the intensity and nature of locomotor activity. The local status of the treatment site was also evaluated. Biopsies and blood samples were collected for biochemical analysis on days 3 and 7.

Animals were euthanized via ether overdose, after which tissue samples and internal organs were harvested for histological and morphological examination. Macroscopic specimens were fixed in 10% formalin solution and embedded in paraffin blocks. Sections, 4-5 μm in thickness, were stained with hematoxylin and eosin (H&E). Microscopic examination was performed using a MIKMED-2 light microscope at magnifications of 40x, 100x, 200x, and 400x.

To assess the biomedical safety and specific activity of the tested tissue-engineered construct for temporary coverage of oral mucosal defects, and of the allofibroblast injections, hematological and biochemical analyses of peripheral blood and serum were performed in the white rats.

Results and discussion. Following implantation of the tissue-engineered construct, both with and without cells, no evidence of endogenous intoxication was observed, indicating the absence of toxic effects on the organism and the safety of using this construct.

The application of cell therapy and fibroblast-based tissue-engineered constructs leads to the induction of epithelial cell and fibroblast differentiation, as well as vascularization, with statistically significant improvements observed primarily on days 3 and 7 of observation. This approach represents a promising method for stimulating healing and regeneration of damaged tissues in cases of oral mucosal injury.

The obtained clinical-experimental data (absence of endogenous intoxication, activation of regeneration at the injury site, including induction of neoangiogenesis), along with the absence of complications during the observation period, demonstrated the efficacy and safety of the developed method for treating oral cavity defects using tissue-engineered constructs based on degummed silk gauze with autologous fibroblasts and autologous fibroblast injections. Analysis of the obtained data revealed that, in the experimental animals, the levels of hemoglobin, erythrocytes, leukocytes, eosinophils, lymphocytes, granulocytes, hematocrit, mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (absolute count), platelet distribution width (PDW), mean platelet volume (MPV), plateletcrit (PCT), absolute lymphocyte count, and the proportions of monocytes, basophils, and eosinophils, as well as the erythrocyte sedimentation rate (ESR), remained within the normal range of control values. This indicates that the hematological parameters of the white rats did not exhibit statistically significant deviations ($P > 0.05$) from either normal values or between the treatment groups throughout the study period. Furthermore, implantation of the silk gauze + fibroblast tissue-engineered construct did not result in endogenous intoxication, further confirming the absence of toxic effects on the organism and the safety of using this construct.

The dynamics of animal weight and the relative weight of internal organs also did not exhibit statistically significant deviations compared to control and reference values, indicating the safety of the cell therapy and tissue engineering methods used for the experimental animals.

The proposed tissue-engineered construct facilitates the maintenance of proliferating dermal fibroblasts within the scaffold, opening up entirely new avenues for the development of advanced tissue and cell engineering techniques and the creation of biomedical cell products (BMCPs) for integration into the comprehensive therapy of a range of socially significant diseases characterized by oral mucosal defects.

Conclusion. The application of cell therapy and fibroblast-based tissue-engineered constructs induces epithelial cell and fibroblast differentiation, as well as vascularization, with statistically significant improvements observed primarily on days 3 and 7 of observation. The obtained clinical-experimental data (absence of endogenous intoxication, activation of regeneration at the injury site, including the induction of neoangiogenesis), along with the absence of complications during the observation period, demonstrated the efficacy and safety of the developed treatment method.

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