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*Research article*

# The effects of Mineral Waters from Slănic Moldova's Spring 1 and Spring 1 bis on Fibroblast activity: An In Vitro Study

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**Abstract:** Primary fibroblast cultures obtained from Wistar rats were investigated with a focus on two vital physiological mechanisms: inflammatory processes and oxidative stress balance. These are believed to be affected by mud and sulfurous natural mineral waters, forming the fundamental biological basis for understanding the therapeutic effects of these substances. Existing scientific research highlights that various cell types, including fibroblasts, are recruited during inflammation. These cells respond to a wide array of intercellular and microenvironmental signals, leading to a regulated production of both pro- and anti-inflammatory mediators. Examples include cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6, as well as chemokines and enzymes like cyclooxygenase (COX)-2. Together, these play vital roles in modulating the inflammatory response.

**Keywords:** Fibroblasts, Sulphurous Mineral Waters, Cytokines, Tumor Necrosis Factor (TNF)-α, Interleukins (IL)-1β and IL-6, Chemokines, Cyclooxygenase (COX)-2

## 1. Introduction

Research data show that different types of cells are recruited during the inflammatory process, including fibroblasts, which respond to different intercellular and microenvironmental signals (1). This leads to the regulated production of various pro- and anti-inflammatory mediators, including cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β and IL-6, chemokines and enzymes such as cyclooxygenase ( COX)-2, which play critical roles in controlling the inflammatory process (2,3).

The concept of oxidative stress caused by free radicals argues for the consideration of biomarkers of oxidative stress (4–8). The oxidative and reductive activity of enzymes that act on glutathione, thioredoxin, and other substrates of interest in the oxidation-reduction process reflect the level of antioxidant protection and are also relevant biomarkers for rheumatic degenerative diseases (9–11).

In the case of natural sulfurous mineral waters, the protective effect of $H_2S$ on neurons is

expressed against oxidative stress by increasing the substrate for producing the antioxidant GSH, including the cystine/glutamate antiporter and intracellular Cys concentrations (12,13). $H_2S$ has vasculoprotective properties in endothelial cells and vascular smooth muscle cells, such as triggering vasorelaxation and decreasing platelet aggregation. $H_2S$ possibly activates plasma membrane voltage-dependent channels (L-type and T-type Ca2+ channels) and mobilizes intracellular Ca2+ stores (14–16). In addition, endogenous $H_2S$ has been found to activate chloride (Cl–) channels and potassium (K+) channels, providing neuroprotective effects. $H_2S$ donors or $H_2S$-rich mineral waters target multiple pathophysiological mechanisms (17–21).

The skin, with an area of almost 2 m2, has an essential role in defending the human body against microorganisms in the environment and also in maintaining water homeostasis (22,23). When the wound damages the integrity of the skin, the body immediately begins the healing process using a sequence of biochemical and biophysical events to restore the integrity of the skin at the site of the wound (24,25).

Cell culture has been and is used as a significant tool to investigate the healing process. This method allows the study of the biological behavior of cells, taking into account either the kinetics of cell proliferation or the biosynthesis of several components of the extracellular matrix (26–28).

Understanding wound healing at the cellular level is essential to prevent unwanted outcomes such as increased scarring or fibrosis. After dermal injury, regenerative processes begin immediately to close the wound and slowly restore tissue integrity rapidly (29). Different cell types tightly regulate wound healing and many cytokines and involve interactions with the extracellular matrix (ECM). After the initial closure of the wound by a fibrin clot, resident dermal fibroblasts, and putative progenitor cells are attracted to the wound site. They deposit ECM proteins and exert forces on the existing ECM, leading to tissue contraction. Thus, the surrounding ECM becomes stiffer, and the prestressed matrix transforms fibroblasts into pro-to-myofibroblasts containing actin stress fibers. Prestressed matrices require stronger traction forces to ensure wound closure (30–33).

For this reason, pro-to-myofibroblasts differentiate into myofibroblasts. These cells possess a pronounced cytoskeleton, enhanced production of ECM molecules (e.g., collagen I and III, fibronectin, and proteoglycans), and a strong capacity for tissue contraction, which is accomplished by incorporating alpha-smooth muscle actin ($\alpha$SMA) in their actin stress fibers. $\alpha$SMA incorporation is one of the most prominent markers of myofibroblast differentiation (34,35).

TGF$\beta$1 is known to be a key player in wound healing, particularly in myofibroblast differentiation. This pro-inflammatory and heparin-binding cytokine is secreted by immune cells, proto-myofibroblasts, and myofibroblasts in a temporally defined paracrine and autocrine manner. But lack of resolution of TGF$\beta$1 release, sustained inflammation, and disrupted Smad signaling (intracellular signal transducers of TGF$\beta$1 signaling) causes myofibroblasts to contract and produce ECM over prolonged periods, leading to hypertrophic scarring, excess fibrous tissue, fibrosis, and associated loss of tissue function. Therefore, TGF$\beta$1 is also used as a target for preventing fibrotic diseases (36,37).

## 2. Brief presentation of the mineral waters from Spring 1 and Spring 1 bis

**Spring 1 from Slanic Moldova** contains a combination of essential electrolytes and minerals, each offering distinctive therapeutic benefits for various medical conditions, especially those related to the digestive system. The key components include:

- **Bicarbonate (HCO3):** Vital for regulating the acid-alkaline balance in the body, bicarbonate may reduce gastric reflux symptoms and improve overall digestive function, potentially aiding in the treatment and prevention of kidney disease (38).
- **Sodium (Na+), Potassium (K+), and Calcium (Ca2+):** These essential electrolytes are crucial for regulating heart rate, muscle, and nerve function (39–43). They can enhance digestive function and alleviate symptoms of biliary dyskinesia and chronic cholecystitis.
- **Chloride (Cl):** An integral contributor to fluid and electrolyte balance, chloride supports the digestive system's functions (44).
- **Sulfate (SO4):** This compound can aid in detoxification, helping to remove toxins and heavy metals (45).
- **Magnesium (Mg):** Involved in over 300 enzyme reactions in the body, magnesium plays roles in metabolizing food, synthesizing fatty acids and proteins, and transmitting nerve impulses (46).
- **Hydrogen Sulfide (H2S) and Carbon Dioxide (CO2):** These dissolved gases possess therapeutic potential, such as improving mitochondrial function and reducing inflammation (47).

The water's therapeutic indications are wide-ranging, extending to the internal treatment of various gastric and hepatobiliary conditions and metabolic diseases like gout, obesity, and dyslipidemias.

**Spring 1bis from Slanic Moldova**, another significant spring, has a specific chemical composition that confers various potential health benefits. Its components include:

- **CO2 (Carbon Dioxide):** Promotes digestion and nutrient absorption by stimulating gastric juice production (48).
- **HCO3 (Bicarbonate):** Helps regulate pH levels and reduce stomach acidity symptoms.
- **Cl (Chloride), Na (Sodium):** Essential for maintaining electrolyte balance and proper cell and nerve functioning.
- **Br (Bromine):** A trace element potentially impacting thyroid function and nervous system health.
- **SO4 (Sulfate), H2S (Hydrogen Sulfide):** Possesses antimicrobial properties and may reduce inflammation (49).
- **K (Potassium), Ca (Calcium), Mg (Magnesium):** Vital for muscle function, bone health, and nerve function (50).

The therapeutic applications of these waters are diverse, ranging from internal treatments for gastric and hepatobiliary disorders (including chronic gastroduodenitis, unspecified enterocolitis, biliary dyskinesia, chronic alithiasis cholecystitis, and post-cholecystectomy disorders) to utilization as sprays and aerosols for chronic respiratory and ENT diseases (51–53).

## 2. Material și Metode

Water Sample Details. The quality of natural factors is substantially determined by their physicochemical properties resulting from the composition of mineral or organic substances, dissolved gases, or suspended particles, and living organisms present.

Primary Fibroblast Cell Cultures and Their Relevance to Study

Fibroblasts have essential roles in various physiological processes, such as extracellular matrix (ECM) synthesis, epithelial differentiation, inflammation regulation, and wound healing. They are also responsible for the secretion of growth factors and act as a scaffold for several other cell types, mediating tissue fibrosis and scar formation (54,55).

Fibroblasts are easy to grow and maintain in vitro, and human fibroblast lines established from patient biopsies have been used in numerous studies to elucidate the pathogenesis of several diseases (56).

Fibroblast collection is done by explant, isolating the dermis from the epidermis with scalpels and scissors. Derm samples are then fragmented into 5.0 mm2 pieces and placed on 100 mm2 Petri dish surfaces in square areas marked by perpendicular lines made with scalpel blades. The Petri dishes are kept semi-open in laminar flow for 40 minutes to adhere the dermis specimens to the culture surface, ensuring the desiccation of dermis samples is avoided by instilling physiological solution over them to maintain cellular viability (57,58).

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) Assay is a cellular viability assay based on the ability of living cells' mitochondria to reduce tetrazolium salts into dark blue formazan compounds (59). This reaction occurs only in living cells, so the color intensity is directly proportional to the number of viable cells. To conduct this test in primary fibroblast culture, the following steps are taken:

Cell Seeding: Seed the cells in 48-well plates, with 10,000 cells per well, in a complete DMEM F12/HAM medium.

- Cell Inoculation: Allow the cells to grow in an incubator until they reach approximately 80% confluence.
- MTT Addition: Add the MTT solution to the culture medium (usually at a final concentration of 0.5 mg/ml) and incubate the cells for 2-4 hours, during which the MTT will be reduced to formazan by the living cells.
- Formazan Dissolution: Remove the MTT solution from the cells and add a solvent (usually DMSO or ethanol) to dissolve the formazan crystals.
- Absorption Measurement: Use a spectrophotometer to measure the absorption at a wavelength of 570 nm. The color intensity will be directly proportional to the number of viable cells.

Electrophoresis is an analytical and preparative method for separating electrically charged particles and particle aggregates under the influence of a uniformly applied external electric field. The technique relies on the physicochemical phenomenon of differential movement or migration of different particles within an electric field (60).

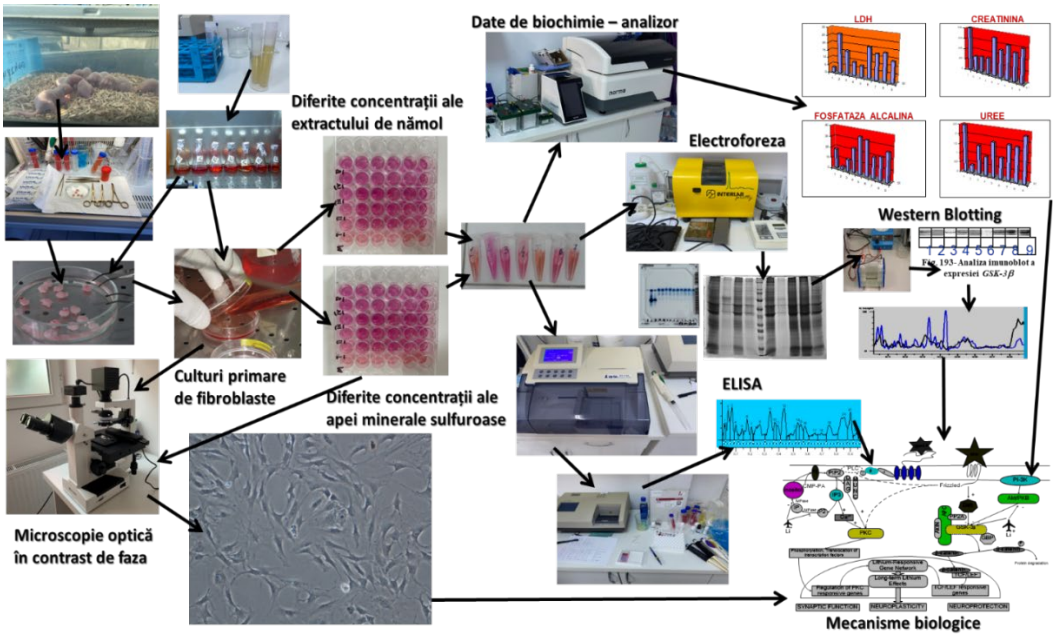
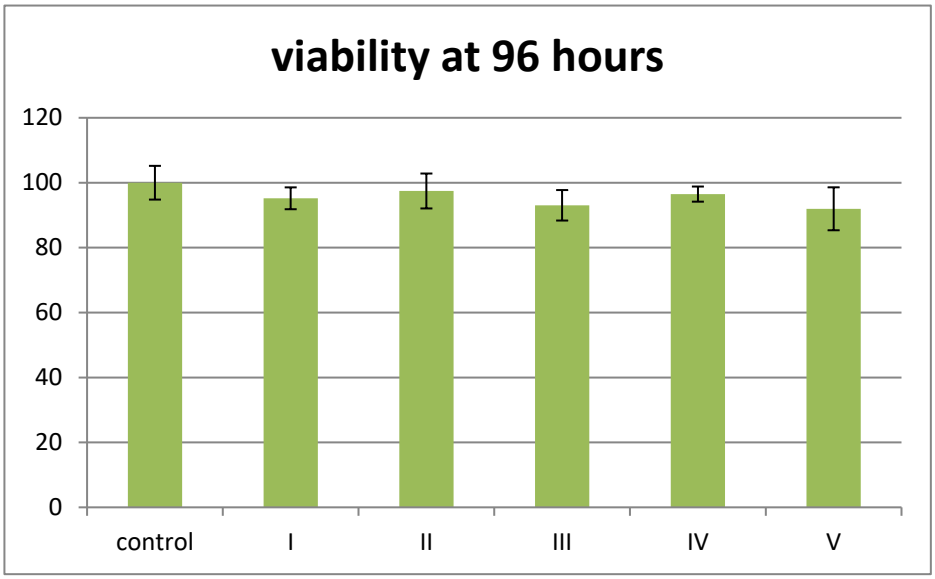



**Figure 1 Original experimental setups on primary fibroblast cell cultures – Biological investigations**

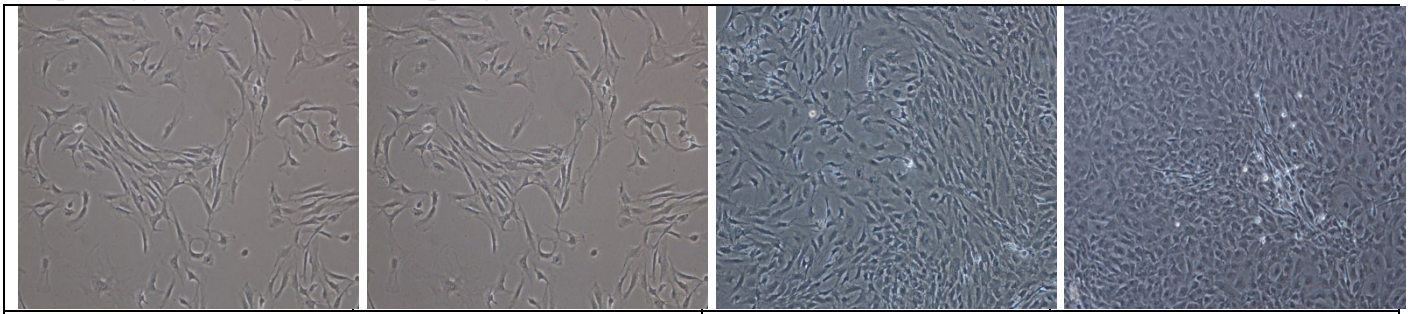
## 3. Results

Primary fibroblasts, 48-well plates, 10,000 cells per well, complete DMEM F12/HAM medium

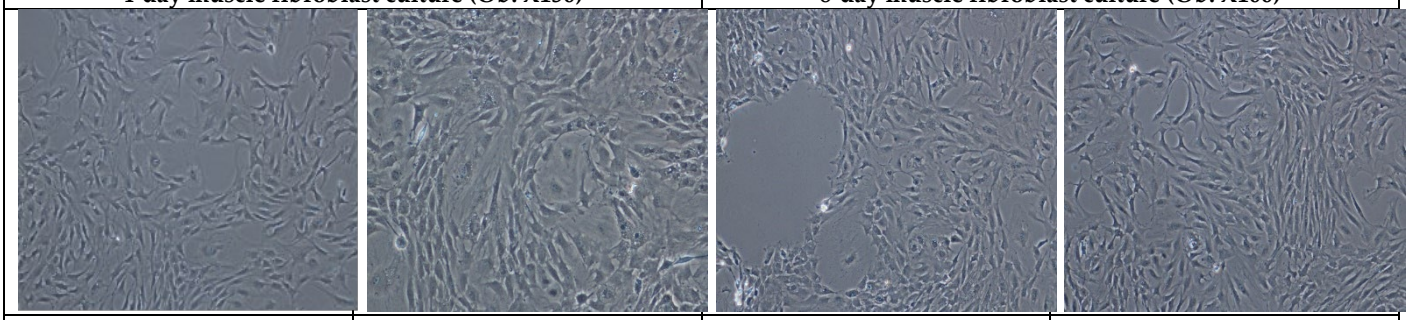
| | viability at 96 hours | Standard error |
|---|---|---|
| Control fibroblast culture | 100 | 5,195607 |
| I - Spring 1 – 15 % | 95,20078 | 3,365599 |
| II- Spring 1 – 10% | 97,45348 | 5,378499 |
| III- Spring 1 – 20% | 93,04603 | 4,696374 |
| IV- Spring 1 bis – 10 % | 96,49363 | 2,328495 |
| V- Spring 1 bis– 20 % | 91,96866 | 6,614756 |



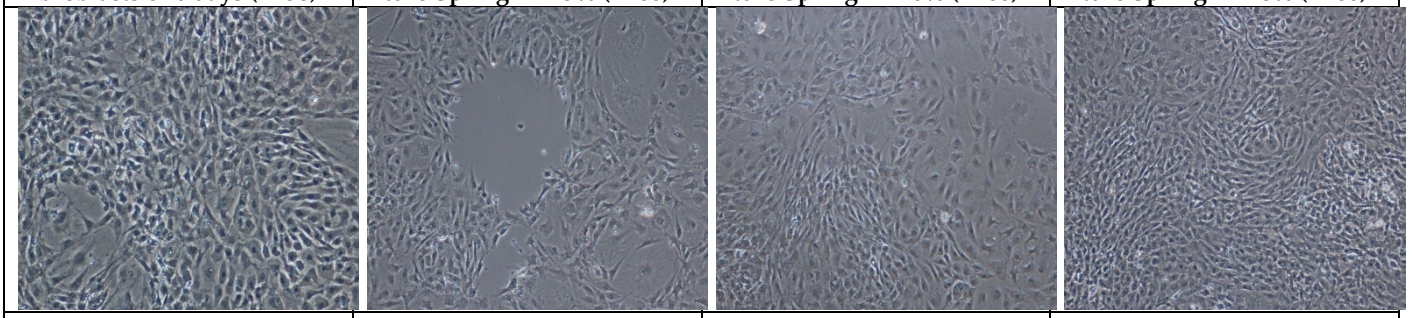
**Morphology results - experiment Spring 1**



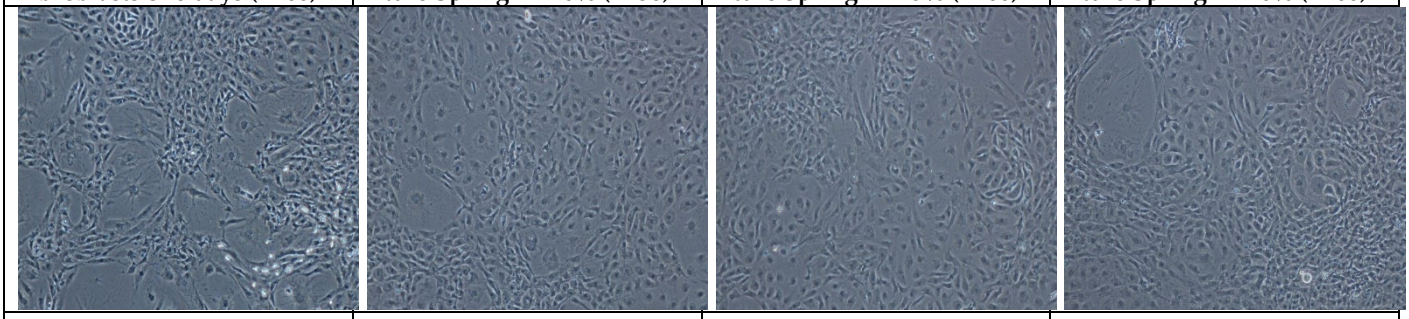
**4-day muscle fibroblast culture (Ob. X150)** | **8-day muscle fibroblast culture (Ob. X100)**



| **Control culture of muscle fibroblasts of 6 days (X150)** | **6-day muscle fibroblast culture Spring 1 – 15% (X200)** | **6-day muscle fibroblast culture Spring 1 – 20% (X100)** | **6-day muscle fibroblast culture Spring 1 – 10% (X100)** |
|---|---|---|---|



| **Control culture of muscle fibroblasts of 8 days (X200)** | **8-day muscle fibroblast culture Spring 1 – 20% (X150)** | **8-day muscle fibroblast culture Spring 1 – 15% (X100)** | **8-day muscle fibroblast culture Spring 1 – 10% (X100)** |
|---|---|---|---|



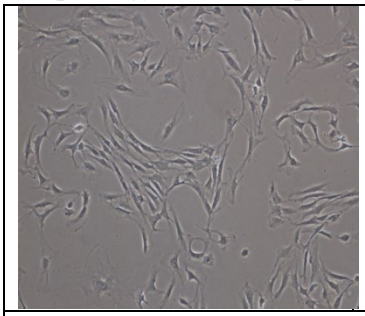
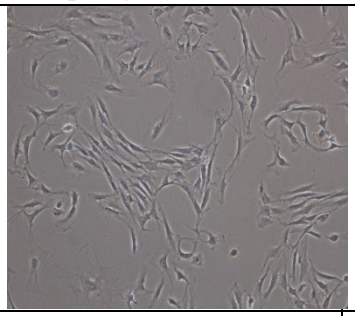
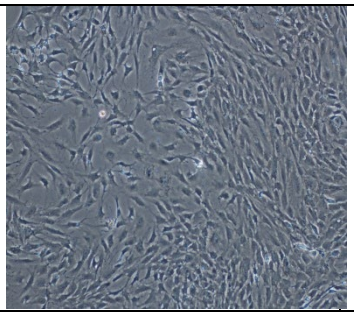
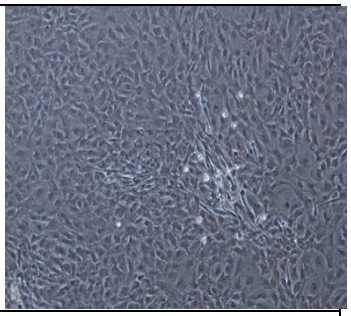
| **Control culture of muscle fibroblasts of 12 days (X100)** | **12-day muscle fibroblast culture Spring 1 – 20% (X100)** | **12-day muscle fibroblast culture Spring 1 – 15% (X100)** | **12-day muscle fibroblast culture Spring 1 – 10% (X100)** |
|---|---|---|---|

**Conclusions:**

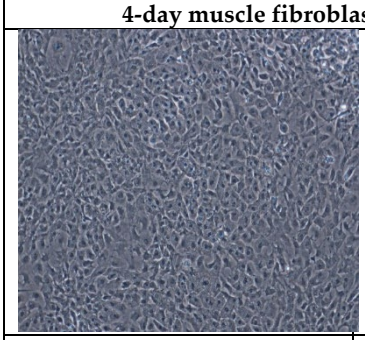
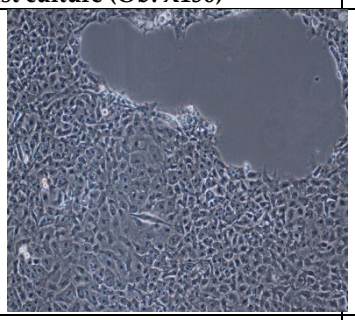
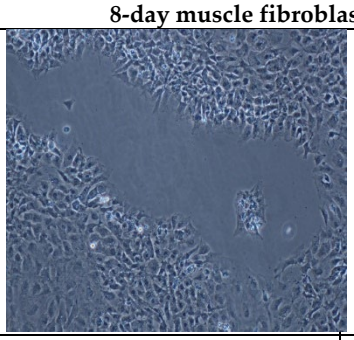
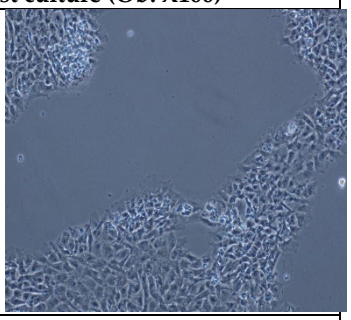
1. Although during the experiment, areas appear where, due to the administered mineral waters, voids are formed in the monolayer, at the end of the investigation, the appearance of the cultures becomes uniform, being similar to that of the control case;
2. The culture of muscle fibroblasts obtained by seeding the cells at the density of 50,000 cells per plate reaches the level of confluence from the 5th day of cultivation;

Morphologically, no significant differences exist between the analyzed cases and the control.

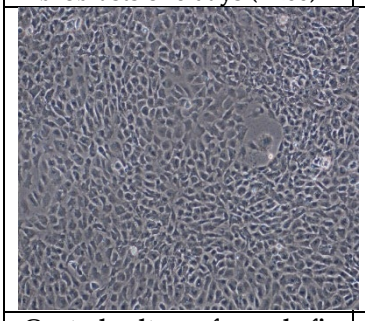
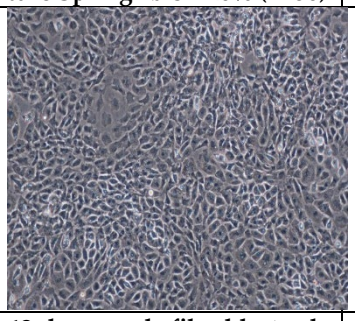
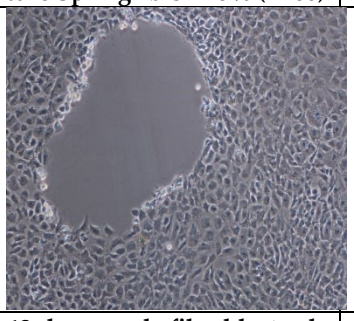
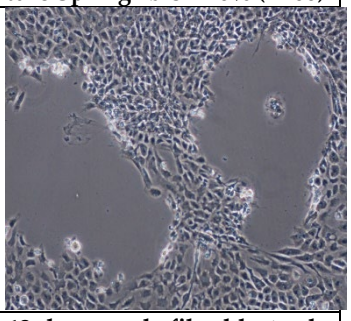
**Morphology results - experiment Spring 1 bis**

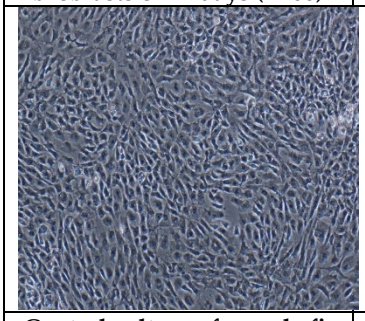
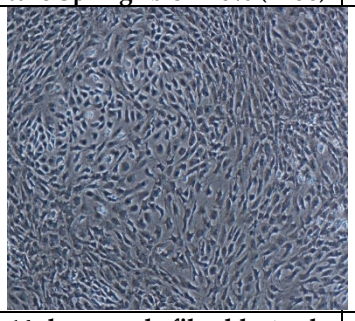
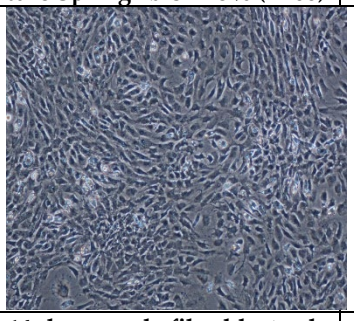
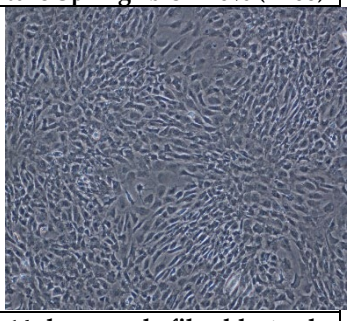
**4-day muscle fibroblast culture (Ob. X150)** | **8-day muscle fibroblast culture (Ob. X100)**

| Control culture of muscle fibroblasts of 8 days (X200) | 8-day muscle fibroblast culture Spring 1bis – 20% (X150) | 8-day muscle fibroblast culture Spring 1bis – 15% (X100) | 8-day muscle fibroblast culture Spring 1bis – 10% (X100) |
|---|---|---|---|

| Control culture of muscle fibroblasts of 12 days (X200) | 12-day muscle fibroblast culture Spring 1bis – 20% (X150) | 12-day muscle fibroblast culture Spring 1bis – 15% (X100) | 12-day muscle fibroblast culture Spring 1bis – 10% (X100) |
|---|---|---|---|

| Control culture of muscle fibroblasts of 14 days (X100) | 14-day muscle fibroblast culture Spring 1bis – 20% (X100) | 14-day muscle fibroblast culture Spring 1bis – 15% (X100) | 14-day muscle fibroblast culture Spring 1 bis – 10% (X100) |
|---|---|---|---|

**Conclusions:**

1. Although during the experiment, areas appear where, due to the administered mineral waters, voids are formed in the monolayer, at the end of the investigation, the morphology of the cultures becomes uniform, being similar to that of the control case;

2. The culture of muscle fibroblasts obtained by seeding the cells at the density of 50,000 cells per plate reaches the level of confluence from the 5th day of cultivation;

Morphologically, no significant differences exist between the analyzed cases and the control.

**Discussion and Conclusion**

Studies by Gambari et al. (2020) (61) have confirmed that in vitro stimulation with sulfurous thermal waters increases the intracellular concentration of $H_2S$ in osteocytes. These waters act as an alternative type of $H_2S$ donor. Tests revealed higher intracellular $H_2S$ levels after treatment with sulfurous thermal waters containing 100 mg/L H2S than those frequently used with 8 mg/L $H_2S$. Furthermore, the intracellular concentration of $H_2S$ can be modulated through different dilutions of sulfurous thermal waters. The analysis demonstrated that these waters significantly increased the expression of osteogenic markers such as BSP, OC, RUNX-2, and OPN and had varying effects on cell viability depending on $H_2S$ concentration (62).

Used for years in balneotherapy and dermatology, La Roche-Posay thermal water has shown protective effects against harmful oxidative species, e.g., UV light. Rich in elements like bicarbonate, silicate, and selenium (53 μg/L), it exhibits antioxidant, immunomodulatory, anti-inflammatory, and anti-irritant effects. It even acts as a probiotic due to its unique microbial composition. These findings justify its therapeutic use in inflammatory skin conditions (51).

Nitrodi water promotes the viability and migration of dermal fibroblasts, stimulating protein expression in skin health. It also reduces reactive oxygen species (ROS), enabling the anti-aging effect and skin protection (53).

A study (52) revealed that Avène mineral water modulates immune responses by affecting the differentiation and maturation of dendritic cells, leading to decreased production of inflammatory molecules. This implies an immunomodulatory potential that limits inflammatory responses.

Studies on human keratinocytes with waters like Comano, BJ1, and BG have shown anti-inflammatory and anti-angiogenic effects, depending on their specific chemical compositions. These findings provide insights into therapeutic benefits for skin diseases such as rosacea and psoriasis (63).

Influence on Endothelial Cells: Hydrogen sulfide affects the migration and survival of endothelial cells to form vascular networks through genes promoting the secretion of VEGF, which in turn inhibits apoptosis (64,65).

Involvement in Inflammatory Processes: Mitogen-activated protein kinases (MAPK) are central to inflammatory processes. Studies with inhibitors and $H_2S$ donors have shown effects on IL-6 and IL-8 expression, indicating potential therapeutic applications (66,67).

Effects on Human Chondrocytes: Research (68) into human chondrocytes and mesenchymal progenitor cells' ability to synthesize $H_2S$ in response to pro-inflammatory mediators shed light on their reactions to exogenous sources of slow-releasing $H_2S$ (69–72).

In conclusion, studies show their potential in modulating biological processes, enhancing skin health, and offering new avenues for treating inflammatory and autoimmune disorders. While some findings are still preliminary, they pave the way for further exploration and clinical applications.

**References:**


1. Wang Z, Qi F, Luo H, Xu G, Wang D. Inflammatory Microenvironment of Skin Wounds. Front Immunol. 2022;13(March):1–17.
2. Landskron G, De La Fuente M, Thuwajit P, Thuwajit C, Hermoso MA. Chronic inflammation and cytokines in the tumor microenvironment. J Immunol Res. 2014;2014.
3. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018;9(6):7204–18.
4. Ghezzi P. Environmental risk factors and their footprints in vivo – A proposal for the classification of oxidative stress biomarkers. Redox Biol [Internet]. 2020;34(January):101442. Available from: https://doi.org/10.1016/j.redox.2020.101442
5. Azzi A. Oxidative Stress: What Is It? Can It Be Measured? Where Is It Located? Can It Be Good or Bad? Can It Be Prevented? Can It Be Cured? Antioxidants. 2022;11(8).
6. Frijhoff J, Winyard PG, Zarkovic N, Davies SS, Stocker R, Cheng D, et al. Clinical Relevance of Biomarkers of Oxidative Stress. Antioxidants Redox Signal. 2015;23(14):1144–70.
7. Tejchman K, Kotfis K, Sieńko J. Biomarkers and mechanisms of oxidative stress—last 20 years of research with an emphasis on kidney damage and renal transplantation. Int J Mol Sci. 2021;22(15).
8. Stoica SI, Onose G, Pitica IM, Neagu AI, Ion G, Matei L, et al. Molecular Aspects of Hypoxic Stress Effects in Chronic Ethanol Exposure of Neuronal Cells. Curr Issues Mol Biol. 2023;45(2):1655–80.
9. Munteanu C, Munteanu D, Hoteteu M. Balneotherapy and healthy ageing - review. Balneo Res J DOI http//dx.doi.org/1012680/balneo20141066 Vol5, No2, May 2014. 2014;5(2):62–82.
10. Yang HY, Lee TH. Antioxidant enzymes as redox-based biomarkers: A brief review. BMB Rep. 2015;48(4):200–8.
11. Stoica SI, Bleotu C, Ciobanu V, Ionescu AM, Albadi I, Onose G, et al. Considerations about Hypoxic Changes in Neuraxis Tissue Injuries and Recovery. Biomedicines. 2022;10(2):1–19.
12. Munteanu C, Dogaru G, Rotariu M, Onose G. Therapeutic gases used in balneotherapy and rehabilitation medicine - scientific relevance in the last ten years (2011 – 2020) - Synthetic literature review. Balneo PRM Res J. 2021;12(Vol.12, 2):111–22.
13. Xie ZZ, Liu Y, Bian JS. Hydrogen Sulfide and Cellular Redox Homeostasis. Oxid Med Cell Longev. 2016;2016.
14. Dai L, Qian Y, Zhou J, Zhu C, Jin L, Li S. Hydrogen sulfide inhibited L-type calcium channels (CaV1.2) via up-regulation of the channel sulfhydration in vascular smooth muscle cells. Eur J Pharmacol [Internet]. 2019;858(January):172455. Available from: https://doi.org/10.1016/j.ejphar.2019.172455
15. Dogaru BG, Munteanu C. The Role of Hydrogen Sulfide ( H 2 S ) in Epigenetic Regulation of Neurodegenerative Diseases : A Systematic Review. 2023;
16. Munteanu C. Hydrogen Sulfide and Oxygen Homeostasis in Atherosclerosis: A Systematic Review from Molecular Biology to Therapeutic Perspectives. Int J Mol Sci. 2023;24(9).
17. Munteanu C, Rotariu M, Turnea M, Dogaru G, Popescu C, Spînu A, et al. Recent Advances in Molecular Research on Hydrogen Sulfide (H2S) Role in Diabetes Mellitus (DM)—A Systematic Review. Int J Mol Sci 2022 Jun 16;23(12)6720 doi 103390/ijms23126720 PMID 35743160; PMCID PMC9223903.
18. Aschner M, Skalny A V., Ke T, da Rocha JB, Paoliello MM, Santamaria A, et al. Hydrogen Sulfide (H2S) Signaling as a Protective Mechanism against Endogenous and Exogenous Neurotoxicants. Curr Neuropharmacol. 2022;20(10):1908–24.
19. Zhang JY, DIng YP, Wang Z, Kong Y, Gao R, Chen G. Hydrogen sulfide therapy in brain diseases: From bench to bedside. Med Gas Res. 2017;7(2):113–9.
20. Munteanu C, Munteanu D, Onose G. Hydrogen sulfide (H2S) - therapeutic relevance in rehabilitation and

balneotherapy Systematic literature review and meta-analysis based on the PRISMA paradig. Balneo PRM Res J. 2021;12(3):176–95.

21. Tabassum R, Jeong NY. Potential for therapeutic use of hydrogen sulfide in oxidative stress-induced neurodegenerative diseases. Int J Med Sci. 2019;16(10):1386–96.
22. Lee HJ, Kim M. Skin Barrier Function and the Microbiome. Int J Mol Sci. 2022;23(21).
23. Skowron K, Bauza-kaszewska J, Kraszewska Z, Wiktorczyk-kapischke N, Grudlewska-buda K, Kwiecińska-piróg J, et al. Human skin microbiome: Impact of intrinsic and extrinsic factors on skin microbiota. Microorganisms. 2021;9(3):1–20.
24. Gonzalez ACDO, Andrade ZDA, Costa TF, Medrado ARAP. Wound healing - A literature review. An Bras Dermatol. 2016;91(5):614–20.
25. Diller RB, Tabor AJ. The Role of the Extracellular Matrix (ECM) in Wound Healing: A Review. Biomimetics. 2022;7(3):14–6.
26. Li N, Tourovskaia A, Folch A. Biology on a Chip: Microfabrication for Studying the Behavior of Cultured Cells. Crit Rev Biomed Eng 2003 ; 31(0) 423–488 [Internet]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf
27. Keira SM, Ferreira LM, Gragnani A, Da Silva Duarte I, Dos Santos IAN. Modelo experimental para cultura de fibroblastos. Acta Cir Bras. 2004;19(SUPPL. 1):11–6.
28. Henrot P, Laurent P, Levionnois E, Leleu D, Pain C, Truchetet ME, et al. A Method for Isolating and Culturing Skin Cells: Application to Endothelial Cells, Fibroblasts, Keratinocytes, and Melanocytes From Punch Biopsies in Systemic Sclerosis Skin. Front Immunol. 2020;11(October):1–12.
29. Anju Verma1 MV and AS. Animal tissue culture principles and applications. Anim Biotechnol DOI https//doi.org/101016/B978-0-12-811710-100012-4 269 [Internet]. 2020; Available from: https://doi.org/10.1016/j.solener.2019.02.027%0Ahttps://www.golder.com/insights/block-caving-a-viable-alternative/%0A???
30. Velnar T, Bailey T, Smrkolj V. The wound healing process: An overview of the cellular and molecular mechanisms. J Int Med Res. 2009;37(5):1528–42.
31. Chhabra S, Chhabra N, Kaur A, Gupta N. Wound Healing Concepts in Clinical Practice of OMFS. J Maxillofac Oral Surg. 2017;16(4):403–23.
32. Durant F, Whited JL. Finding Solutions for Fibrosis: Understanding the Innate Mechanisms Used by Super-Regenerator Vertebrates to Combat Scarring. Adv Sci. 2021;8(15):1–18.
33. Thiruvoth F, Mohapatra D, Sivakumar D, Chittoria R, Nandhagopal V. Current concepts in the physiology of adult wound healing. Plast Aesthetic Res. 2015;2(5):250.
34. Ansorge M, Sapudom J, Chkolnikov M, Wilde M, Anderegg U, Möller S, et al. Mimicking Paracrine TGFβ1 Signals during Myofibroblast Differentiation in 3D Collagen Networks. Sci Rep. 2017;7(1):1–8.
35. Grigorieva O, Basalova N, Vigovskiy M, Arbatskiy M, Dyachkova U, Kulebyakina M, et al. Novel Potential Markers of Myofibroblast Differentiation Revealed by Single-Cell RNA Sequencing Analysis of Mesenchymal Stromal Cells in Profibrotic and Adipogenic Conditions. Biomedicines. 2023;11(3).
36. Ansorge M, Sapudom J, Chkolnikov M, Wilde M, Anderegg U, Möller S, et al. Mimicking Paracrine TGFβ1 Signals during Myofibroblast Differentiation in 3D Collagen Networks. Sci Rep. 2017;7(1).
37. Bochaton-Piallat ML, Gabbiani G, Hinz B. The myofibroblast in wound healing and fibrosis: Answered and unanswered questions. F1000Research. 2016;5(0):1–8.
38. Quade BN, Parker MD, Occhipinti R. The therapeutic importance of acid-base balance. Biochem Pharmacol 2021 Jan;183114278 doi 101016/j.bcp2020114278 Epub 2020 Oct 9 PMID 33039418; PMCID PMC7544731.

39. Pop MS, Cheregi DC, Onose G, Munteanu C, Popescu C, Rotariu M, et al. Exploring the Potential Benefits of Natural Calcium-Rich Mineral Waters for Health and Wellness: A Systematic Review. Nutrients. 2023;15(14):1–18.
40. Haddy FJ, Vanhoutte PM, Feletou M. Role of potassium in regulating blood flow and blood pressure. Am J Physiol - Regul Integr Comp Physiol. 2006;290(3):546–52.
41. Munteanu C, Munteanu D. Metabolic Fingerprinting in Balneotherapy. Balneo Res J [Internet]. 2013;4(2):87–97. Available from: http://10.0.49.136/balneo.2013.1046%5Cnhttp://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=90645874&site=ehost-live
42. Rafaqat S, Rafaqat S, Khurshid H, Rafaqat S. Electrolyte's imbalance role in atrial fibrillation: Pharmacological management. Int J Arrhythmia [Internet]. 2022;23(1). Available from: https://doi.org/10.1186/s42444-022-00065-z
43. Munteanu C, Iliuţã A. The role of sodium in the body. Balneo-Research J [Internet]. 2011;2(1):70–4. Available from: http://www.bioclima.ro/J226eng.pdf
44. Berend K, Van Hulsteijn LH, Gans ROB. Chloride: The queen of electrolytes? Eur J Intern Med [Internet]. 2012;23(3):203–11. Available from: http://dx.doi.org/10.1016/j.ejim.2011.11.013
45. Rahim HU, Qaswar M, Wang M, Jing X, Cai X. Environmental applications of reduced sulfur species and composites in transformation and detoxification of contaminants. J Environ Chem Eng [Internet]. 2021;9(6):106696. Available from: https://doi.org/10.1016/j.jece.2021.106696
46. Al Alawi AM, Majoni SW, Falhammar H. Magnesium and Human Health: Perspectives and Research Directions. Int J Endocrinol. 2018;2018.
47. Wang R. Physiological implications of hydrogen sulfide: A whiff exploration that blossomed. Physiol Rev. 2012;92(2):791–896.
48. Hornbuckle WE, Simpson KW, Tennant BC. Gastrointestinal Function. Clin Biochem Domest Anim Sixth Ed. 2008;(1):413–57.
49. Stummer N, Feichtinger RG, Weghuber D, Kofler B, Schneider AM. Role of Hydrogen Sulfide in Inflammatory Bowel Disease. 2023;1–20.
50. Theobald H. Dietary calcium and health. Nutr Bull. 2005;30(3):237–77.
51. Ravelli FN, Curi T, de Mendonça Reis Filho EG, Correia PGN, Junior JEG, Sant'Anna B. Thermal spring waters: From balneotherapy to genomics. Surg Cosmet Dermatology [Internet]. 2021;12(4):141–8. Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-85109439225&doi=10.5935%2FSCD1984-8773.20201242568&partnerID=40&md5=c99a9d7fe89ea4f5af37d7b3d8355f41
52. Eliasse Y, Galliano MF, Redoules D, Espinosa E. Effect of thermal spring water on human dendritic cell inflammatory response. J Inflamm Res. 2019;12:181–94.
53. Napolitano F, Postiglione L, Mormile I, Barrella V, de Paulis A, Montuori N, et al. Water from Nitrodi's Spring Induces Dermal Fibroblast and Keratinocyte Activation, Thus Promoting Wound Repair in the Skin: An In Vitro Study. Int J Mol Sci. 2023;24(6).
54. Mignon C, Uzunbajakava NE, Raafs B, Botchkareva N V., Tobin DJ. Photobiomodulation of human dermal fibroblasts in vitro: Decisive role of cell culture conditions and treatment protocols on experimental outcome. Sci Rep. 2017;7(1):1–14.
55. Rhee S. Fibroblasts in three dimensional matrices: Cell migration and matrix remodeling. Exp Mol Med. 2009;41(12):858–65.
56. Place TL, Domann FE, Case AJ. Limitations of oxygen delivery to cells in culture: An underappreciated problem in basic and translational research. Free Radic Biol Med [Internet]. 2017;113(October):311–22. Available from:

https://doi.org/10.1016/j.freeradbiomed.2017.10.003
57. Fernandes IR, Russo FB, Pignatari GC, Evangelinellis MM, Tavolari S, Muotri AR, et al. Fibroblast sources: Where can we get them? Cytotechnology. 2016;68(2):223–8.
58. Seluanov A, Vaidya A, Gorbunova V. Establishing primary adult fibroblast cultures from rodents. J Vis Exp. 2010;(44):1–4.
59. Kuterbekov M, Machillot P, Baillet F, Jonas AM, Glinel K, Picart C. Design of experiments to assess the effect of culture parameters on the osteogenic differentiation of human adipose stromal cells. Stem Cell Res Ther. 2019;10(1):1–9.
60. Weiskirchen S, Schröder SK, Buhl EM, Weiskirchen R. A Beginner's Guide to Cell Culture: Practical Advice for Preventing Needless Problems. Cells. 2023;12(5).
61. Gambari L, Grigolo B, Filardo G, Grassi F. Sulfurous thermal waters stimulate the osteogenic differentiation of human mesenchymal stromal cells – An in vitro study. Biomed Pharmacother [Internet]. 2020;129(April):110344. Available from: https://doi.org/10.1016/j.biopha.2020.110344
62. Carubbi C, Gobbi G, Bucci G, Gesi M, Vitale M, Mirandola P. Skin, inflammation and sulfurous waters: What is known, what is believed. Eur J Inflamm. 2013;11(3):591–9.
63. Coavoy-Sánchez SA, Costa SKP, Muscará MN. Hydrogen sulfide and dermatological diseases. Br J Pharmacol. 2020;177(4):857–65.
64. Sanmartin C, Plano D, Font M, A. Palop J. Kinase Regulation by Sulfur and Selenium Containing Compounds. Curr Cancer Drug Targets. 2011;11(4):496–523.
65. Chiarini A, Dal Pra I, Pacchiana R, Zumiani G, Zanoni M, Armato U. Comano's (Trentino) thermal water interferes with interleukin-6 production and secretion and with cytokeratin-16 expression by cultured human psoriatic keratinocytes: Further potential mechanisms of its anti-psoriatic action. Int J Mol Med. 2006;18(6):1073–9.
66. Kloesch B, Liszt M, Krehan D, Broell J, Kiener H, Steiner G. High concentrations of hydrogen sulphide elevate the expression of a series of pro-inflammatory genes in fibroblast-like synoviocytes derived from rheumatoid and osteoarthritis patients. Immunol Lett. 2012;141(2):197–203.
67. Cheleschi S, Gallo I, Tenti S. A comprehensive analysis to understand the mechanism of action of balneotherapy: why, how, and where they can be used? Evidence from in vitro studies performed on human and animal samples. Int J Biometeorol. 2020;64(7):1247–61.
68. Fox B, Schantz JT, Haigh R, Wood ME, Moore PK, Viner N, et al. Inducible hydrogen sulfide synthesis in chondrocytes and mesenchymal progenitor cells: Is H2S a novel cytoprotective mediator in the inflamed joint? J Cell Mol Med. 2012;16(4):896–910.
69. Karagülle MZ, Karagülle M, Kılıç S, Sevinç H, Dündar C, Türkoğlu M. In vitro evaluation of natural thermal mineral waters in human keratinocyte cells: a preliminary study. Int J Biometeorol. 2018;62(9):1657–61.
70. Kloesch B, Liszt M, Steiner G, Bröll J. Inhibitors of p38 and ERK1/2 MAPkinase and hydrogen sulphide block constitutive and IL-1β-induced IL-6 and IL-8 expression in the human chondrocyte cell line C-28/I2. Rheumatol Int. 2012;32(3):729–36.
71. Liu YH, Lu M, Xie ZZ, Hua F, Xie L, Gao JH, et al. Hydrogen sulfide prevents heart failure development via inhibition of renin release from mast cells in isoproterenol-treated rats. Antioxidants Redox Signal. 2014;20(5):759–69.
72. Miller TW, Wang EA, Gould S, Stein E V., Kaur S, Lim L, et al. Hydrogen sulfide is an endogenous potentiator of T cell activation. J Biol Chem. 2012;287(6):4211–21.