In vitro experimental evaluation of wound and burns healing capacity after exposure to salty microclimate from Dej and Cacica

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Abstract

Objective: **To investigate the influence of Cacica and Dej salt mines microclimate on the morphology and electrophoresis protein expression of *in vitro* dermal fibroblasts from hypodermic tissue of Wistar rats in normal conditions and after experimental induced wounds and burns.**

Materials and methods: **Dermal fibroblast cultures were obtained from hypodermic tissue collected from Wistar rats. Grow in cultures derived fibroblast monolayer is attached to the cultivation flask. Before initiation of cultures, Wistar rats weighing 75-100 g were separated into three groups: control, wounds and burns. Five animals from each group were sent to Cacica and Dej salt mines, for 14 days and kept in saline environment, as in speleotherapy.**

Results: **Speleotherapy applied to Wistar rats caused significant differences in cell morphology and electrophoretic expression of dermal fibroblasts in primary culture completed. The data obtained confirm the therapeutic effects of speleotherapy compared with experimental data from control animals.**

Conclusions: **The results of this study indicate that speleotherapy induces changes in morphology and protein expression of *in vitro* dermal fibroblasts and therapeutic effects of these changes are present in the wounds and burns cases.**

Keywords : **speleotherapy, dermal fibroblasts, wounds and burns, salt mine.**

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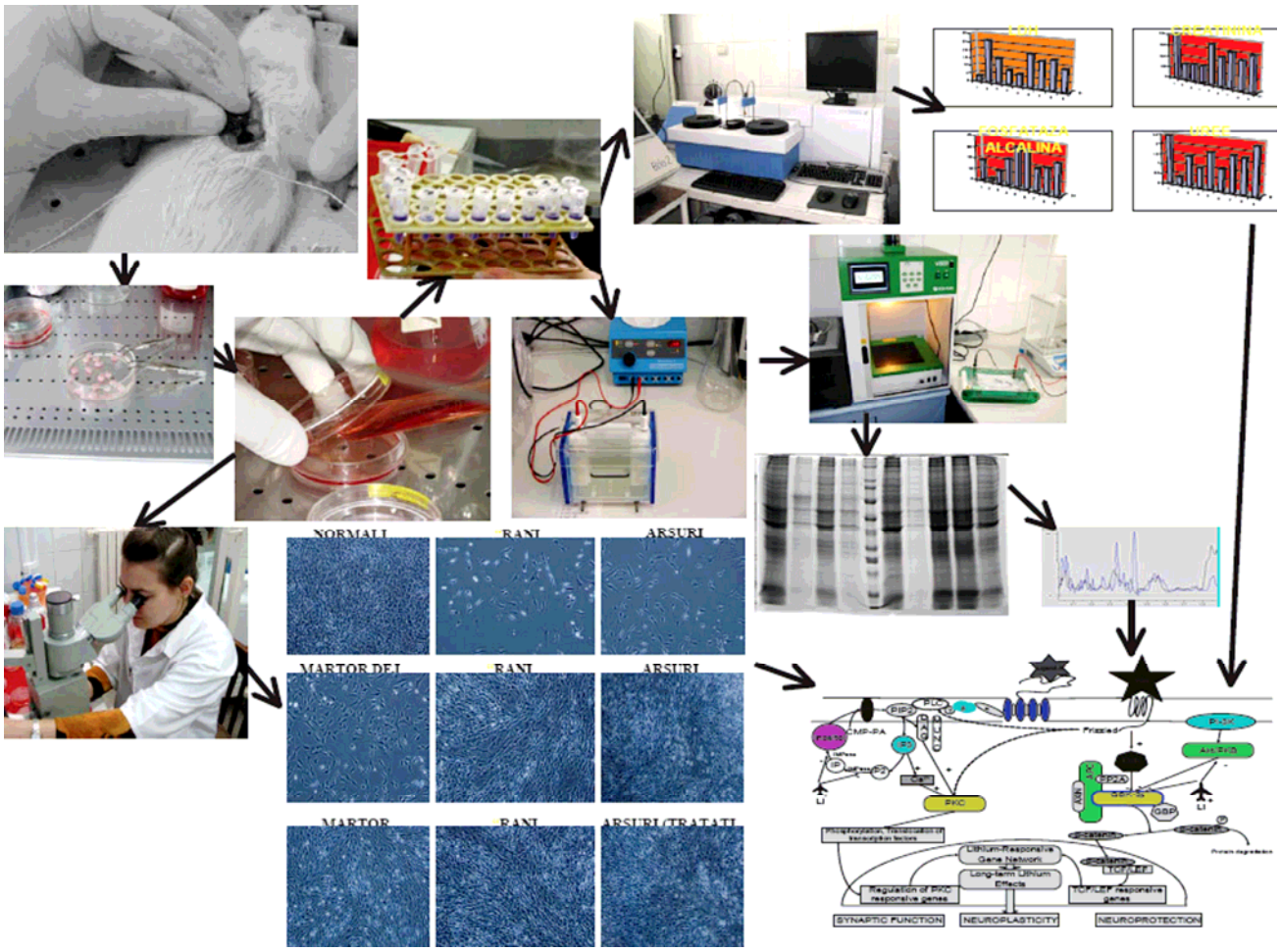


INTRODUCTON

The skin is the primary interface between body and environment. Spectrum in which aggression is likely included skin conditions caused by chemical and microbial, thermal and electromagnetic radiation and mechanical trauma. Skin damage is the consequence of the invasion of pathogenic microorganisms, which may affect human life.

Wound healing is a restorative natural responded to tissue damage - which consists of a cascade of cellular events whose nature depends on the characteristics of the wound. There are acute injuries resulting from surgery, penetration of sharp objects, amputation of phalanx, fray, burns, animal bites, etc.. and chronic wounds as arterial ulcers, venous ulcers, limfedemul, pressure ulcers and neuropathic ulcers.

Regenerative medicine is widely seen as one of the next revolutions in medical treatment. It draws heavily from the fields of tissue science, biology, biochemistry, physics, chemistry, materials science, applied engineering and other fields and is a highly interdisciplinary new discipline. The general aim of regenerative medicine is to repair, replace or regenerate lost or damaged tissues and organs *in vivo* through techniques that stimulate them into healing themselves. Tissues and organs can also be grown *in vitro* for subsequent implantation into the body.

Using skin cell cultures to verify the therapeutic properties of saline mines medium, described as speleotherapy, represents an innovative and scientific new way to establish the medical methodology of preventing, treating and recovery of patients with various dermatological problems.

MATERIALS AND METHODS

Materials

Phosphate Buffer Solution (PBS: NaCl 0,13M + KCl 2,6mM + Na_2HPO_4 x12 H_2O 8mM + KH_2PO_4 1,4mM); HAM-F12 culture medium (Sigma); penicillin 100 U/ml, streptomycin 100µg/ml; neomycin 50µg/ml (Sigma); fetal bovine serum (Sigma).

Animal model - Wistar rats with experimental wounds and burns: Wistar rats of 75-100 g weight were subjected to burns and wounds of 1 cm square on the back.

Dermal fibroblasts culture

After anaesthesia with chloroform, rats were killed. After hair removing, a patch of 1 cm^2 of skin was detached en bloc in a laminar flow hood using sterile technique and put into ice-cold sterile Phosphate Buffer Solution (PBS: NaCl 0,13M + KCl 2,6mM + Na_2HPO_4 x12 H_2O 8mM + KH_2PO_4 1,4mM). 1mm tissue pieces were suspended in 0.125% trypsin and 0.001% DNase and repeatedly stirred for 6 minutes and centrifuged at 1000g. The pellet was resuspended in HAM-F12 medium with 4500mg/l glucose, 25 mM HEPES, 100 U/ml penicillin, 100 µg/ml streptomycin and 50 µg/ml neomycin and 10% fetal bovine serum.

Phase Contrast Microscopy

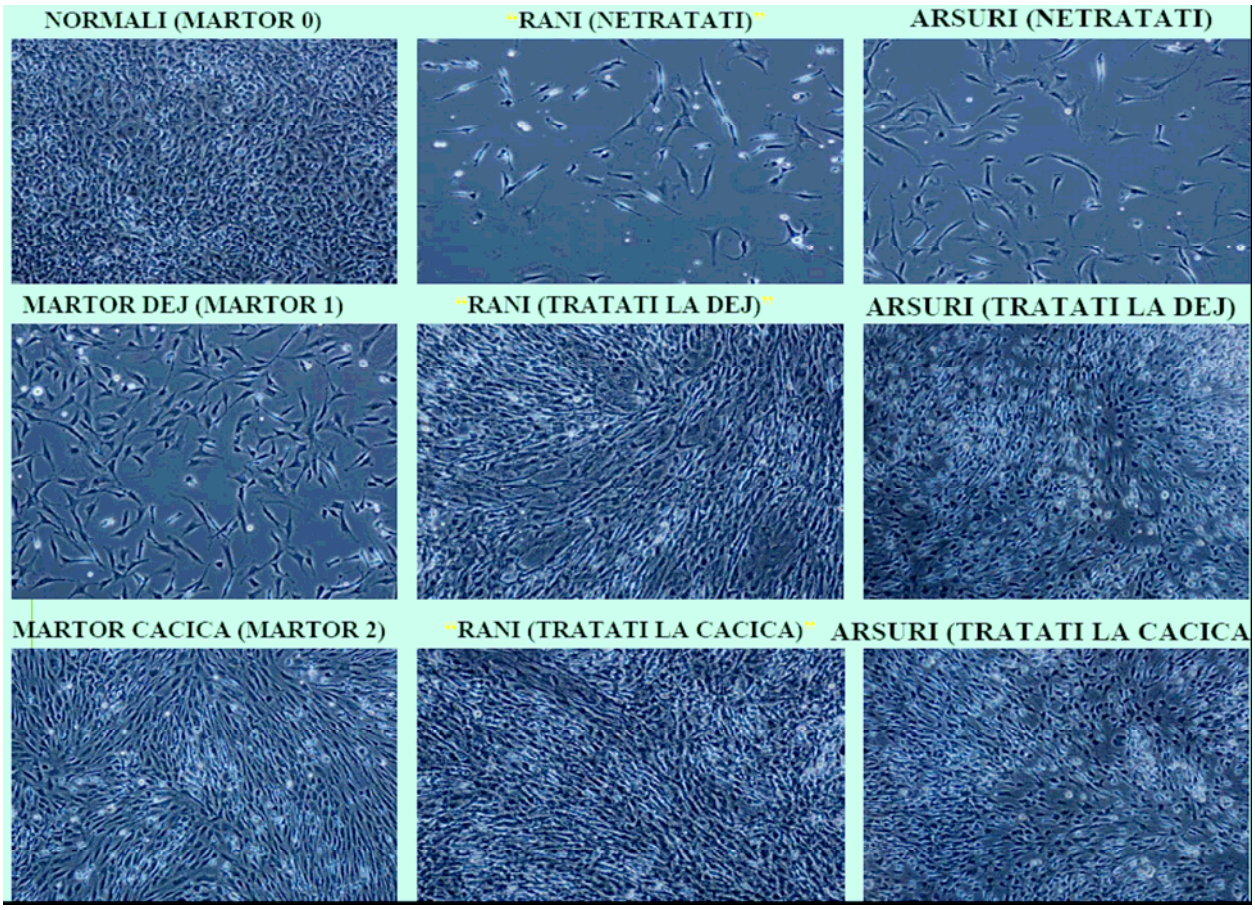
Phase contrast microscopy, first described in 1934 by Dutch physicist Frits Zernike, is a contrast-enhancing optical technique that can be utilized to produce high-contrast images of transparent specimens, such as living cells.

SDS-PAGE Electrophoresis

The proteins electrophoresis from the total homogenate has as the purpose to establish the changes, which are revealed at the proteic level of fibroblasts cultures obtained from rats held on speleotherapy.

The proteins electrophoresis in gel of polyacrylamide was done in the denaturated conditions in the conformity with the techniques described by Laemmli (1979). The cultures have been washed with PBS, curetted from the culture plate and lyzed in buffer containing 0,5M Tris-HCl, pH 6,8 + 0,05% BPB + 10% glycerol + SDS 10%.

Analysis with GeneTools version 4 software from SynGene of each track of the electrophoresis allowed us to compare the profiles of the total proteins expression.



RESULTS and DISCUTIONS

Control dermal fibroblasts culture of 7 days has a heterogenic aspect with a high pre-confluence level. The cell division is to a high level and the cell morphology shows a typical microscopic view, described in the specific literature. There are mainly two types of cells: epithelial and fibroblastic cells.

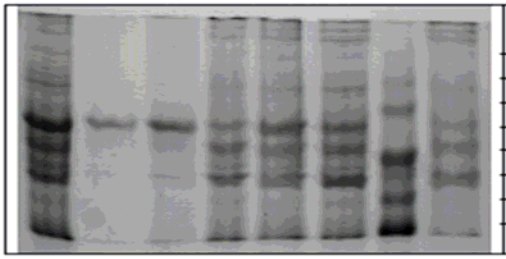
Dermal cells cultures of 7 days obtained from negative control untreated rats with wounds and burns presents many morphological changes from the control skin cell culture, being observed an sensible number reducing of dermal fibroblasts in culture, the diminished cellular dividing frequency and an accentuated cellular morphopathology of the cells in culture. After 7 days of culturing, the pre-confluence level is much lower than in the Biobase control case.

Dermal fibroblasts cultures of 7 days obtained from positive control rats treated by speleotherapy in Cacica Salt Mine shows an improvement of the morphological parameters of the cells comparative with the cultures obtained from negative control rats. By phase contrast microscopy, it is possible to observe a rising of the cells number.

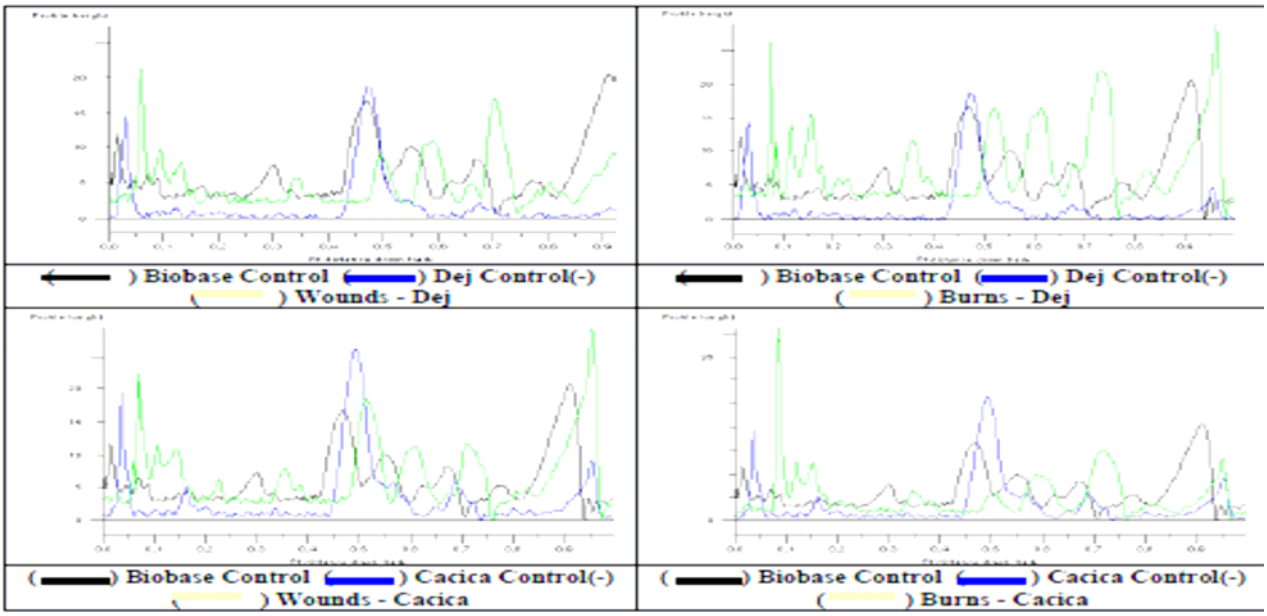
Dermal fibroblasts cultures of 7 days obtained from rats with wounds and burns and treated by speleotherapy in Dej and Cacica Salt Mines show an improvement of the morphological parameters of the cells comparative with the cultures obtained from negative control rats. It is observed the rising of the cell population density and that of cell viability.

Following electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250, that allowed visualization of the separated proteins. After staining, different proteins appeared as distinct bands within the gel (Towbin *et al.,* 1979).

The morphological observations are confirmed by the electrophoretic analyses, which demonstrate through rising of the expression of many proteins and of total protein amount that the exposure of animals to the saline medium from Dej and Cacica Salt Mines is reversing the cells morphopathology of dermal fibroblasts in cultures.



Sample	Protein quantity per 10µl sample
1. Biobase Control	11,00 µg
2. Dej Control (-)	3,51 µg
3. Cacica Control (-)	6,24 µg
4. Wounds – Dej	9,40 µg
5. Wounds – Cacica	11,52 µg
6. Burns – Dej	13,88 µg
7. Molecular Weight markers	13,27 µg
8. Burns - Cacica	8,94 µg



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