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JOURNAL CLUB

• Intentions, plans, thoughts – Constantin Munteanu
Editorial

Constantin Munteanu

The new conceptions and the new orientations of the modern medicine that approach the health as a fundamental right of the human being, the introduction on the evaluation criteria of notions as the quality of life, lifestyle, welfare determined a re-evaluation of the medical assistance offered in balneary resorts, of balneology generally speaking, and of traditional tourism.

In many countries of the world there is an interest and a preoccupation for the limit of negative effects that were created by the economical development on the population health. One of the efficient ways to fight against the modern civilization diseases is offered by the balneary tourism.

The international trend is to come back to the nature for treatment and recreation, and from this point of view the balneary health resorts are obviously a proper destination.

Due to this fact and favorable effects on the social and economical plans, the countries that have natural cure factors for health resorts and have traditions in the exploiting these, same as Romania, they established for the balneary tourism strategies for diversification and launching, for development and modernization.

The attention given to this form of tourism at the international level is point out by the declaration of the 2000 year as “The International Year of Balneology” and the scientific manifestations organized: The International Congress of Balneology from Rome, 2000, with the theme “Balneology for the Third Millennium”, where specialists from different countries presented points of view and valuable solutions for the future of European balneology; The Congress of International Society of Technical Balneology”, from Levico Terme – Italy, 2002, with the theme “The tourism for Health and Medical Treatment in Balneary Resorts”.

The new criteria of evaluation show the impressive leap registered on the therapy with natural cure factors, from the crisis periods when there were no solid scientific substantiation, the methodology used being based more likely on the empiricism, to the multiple studies and scientific examinations which proved the efficiency of therapeutic and rehabilitation cures for different groups of diseases, based on a complex methodology.

The modern evaluation of the health, the welfare, and the quality of life imposes the continuation and development of scientific study for the establishment of action mechanisms and curative effects of the natural therapeutic factors. There are considered as a priority the studies on the methodology and effects of “health cures”, which represent the most important domain of the primary prophylaxis of the major diseases from the pathology related to the life style of civilization from the new millennium.

The contribution of the Romanian specialists to the scientific substantiation of the therapy with natural factors is remarkable. Since 1949, when the Institute of Balneology and Climatology was founded in Bucharest, the balneoclimatology have made impressive efforts for the complex study of natural therapeutic factors, so much physics chemical, microbiology, and pharmacology experimented as much as clinical therapeutic.

The medical and technical personnel performed systematic researches in clinics, laboratories and balneary resorts. There were organized complex studies and researches; there were discovered, studied and exploited new valuable resources of the cure natural factors.

The results of these studies were published in a collection of 11 volumes of “Studies and researches of balneology and physical therapy”, in a monograph of 3 volumes “The mineral waters and mud from Romania” during the 1960-1972 and also numerous “Methodological guides” and “Indications for the treatment in balneoclimatology resorts” published on 1960, 1965, 1975 and 1986.

The boost of balneary tourism from our country and the alignment of our health resort to the existing standards in other European Union countries depend on a complex set of factors including scientific research as an important milestone.
Agenda of the VIII-th National Conference of Balneology

**A VIII-a CONFERINȚĂ NAȚIONALĂ DE BALNEOLOGIE**

**Agenda evenimentului**

**Vineri, 5 Noiembrie 2010**

10.00 - 16.00  Inscrierea participanților și primirea mapelor de participare, cazare.

18.00 - 19.00  Deschiderea lucrărilor Conferinței.

20.00  Cină festivă.

**Sâmbătă, 6 Noiembrie 2010**

09.00 - 11.00  Simpozion „Asistența medicală în stațiunile balneare din România“.

11.00 - 11.30  Pauză cafea.

11.30 - 13.30  Simpozion „Cercetarea științifică balneară: principii și metodologie“.

13.30 - 15.30  Pauză prânz.

15.30 - 17.30  Simpozion „Lucrări științifice (rapoarte și comunicări)“.

17.30 - 18.00  Discuții finale.

Concluzii.

Închiderea lucrărilor.

**Lectori anunțați:**

Medic primar Dr. Al Jashi Isam
Prof. Dr. M. Zeki Karagülle (ISMH)
Prof. Dr. Nica Sarah Adriana
Dr. Mariana Florian
Dr. Iaroslav Kiss
Conf. Dr. Delia Cinteză
Conf. Dr. Olga Surdu
Experimental Methodology used by Cell Cultures Laboratory from National Institute of Rehabilitation, Physical Medicine and Balneoclimatology, Bucharest, Romania to assess the therapeutic effect of natural factors

Constantin Munteanu¹, Diana Munteanu

Abstract

The experimental study design on cell cultures allows the direct biological evaluation at the cellular level, of the therapeutic effect that natural factors can play over the organism.

Techniques for obtaining cell cultures requires a complex and laborious task that starts from live tissue sampling, continuous with isolation of cells and their preparation for sowing a culture plate.

This preparation involves mechanical and enzymatic action from the researcher on biological material.

Derived cell cultures are monitored morphologically by high-performance inverted biological microscope, with video camera for image acquisition.

In the final stage, the cells are scraped, and through biochemical and molecular techniques, the therapeutic efficiency hypothesis of the investigated natural factor is verified experimentally.

The cell cultures can be crioconservated in special containers with liquid nitrogen.

¹ Corresponding author: Constantin Munteanu, tel./fax: +40213186458, e-mail: constantin2378@yahoo.com,
Mailing address: B-dul Ion Mihalache, nr.11A, District 1, cod 79173, Bucharest, Romania,
http://www.cell-culture.xhost.ro/
Cell Cultures Laboratory

Periodical certification of natural factors quality used in therapeutic baths is an attribute of the National Institute of Rehabilitation, Physical Medicine and Balneoclimatology (INRMFB). Scientific arguments done by the research activity conducted at the institute, through the fundamental and applied research sector, will be to base for an evaluation of a resort and for promotion and motivation of that resort.

The history of cell culture dates back to early twentieth century. The original impetus for the development of cell culture was to study, under the microscope, normal physiological events such as nerve development.

Natural therapeutic factors

The natural cure factors: mineral waters and thermo, salt lakes, mud and moifette, mines, climate, herbs, etc. are the fundamental condition, the starting point for designing and realizing any spa offers.

Primarily therapeutic minerals, with physico-chemical properties that meet the needs of medical and preventive maintenance, enhancement, restoration to health, the work capacity and physical and mental comfort of the individual, represent spas resources.

Experimental methodology

Cell culture is the complex process by which cells are grown under controlled conditions. In practice, the term „cell culture” has come to refer to the culturing of cells derived from multicellular eukaryotes, especially animal cells. However, there are also cultures of plants, fungi and microbes, including viruses, bacteria and protists. The historical development and methods of cell cultures are closely interrelated to those of tissue culture and organ culture.

The growth rate of animal cells is relatively slow compared with bacteria. Whereas bacteria can double every 30 minutes or so, animal cells require anywhere from 18 to 24 hr to double. This makes the animal culture vulnerable to contamination, as a small number of bacteria would soon outgrow a larger population of animal cells. Consequently, animal cell culture did not become a routine laboratory technique until the 1950s.

Cultured mammalian cells are used extensively in cell biology studies. It requires a number of special skills in order to be able to preserve the structure, function, behavior, and biology of the cells in culture. This unit describes the basic skills required to maintain and preserve cell cultures: maintaining aseptic technique, preparing media with the appropriate characteristics, passaging, freezing and storage, recovering frozen stocks, and counting viable cells.

The cells are grown in an atmosphere of 5-10% CO₂ because the medium used is buffered with sodium bicarbonate/carbonic acid and the pH must be strictly maintained. Culture flasks should have loosened caps to allow for sufficient gas exchange. Cells should be left out of the incubator for as little time as possible and the incubator doors should not be opened for very long. The humidity must also be maintained for those cells growing in tissue culture dishes so a pan of water is kept filled at all times.

 Cultures should be examined daily, observing the morphology, the color of the medium and the density of the cells.
A tissue culture log should be maintained that is separate from your regular laboratory notebook. The log should contain: the name of the cell line, the medium components and any alterations to the standard medium, the dates on which the cells were split and/or fed, a calculation of the doubling time of the culture (this should be done at least once during the semester), and any observations relative to the morphology, etc.

On *in vitro* studies can be monitored: cell morphology, protein synthesis, secretion of certain substances, cellular metabolism, cells interaction through cellular receptors with different ligands, capture or release of electrolytes or other substances that enter the cellular environment.

The system of cell cultures defined the following categories: tissue culture, organ culture, adherent cell cultures and in suspension cultures, histiotype or organotype cultures, mass culture and clonal primary cultures, finite cell cultures, continuous cell lines.

Cells will initially go through a quiescent or lag phase that depends on the cell type, the seeding density, the media components, and previous handling. The cells will then go into exponential growth where they have the highest metabolic activity. The cells will then enter into stationary phase where the number of cells is constant; this is characteristic of a confluent population (where all growth surfaces are covered).

Response tracking and characterization of cells cultivated in vitro and subjected to direct action of natural therapeutic factors will be used for cell cultures obtained through the specific processes within the cell culture laboratories INRMFB.

Assessing changes in cellular and molecular level can be achieved by optical microscopy studies, which is tracked cell morphology, cell viability studies, immunohistochemistry studies, studies conducted by proteomic techniques, including electrophoresis and Western blotting, determination of biochemical parameters based on the culture medium, cell physiology studies, studies on cellular senescence, cell signaling studies.

Optical microscopy studies. After each experimental variant cells are fixed with 10% paraformaldehyde and stained with hematoxylin-eosine (for general histology). Microscopy studies allow the assessment of cellular morphology throughout the experimental period.

Immunohistochemistry studies. These studies will aim to assess changes in cell markers, stress proteins (HSP 70, HSP 90), pro-and antiapoptotic proteins (p53, Bax, Bcl-2, caspase 3 and cytochrome c) and the main matrix constituents (fibronectin, laminin, collagen).

Our general experimental design for testing natural factors effects on cells in cultures implies three major steps. After the cell culture obtaining, the media for cells nutrition is prepared using the main chemicals found in the composition of the therapeutic factor.

In the second stage, the cells are grown in cultures using the medium prepared as described earlier for cells nutrition, mean time the cell morphology is monitored on microscope.

The last stage of the experimental design consists of entire cell culture lyses and the biochemical and molecular analyses of culture medium and of cellular lysate.
Experimental design for cell biology effects testing of natural therapeutic factors.
Morphological and electrophoretic data about heterogeneous primary skin cells cultures obtained from normal and Ovalbumin-Challenged Wistar rats after treatment by speleotherapy in the Cacica and Dej Romanian Salt Mines

Constantin Munteanu, PhD, ¹a Diana Munteanu, MBiol a, Iuri Simionca, PhD a, Mihai Hoteteu, a

¹(a) National Institute of Rehabilitation, Physical Medicine and Balneoclimatology, Bucharest, Romania

Abstract

Objective: To investigate the influence of salt mine medium from the Romanian Cacica and Dej Salt Mines upon the cell morphology and electrophoretic expression of heterogeneous skin cell cultures obtained from Wistar rats’ abdominal skin, on normal and Ovalbumin-sensitized animals.

Materials and methods: Heterogeneous skin cell cultures were prepared from Wistar abdominal skin. Cultures derived from skin rat develop with a monolayer of fibroblasts and epithelial cells attached to the culture dish. Before cultures initiation, Wistar rats of 75-100 g weight were divided in two lots: control and ovalbumin challenged animals. Ten animals of each lot were send to Cacica and Dej Salt Mine for 14 days and maintained in the salt mine medium, as in speleotherapy treatment.

Results: Speleotherapy of Wistar rats had induced significant differences in cell morphology and electrophoretic expression of primary dermal cells cultures. The data obtained support the protective effects of speleotherapy by comparing with ovalbumin sensibilised animals.

Conclusions: The results of this study indicate the fact that speleotherapy induces changes on the morphology and protein expression of dermal cells in vitro, and these changes support the beneficial effects of speleotherapy.

Key words: speleotherapy, dermal cells culture, salt mine

¹ Corresponding author: Constantin Munteanu, tel./fax: +40213186458, e-mail: constantin2378@yahoo.com, mailing address: B-dul Ion Mihalache, nr.11A, District 1, cod 79173, Bucharest, Romania, http://www.cell-culture.xhost.ro/
INTRODUCTION

Primary cell cultures can readily be obtained from human and animal skin using the explant method or trypsinisation.

Full thickness skin, also called the integument, is a composite of three tissues (epidermis, dermis and subcutaneous tissue), none of which constitutes a homogenous entity. Epidermis normally is composed of keratinocytes, which represent the largest population numerically, and lesser numbers of melanocytes, Langerhans’cells, and occasional cells of the lympho-reticular system, which are, however, transient members of the community.

Although the bulk of the dermis is noncellular (collagen and ground substance), within this compartment is also a variety of cell types, including fibroblasts, histicytes, mast cells, macrophages, lymphocytes and Schwann cells, endothelial cells of blood vessels and lymphatics, striated muscle cells of erector pili muscles, and smooth muscle of blood vessels.

The subcutaneous tissue includes most of the dermal cell types and fat cells as well (Flaxman, 1974).

The current study was designed to investigate the influence of salt mine medium from Cacica and Dej Salt Mines upon the cell morphology and electrophoretic expression of skin cells in vitro obtained from Wistar rats’ skin, in normal and Ovalbumin challenged conditions.

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$_2$HPO$_4$ x12 H$_2$O 8mM + KH$_2$PO$_4$ 1,4mM); HAM-F12 culture medium (Sigma); penicillin 100 U/ml, streptomycin 100µg/ml; fetal bovine serum (Sigma).

Rat Wistar Model of Allergic Asthma

Wistar rats of 75-100g weights were sensitized to Ovalbumin by subcutaneous injections with 100 µg of Ovalbumin.

Primary 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$_2$HPO$_4$ x12 H$_2$O 8mM + KH$_2$PO$_4$ 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 (usually in culture), microorganisms, thin tissue slices, lithographic patterns, fibers, latex dispersions, glass fragments, and subcellular particles (including nuclei and other organelles).
**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 skin cells cultures obtained from rats held on saline mine medium for the 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%.

**RESULTS**

Control skin cells 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 (fig.11). There are two types of cells: epithelial and fibroblastic.

Skin cells cultures of 7 days obtained from Ovalbumin sensitized rats 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 control case (fig.12).

Skin cells cultures of 7 days obtained from Ovalbumin-challenged rats. By phase contrast microscopy, it is possible to observe a rising of the cells number (fig.13).

Skin cells cultures of 7 days obtained from Ovalbumin sensitized and treated by speleotherapy in Dej Salt Mine shows also an improvement of the morphological parameters of the cells comparative with the cultures obtained from Ovalbumin-challenged rats. It is observed the rising of the cell population density and that of cell viability (fig.14).

Skin cells cultures were homogenized with Laemmli buffer pH 6.8, and the proteins of the obtained homogenate were separated by 10% SDS polyacrylamide gel electrophoresis that maintains polypeptides in a denatured state once they have been treated with strong reducing agents to remove secondary and tertiary structure.

Samples of 10µl were loaded into wells in the gel. One lane was reserved for Sigma molecular markers mixture of 205; 116; 97; 66; 55; 45; 36; 29; 24; 20,1; 14,2 and 6,5 KDa.

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).

Analysis with GeneTools version 4 software from SynGene of each track of the electrophoresis (fig.1), allowed us to compare the profiles of the total proteins expression (fig. 2- fig.10)

**DISCUSSION**

The present study evaluated morphological phenotypes related to repair and remodeling in fibroblasts and epithelial cells obtained from control Wistar rats and from Ovalbumin-sensitized and -challenged rats.
Compared with control culture, skin cells cultures from Ovalbumin-sensitized rats and Ovalbumin-sensitized treated in Cacica and Dej Salt Mines rats demonstrated the positive role of the saline medium for the sensitized rats.

The current study focused on skin cells, which are believed to play a major role in the organism – environment interaction. In this context, fibroblasts are believed to play a key role in maintaining and altering tissue structure. The ability of fibroblasts to migrate in response to chemotactic stimuli and to proliferate in response to specific growth factors is believed to control their accumulation at sites undergoing tissue repair. The ability of fibroblasts to produce and remodel extracellular matrix is thought to contribute to tissue structural changes. Remodeling of tissues likely involves fibroblast contractile activity.

In summary, the present study supports the concept that phenotypically altered fibroblasts can contribute to lesion repair in dermatological problems. Cells cultured from the skin of chronically OVA-sensitized and -challenged animals demonstrated consistently augmented repair responses for a number of functional assays (Sugiura et al., 2007).

CONCLUSIONS

- Phase contrast microscopy analyses of primary skin cells cultures reveals an cellular regeneration after animal exposure to saline medium in Cacica and Dej Salt Mines, comparative with the cells morphology of cultures from Ovalbumin sensitised rats.
- 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 Ovalbumin-sensitised animals to the saline medium from Cacica and Dej Salt Mines is reversing the cells morphopathology of skin cells in cultures;
- Wistar rats sensitised with Ovalbumin have a low number fibroblasts in skin cells cultures, with a more sensitive morphopathologic level.

Acknowledgments

This study will be finished in 2011 and is granted by The National Authority for Research - CNMP, contract nr. 42120/2008, project: Complex of medical-biological study of potential therapeutic factors related to salt mines and karst environments for effective use in health and balneo-turism; development and modelling solutions of these factors”, coordinated by Dr. Iuri Simionca.

References

TABLE 1: SDS polyacrylamide gel electrophoresis of the dermal cells cultures

<table>
<thead>
<tr>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Déj Salt Mine</td>
</tr>
<tr>
<td>2- 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine</td>
</tr>
<tr>
<td>3- 7 days skin cells culture from Ovalbumin-sensitised rats</td>
</tr>
<tr>
<td>4- 7 days Control skin cells culture</td>
</tr>
<tr>
<td>5- Sigma molecular markers</td>
</tr>
</tbody>
</table>

Fig. 1 - Electrophoretic profile of skin cells cultures

Track 4 7 days Control skin cells culture

Fig. 2 Densitogram of 7 days Control skin cells culture

Fig. 6 Profile matching for CONTROL (---) - OVALBMN (---)

Track 3 9 days skin cells culture from Ovalbumin-sensitised rats

Fig. 3 Densitogram of 7 days skin cells culture from Ovalbumin-sensitised rats

Fig. 7 Profile matching for CONTROL (---) - DEJ (---) - CACICA (---)

Track 2 9 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine

Fig. 4 Densitogram of 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine

Fig. 8 Profile matching for OVALBUMIN (---) - DEJ (---) - CACICA (---)

Track 1 9 days dermal cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Déj Salt Mine

Fig. 5 Densitogram of 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Déj Salt Mine

Fig. 9 Profile matching for CONTROL (---) OVALBUMIN (---) - DEJ (---) - CACICA (---)
### TABLE 2 Protein expression analysis of the skin cells cultures

<table>
<thead>
<tr>
<th>Peak Nr.</th>
<th>Peak weights molecular limits (KD)</th>
<th>CONTROL Quantity (µg/10µl)</th>
<th>OVALBUMIN Quantity (µg/10µl)</th>
<th>CACICA Quantity (µg/10µl)</th>
<th>DEJ Quantity (µg/10µl)</th>
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<tr>
<td>1</td>
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<td>24.72</td>
<td>18.30</td>
<td>23.20</td>
<td>30.22</td>
</tr>
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</table>

**TOTAL amount of proteins in 10 µl of sample:**

- CONTROL: 116.5
- OVALBUMIN: 79.45
- CACICA: 108.19
- DEJ: 85.74

**Fig. 10 TOTAL amount of proteins in 10 µl of sample**
Fig. 11 Control skin cells culture of 7 days, A-B X 150, C-D X 300

Fig. 12 7 days skin cells culture from Ovalbumin-sensitised rats, A-B X 150, C-D X 300
Fig. 13 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine, A-B X 150, C-D X 300

Fig. 14 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine, A-B X 150, C-D X 300
Morphological and electrophoretic data of primary pulmonary fibroblasts cultures obtained from normal and Ovalbumin-Challenged “Asthmatic” Wistar rats treated by speleotherapy in Cacica and Dej Romanian Salt Mines

Constantin Munteanu, PhD, Diana Munteanu, MBiol, Iuri Simionca, PhD, Mihai Hoteteu,

(a) National Institute of Rehabilitation, Physical Medicine and Balneoclimatology, Bucharest, Romania

Abstract

Objective: To investigate the influence of salt mine medium from the Romanian Cacica and Dej Salt Mines upon the cell morphology and electrophoretic expression of pulmonary fibroblasts in vitro obtained from Wistar rats’ lung, in normal and Ovalbumin challenged “asthmatic” conditions.

Materials and methods: Pulmonary fibroblasts cultures were prepared from Wistar rat lung. Cultures derived from lung rat develop with a monolayer of fibroblasts attached to the culture dish. Before cultures initiation, Wistar rats of 75-100 g weight were divided in two lots: control and ovalbumin challenged animals. Ten animals of each lot were send to Cacica and Dej Salt Mine for 14 days and maintained in the salt mine medium, as in speleotherapy treatment.

Results: Speleotherapy of Wistar rats had induced significant differences in cell morphology and electrophoretic expression of primary pulmonary fibroblasts cultures. The data obtained support the protective effects of speleotherapy by comparing with ovalbumin sensibilised animals.

Conclusions: The results of this study indicate the fact that speleotherapy induces changes on the morphology and protein expression of pulmonary fibroblasts in vitro, and these changes support the beneficial effects of speleotherapy.

Key words: speleotherapy, fibroblasts, salt mine

1 Corresponding author: Constantin Munteanu, tel./fax: +40213186458, e-mail: constantin2378@yahoo.com, mailing address: B-dul Ion Mihalache, nr.11A, District 1, cod 79173, Bucharest, Romania, http://www.cell-culture.xhost.ro/
INTRODUCTION

Asthma is a disorder characterized by chronic inflammation of the airways, airways hyper-responsiveness, and changes in airway architecture, termed remodeling. The cells responsible for maintenance of lung structure are the parenchymal cells of the lung, including epithelial cells, mesenchymal cells, and endothelial cells. Recent studies have suggested that the function of epithelial cells, smooth muscle cells, and fibroblasts cultured from lungs of individuals with asthma differs from the function of cells similarly cultured from individuals without asthma. These functional differences, particularly as they relate to repair and remodeling, could contribute airway structural alterations (Sugiura et al., 2007).

The current study was designed to investigate the influence of salt mine medium from Cacica and Dej Salt Mines upon the cell morphology and electrophoretic expression of pulmonary fibroblasts in vitro obtained from Wistar rats’ lung, in normal and Ovalbumin challenged “asthmatic” conditions.

Fibroblasts were cultured from lung parenchyma of control, ovalbumin-sensitized, and speleotherapy treated rats after ovalbumin-sensitization. Fibroblasts shape in culture can vary in accordance with the substrate, which on they is growing, and the space they have for movement.

Using pulmonary fibroblasts 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 pulmonary problems.

MATERIALS AND METHODS

**Materials:** Phosphate Buffer Solution (PBS: NaCl 0,13M + KCl 2,6mM + Na₂HPO₄ x12 H₂O 8mM + KH₂PO₄ 1,4mM); HAM-F12 culture medium (Sigma); penicillin 100 U/ml, streptomycin 100µg/ml; neomycin 50µg/ml, fetal bovine serum (Sigma).

**Rat Wistar Model of Allergic Asthma**

Wistar rats of 75-100g weights were sensitized to Ovalbumin by i.m. injections.

**Primary fibroblasts culture**

After anaesthesia with chloroform, rats were killed. The thorax was opened and then the lungs were removed 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 + Na₂HPO₄ x12 H₂O 8mM + KH₂PO₄ 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 (Sugiura et al, 2007; Foster et al, 1990; Nunez et al, 1995).

**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 (usually in culture), microorganisms, thin tissue slices, lithographic patterns, fibers, latex dispersions, glass fragments, and subcellular particles (including nuclei and other organelles).
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 saline mine medium for the 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%.

RESULTS
Control pulmonary fibroblasts culture of 9 days has a homogenic 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.

Pulmonary fibroblasts cultures of 9 days obtained from Ovalbumin sensitized rats presents many morphological changes from the control pulmonary fibroblasts culture, being observed an sensible number reducing of pulmonary fibroblasts in culture, the diminished cellular dividing frequency and an accentuated cellular morphopathology of the cells in culture. After 9 days of culturing, the pre-confluence level is much lower than in the control case.

Pulmonary fibroblasts cultures of 9 days obtained from Ovalbumin sensitized rats and treated by speleotherapy in Cacica Salt Mine shows an improvement of the morphological parameters of the cells comparative with the cultures obtained from Ovalbumin-challenged asthmatic rats. By phase contrast microscopy, it is possible to observe a rising of the cells number.

Pulmonary fibroblasts cultures of 9 days obtained from Ovalbumin sensitized and treated by speleotherapy in Dej Salt Mine shows also an improvement of the morphological parameters of the cells comparative with the cultures obtained from Ovalbumin-challenged asthmatic rats. It is observed the rising of the cell population density and that of cell viability.

Pulmonary fibroblasts were homogenized with Laemmli buffer pH 6.8, and the proteins of the obtained homogenate were separated by 10 % SDS polyacrylamide gel electrophoresis that maintains polypeptides in a denatured state once they have been treated with strong reducing agents to remove secondary and tertiary structure.

Samples of 10µl were loaded into wells in the gel. One lane was reserved for Sigma molecular markers mixture of 205; 116; 97; 66; 55; 45; 36; 29; 24; 20; 14,2 and 6,5 KDa.

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 in the gel (Towbin et al., 1979).

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.

DISCUSSION
The present study evaluated morphological phenotypes related to repair and remodeling in fibroblasts obtained from control Wistar rats and from Ovalbumin-sensitized and -challenged rats, a model of asthma that results in airway hyperresponsiveness.
and chronic airway remodeling, as other authors had presented.

Compared with control fibroblasts, fibroblasts obtained from lung parenchyma of the "asthmatic" rats and Ovalbumin-sensitized rats treated in Cacica and Dej Salt Mines demonstrated the positive role of the saline medium for the “asthmatic” rats.

The current study focused on fibroblasts, which are believed to be cells that play a major role in the maintenance and remodeling of interstitial connective tissue. In this context, fibroblasts are believed to play a key role in maintaining and altering tissue structure. The ability of fibroblasts to migrate in response to chemotactic stimuli and to proliferate in response to specific growth factors is believed to control their accumulation at sites undergoing tissue repair. The ability of fibroblasts to produce and remodel extracellular matrix is thought to contribute to tissue structural changes. Remodeling of tissues likely involves fibroblast contractile activity.

In summary, the present study supports the concept that phenotypically altered fibroblasts can contribute to airway remodeling in asthma. Fibroblasts cultured from the lungs of chronically OVA-sensitized and -challenged animals demonstrated consistently augmented repair responses for a number of functional assays (Sugiura et al., 2007).

CONCLUSIONS

• 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 Ovalbumin-sensitized animals to the saline medium from Cacica and Dej Salt Mines is reversing the cells morphopathology of pulmonary fibroblasts in cultures;

• Wistar rats sensitized with Ovalbumin have a low number pulmonary fibroblasts output cultures, with a more sensitive morphopathologic level.

Acknowledgments This study will be finished in 2011 and is granted by The National Authority for Research- CNMP, contract nr. 42120/2008, project: Complex of medical-biological study of potential therapeutic factors related to salt mines and karst environments for effective use in health and balneo-turism; development and modeling solutions of these factors.

References


TABLE 2 Protein expression analysis of the pulmonary fibroblasts cultures

<table>
<thead>
<tr>
<th>Peak Nr.</th>
<th>Peak weights molecular limits (kDa)</th>
<th>CONTROL Quantity (μg/10μl)</th>
<th>OVALBUMIN Quantity (μg/10μl)</th>
<th>CACICA Quantity (μg/10μl)</th>
<th>DEJ Quantity (μg/10μl)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>225 – 240</td>
<td>5.47</td>
<td>5.18</td>
<td>2.98</td>
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<td>2</td>
<td>220 – 225</td>
<td>3.37</td>
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<td>0.99</td>
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<td>3</td>
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<td>3.08</td>
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<td>6 – 19</td>
<td>18.64</td>
<td>12.62</td>
<td>16.80</td>
<td>15.94</td>
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TOTAL amount of proteins in 10 μl of sample:

- CONTROL: 80.66 μg
- OVALBUMIN: 67.41 μg
- CACICA: 81.95 μg
- DEJ: 88.72 μg

**Fig. 10** TOTAL amount of proteins in 10 μl of sample
TABLE 1: SDS polyacrylamide gel electrophoresis of the pulmonary fibroblasts cultures

<table>
<thead>
<tr>
<th>Samples</th>
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<tr>
<td>5- 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine</td>
</tr>
<tr>
<td>4- 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine</td>
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<tr>
<td>3- 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats</td>
</tr>
<tr>
<td>2- 9 days Control pulmonary fibroblasts culture</td>
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</table>

Fig. 1 - Electrophoretic profile of pulmonary fibroblasts cultures

Track 2 9 days Control pulmonary fibroblasts culture

Fig. 2 Densitogram of 9 days Control pulmonary fibroblasts culture

Fig. 6 Profile matching for
CONTROL ( ) - OVALBUMIN ( )

Track 3 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats

Fig. 3 Densitogram of 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats

Fig. 7 Profile matching for
CONTROL ( ) - DEJ ( ) - CACICA ( )

Track 4 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine

Fig. 4 Densitogram of 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine

Fig. 8 Profile matching for
OVALBUMIN ( ) - DEJ ( ) - CACICA ( )

Track 5 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine

Fig. 5 Densitogram of 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine

Fig. 9 Profile matching for
CONTROL ( )
OVALBUMIN ( ) - DEJ ( ) - CACICA ( )
Fig. 11 Control pulmonary fibroblasts cultures of 9 days, A-B X 150, C-D X 300

Fig. 12 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats, A-B X 150, C-D X 300
Fig. 13 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine, A-B X 150, C-D X 300

Fig. 14 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine, A-B X 150, C-D X 300
Therapeutical evaluation of Turda Salt Mine microclimate on pulmonary fibroblasts cultures

- Constantin Munteanu ¹, Diana Munteanu, Iuri Simionca, Mihai Hoteteu, Ovidiu Mera

Abstract
Objective: To investigate the influence of salt mine medium from the Turda Salt Mine upon the cell morphology and electrophoretic expression of pulmonary fibroblasts in vitro obtained from Wistar rats’ lung, in normal and Ovalbumin challenged - “asthmatic” conditions.

Materials and methods: Pulmonary fibroblasts cultures were prepared from Wistar rat lung. Cultures derived from lung rat develop with a monolayer of fibroblasts attached to the culture dish. Before cultures initiation, Wistar rats of 75-100 g weight were divided in two lots: control and ovalbumin challenged animals. Five animals of each lot were send to Turda Salt Mine for 14 days and maintained in the salt mine medium, as in speleotherapy treatment.

Results: Speleotherapy of Wistar rats had induced significant differences in cell morphology and electrophoretic expression of primary pulmonary fibroblasts cultures. The data obtained support the protective effects of speleotherapy by comparing with ovalbumin sensibilised animals.

Conclusions: The results of this study indicate the fact that speleotherapy induces changes on the morphology and protein expression of pulmonary fibroblasts in vitro, and these changes support the therapeutical properties of Turda Salt Mine medium.

Key words: speleotherapy, fibroblasts, salt mine.

¹ Corresponding author: Constantin Munteanu, tel./fax: +40213186458, e-mail: constantin2378@yahoo.com, mailing address: B-dul Ion Mihalache, nr.11A, District 1, cod 79173, Bucharest, Romania, http://www.cell-culture.xhost.ro/
INTRODUCTION

Asthma is a disorder characterized by chronic inflammation of the airways, airways hyper-responsiveness, and changes in airway architecture, termed remodeling. The cells responsible for maintenance of lung structure are the parenchymal cells of the lung, including epithelial cells, mesenchymal cells, and endothelial cells. Recent studies have suggested that the function of epithelial cells, smooth muscle cells, and fibroblasts cultured from lungs of individuals with asthma differs from the function of cells similarly cultured from individuals without asthma. These functional differences, particularly as they relate to repair and remodeling, could contribute airway structural alterations (Sugiura et al., 2007).

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Fibroblasts were cultured from lung parenchyma of control, ovalbumin-sensitized, and speleotherapy treated rats after ovalbumin-sensitization. Fibroblasts shape in culture can vary in accordance with the substrate, which on they is growing, and the space they have for movement.

Using pulmonary fibroblasts cultures to verify the therapeutic properties of Turda Salt Mine medium, described as speleotherapy, represents an innovative and scientific way to establish the medical methodology of preventing, treating and recovery of patients with various pulmonary problems.
MATERIALS AND METHODS

Materials

Phosphate Buffer Solution (PBS: NaCl 0.13M + KCl 2.6mM + Na₂HPO₄ x12 H₂O 8mM + KH₂PO₄ 1.4mM); HAM-F12 culture medium (Sigma); penicillin 100 U/ml, streptomycin 100µg/ml; neomycin 50µg/ml (Sigma); fetal bovine serum (Sigma).

Rat Wistar Model of Allergic Asthma

Wistar rats of 75-100g weights were sensitised to Ovalbumin by i.m. injections.

Primary fibroblasts culture

After anaesthesia with chloroform, rats were killed. The thorax was opened and then the lungs were removed 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 + Na₂HPO₄ x12 H₂O 8mM + KH₂PO₄ 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 (Sugiura et al, 2007; Foster et al, 1990; Nunez et al, 1995).

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 Turda Saline Mine medium for the 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%.
RESULTS

Control pulmonary fibroblasts culture of 7 days has a homogenic 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.

Pulmonary fibroblasts cultures of 7 days obtained from Ovalbumin sensitized rats presents many morphological changes from the control pulmonary fibroblasts culture, being observed an sensible number reducing of pulmonary 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 control case.

Pulmonary fibroblasts cultures of 7 days obtained from Ovalbumin sensitized rats and treated by speleotherapy in Turda Salt Mine shows an improvement of the morphological parameters of the cells comparative with the cultures obtained from Ovalbumin-challenged asthmatic rats.

Pulmonary fibroblasts were homogenized with Laemmli buffer pH 6,8, and the proteins of the obtained homogenate were separated by 10 % SDS polyacrylamide gel electrophoresis that maintains polypeptides in a denatured state once they have been treated with strong reducing agents to remove secondary and tertiary structure.

Samples of 10µl were loaded into wells in the gel. One lane was reserved for Sigma molecular markers mixture of 205; 116; 97; 66; 55; 45; 36; 29; 24; 20; 14,2 and 6,5 KDa

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).
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.

The data obtained confirm our observations of optical microscopy, was detected a lower level of 130µg total protein from induced asthma for as against 160 mg in control. Speleotherapy cure in Turda Salt Mine restored this parameter to a value close to control, ie 155 mg total protein.

CONCLUSIONS

Microscopy analyses of primary fibroblasts cultures reveal a cellular regeneration after animal exposure to saline medium in Turda Salt Mine, comparative with the cells morphology of cultures from Ovalbumin sensitized rats.

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 Ovalbumin-sensitized animals to the saline medium from Turda Salt Mine is
reversing the cells morphopathology of pulmonary fibroblasts in cultures;

**DISCUSSION**

The present study evaluated morphological phenotypes related to repair and remodeling in fibroblasts obtained from control Wistar rats and from Ovalbumin-sensitized and -challenged rats, a model of asthma that results in airway hyperresponsiveness and chronic airway remodeling, as other authors had presented.

Compared with control fibroblasts, fibroblasts obtained from lung parenchyma of the "asthmatic" rats and Ovalbumin-sensitized rats treated in Turda Salt Mine demonstrated the positive role of the saline medium for the “asthmatic” rats.

The current study focused on fibroblasts, which are believed to be cells that play a major role in the maintenance and remodeling of interstitial connective tissue. In this context, fibroblasts are believed to play a key role in maintaining and altering tissue structure. The ability of fibroblasts to migrate in response to chemotactic stimuli and to proliferate in response to specific growth factors is believed to control their accumulation at sites undergoing tissue repair. The ability of fibroblasts to produce and remodel extracellular matrix is thought to contribute to tissue structural changes. Remodeling of tissues likely involves fibroblast contractile activity.

In summary, the present study supports the concept that phenotypically altered fibroblasts can contribute to airway remodeling in asthma. Fibroblasts cultured from the lungs of chronically OVA-sensitized and -challenged animals demonstrated consistently augmented repair responses for a number of functional assays (Sugiura et al., 2007).

**Acknowledgments**

This study was funded by contract nr. 5366 bis./2010 with SC Turda Salina Durgău SA, as the beneficiary of the results.

**References**

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

- Constantin Munteanu 1, Diana Munteanu, Iuri Simionca, Mihai Hoteteu

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.

1 Corresponding author: Constantin Munteanu, tel./fax: +40213186458, e-mail: constantin2378@yahoo.com, Mmailing address: B-dul Ion Mihalache, nr.11A, District 1, cod 79173, Bucharest, Romania, http://www.cell-culture.xhost.ro/
INTRODUCTION

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 + Na₂HPO₄ x12 H₂O 8mM + KH₂PO₄ 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 cm² 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 + Na₂HPO₄ x12 H₂O 8mM + KH₂PO₄ 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).
RESULTS

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.

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References
Balneary PUBLISHING HOUSE serves to disseminate and promote research conducted by the National Institute of Rehabilitation, Physical Medicine and balneoclimatology in order to contribute to validating the therapeutic ability of natural factors spas.

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The manuscripts will be submitted as attachment to the email in Word format (to culturi@gmail.com). Photo processing, scanning, graph processing – if needed – are the responsibility of the editing team. Language of papers is English. Articles can be published with translation into Romanian.

After manuscript receipt, the corresponding author will receive a short e-mail confirming the receipt which will contain the registration number, the date the manuscript was received and the fact that the manuscript was handed out to the Editorial Board. The Journal Editor chooses 2 peer-reviewers (from the Editorial and Peer-review Board) and sends them by e-mail the manuscript.

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