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UNIVERSITÀ di VERONA

Do oxygen-ozone mixtures affect cell proliferation and death?

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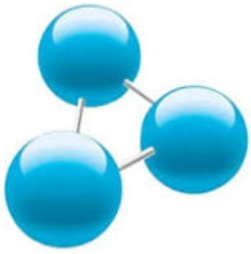
In the clinical practice, the oxygen-ozone therapy based on low ozone concentrations proved to be beneficial in many diseases, thanks to multiple effects, among which:

- Anti-inflammatory action
- Painkilling action
- Immunomodulating action
- Wound healing/Tissue regeneration action
- Activating-improving action on microcirculation
- Anti-oedemigenic action
- Anti-bacterial and anti-mycotic action



2

However, the cellular and molecular mechanisms accounting for these multiple effects need to be further studied.

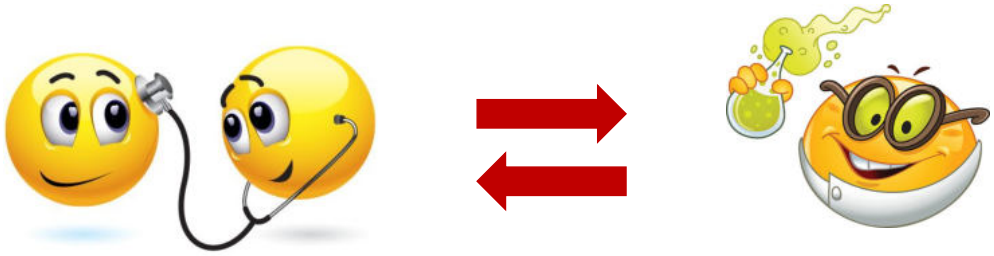
Our research aims at identifying these mechanisms by analyzing the structural and functional changes occurring at the cellular level after treatment with low ozone concentrations.

By providing a mechanistic explanation, our results are immediately functional to ozone therapists to improve the efficacy of the therapeutic procedures presently used.

3

Translational research:

from bedside to bench, to bedside again



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Do oxygen-ozone mixtures affect
cell proliferation and death?

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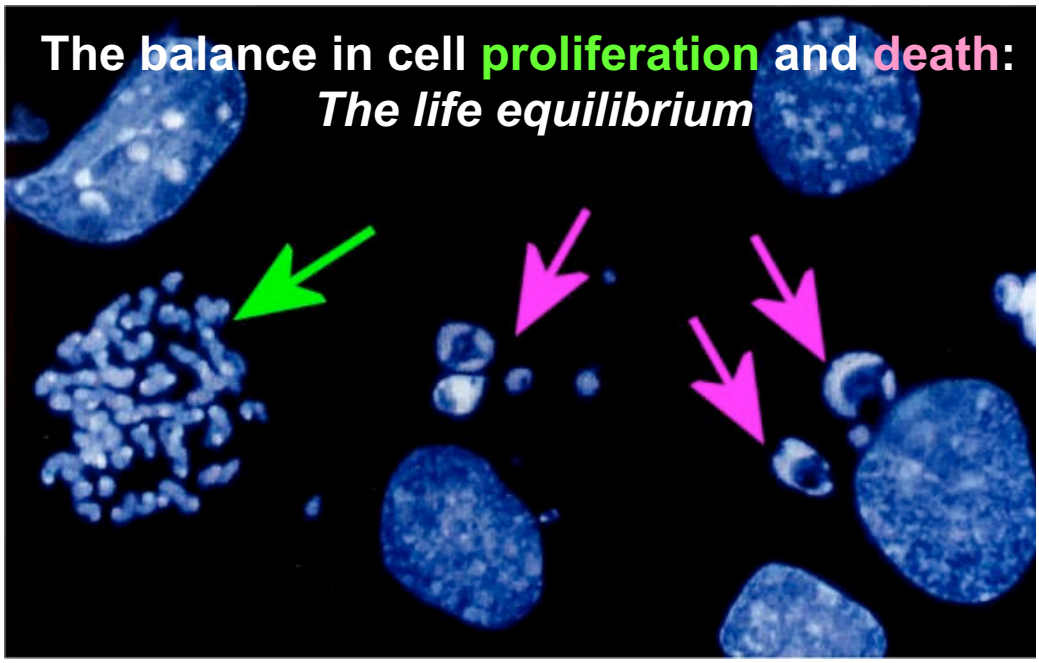

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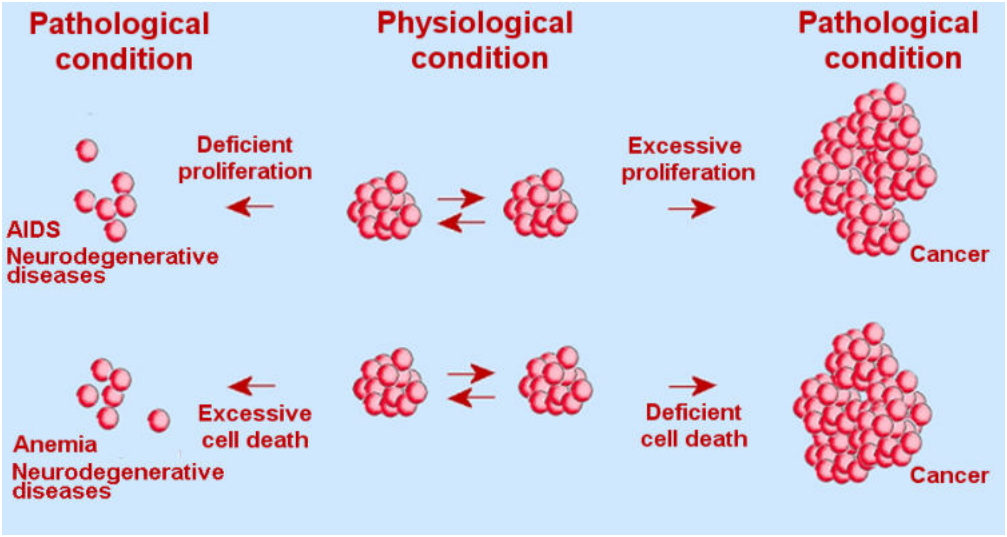
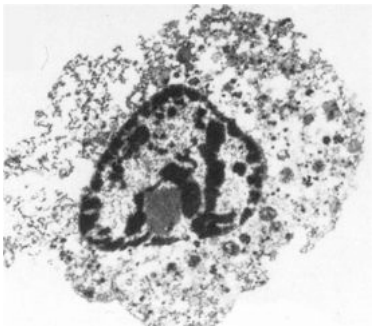
Cell proliferation and death are essential during the embryo development as well as at the end of histogenesis, to maintain tissue homeostasis

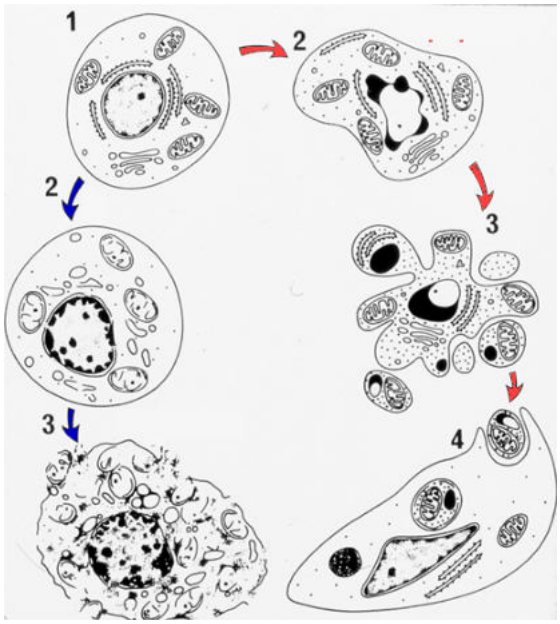


7

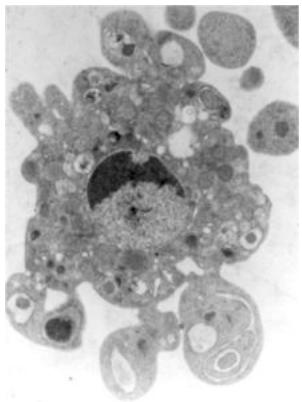
Cells may die by either uncontrolled (necrotic) or genetically-driven (apoptotic) death

Necrosis





Apoptosis



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Experimental method

We used cell systems in vitro that allow analyzing the structural, functional and kinetic features under strictly controlled and standardized experimental conditions suitable for the application of refined multimodal imaging and biomolecular analyses.

The cells were exposed to O_2-O_3 mixtures with different concentration of O_3 (generally 10 to 35 μg/mL O_2).

As a control, the cells were exposed to air.

Exposure to pure O_2 was used distinguish the effects of O_3 from those induced by O_2.

All samples were made of an established cell number in a same amount of medium.

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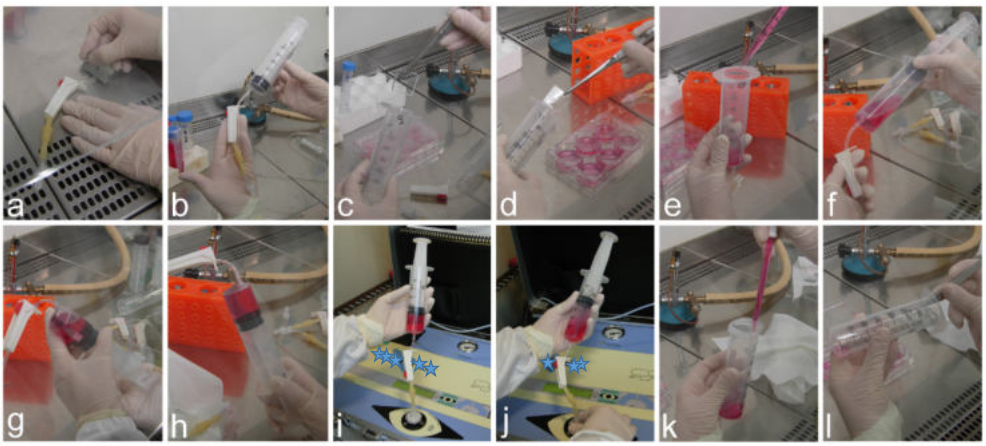
To analyze the effects of treatment, an amount of gas corresponding to the amount of medium (1:1) was insufflated into a syringe containing the cells and, after 10 min exposure, the sample was transferred into a flask or dish, and maintained in an incubator until analysis.



Costanzo et al., Microscopie (2015)

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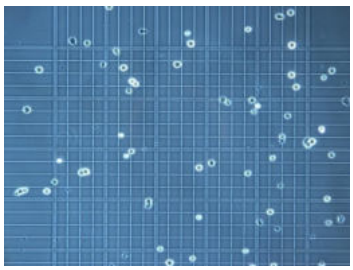
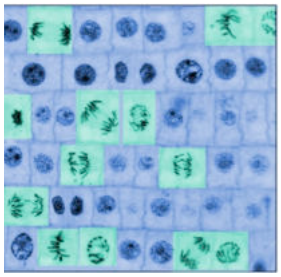
Cell proliferation assessment

By counting the total number of cells at increasing time points cell growth can be measured



Cell proliferation may be estimated by the so-called «mitotic index» i.e., the percentage of cells in mitosis



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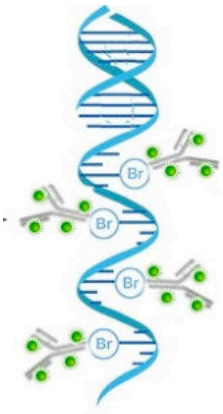
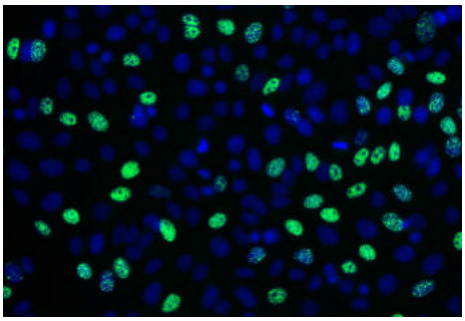
Cell proliferation assessment

Evaluation of the S phase fraction: Bromodeoxyuridine (BrdU) is a thymidine analog that is incorporated into DNA of proliferating cells during S phase. Incorporated BrdU may be visualized by immunohistochemistry.



Antibody detection



Green: S phase cells
Blue: non DNA-duplicating cells

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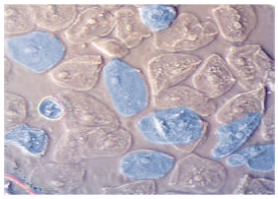
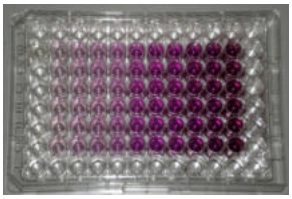
Cell death assessment

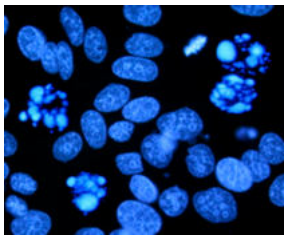
Trypan blue exclusion test: only dead cells are stained.





Enzymatic assays are based on the measurement of a specific analyte produced by dead cells (e.g. LDH).

Chromatin condensation and nuclear fragmentation (karyorrhexis) are the most easily detectable features of apoptotic cells at light microscopy



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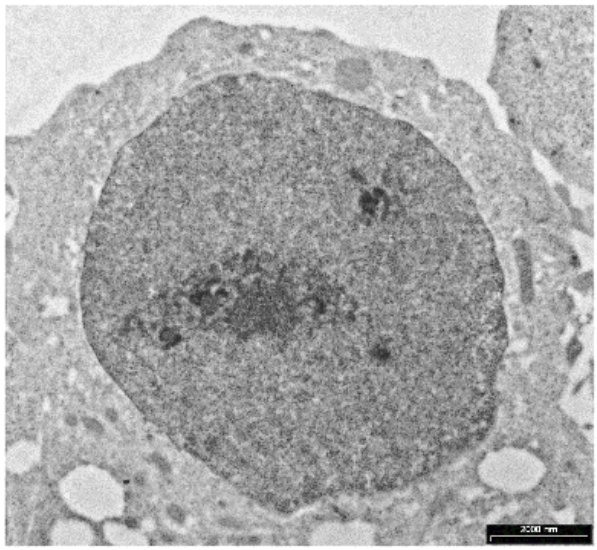
Neuronal cell line



Scassellati et al., Toxicol. In Vitro (2017)

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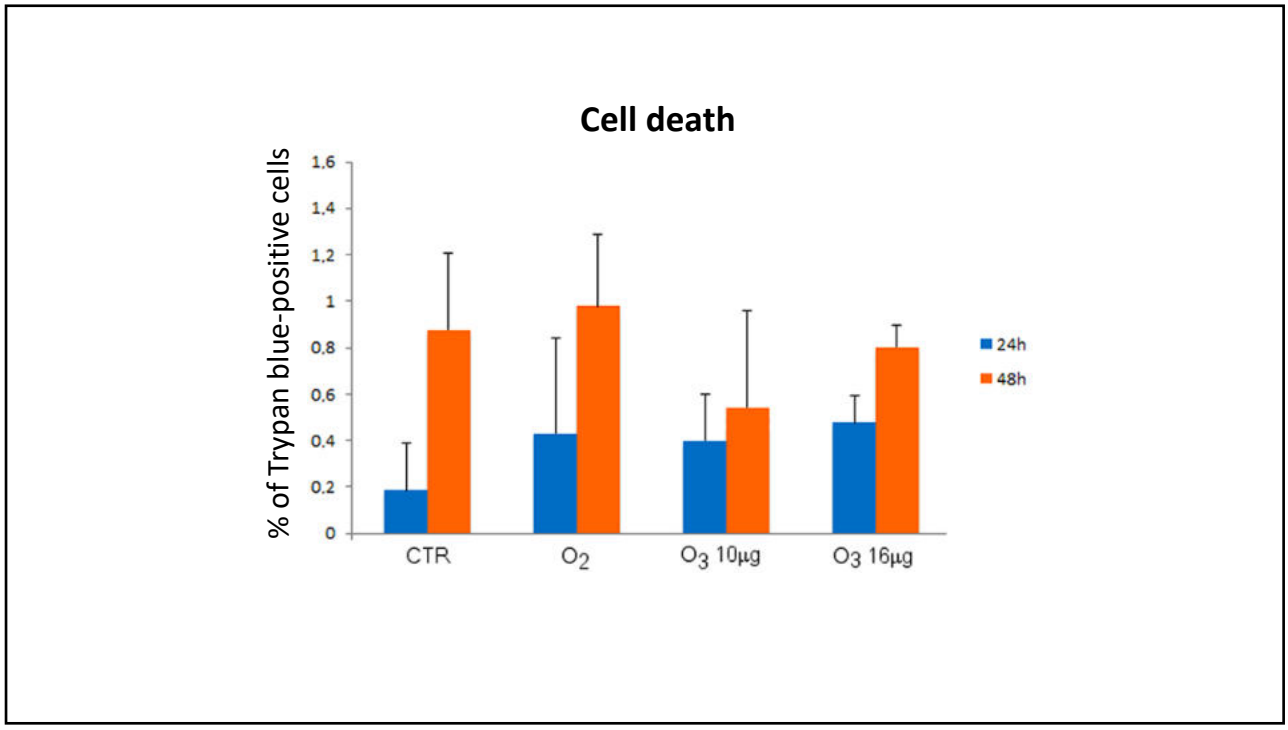
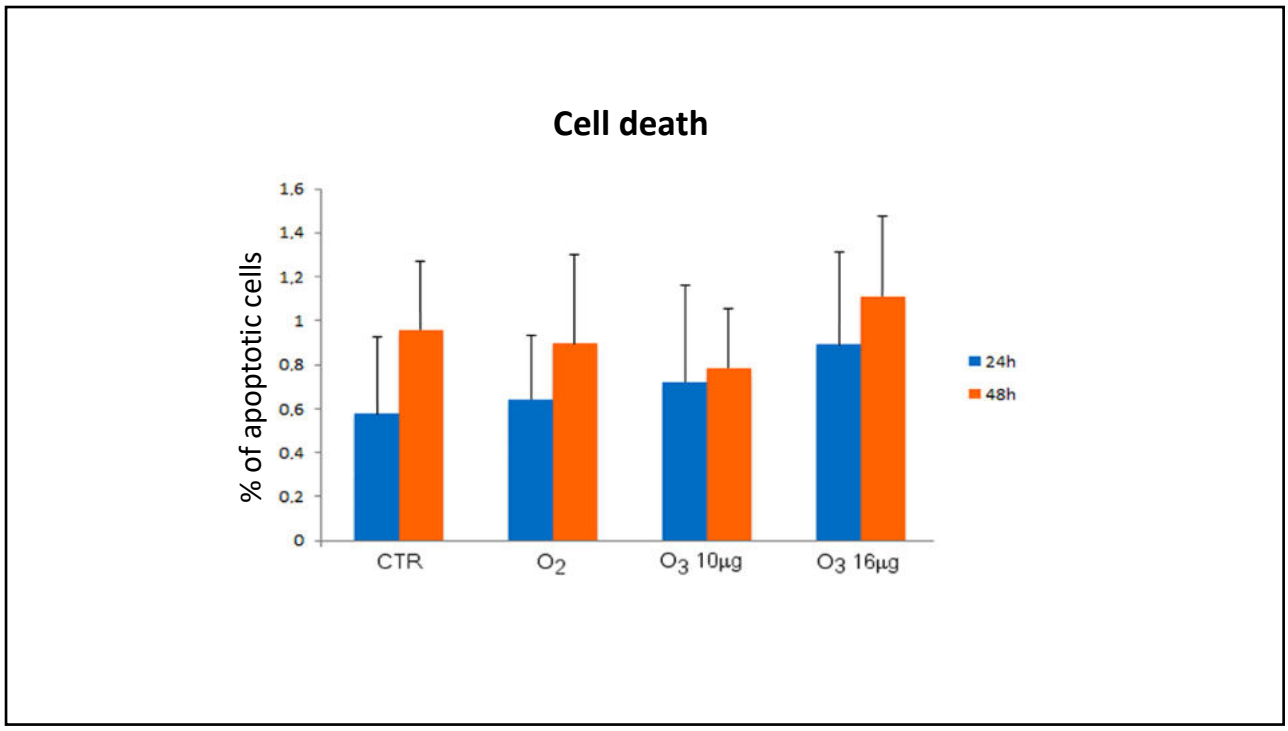
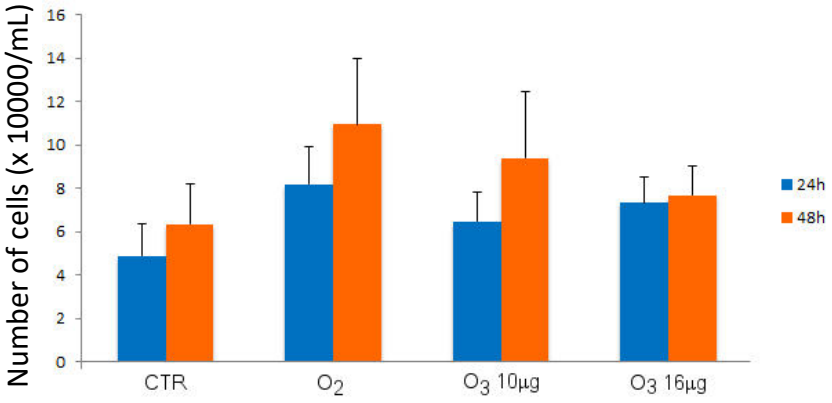

Cell death
% of Trypan blue-positive cells
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
CTR
O2
O3 10µg
O3 16µg
24h
48h

15


Cell death
% of apoptotic cells
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
CTR
O2
O3 10µg
O3 16µg
24h
48h

16

Cell proliferation



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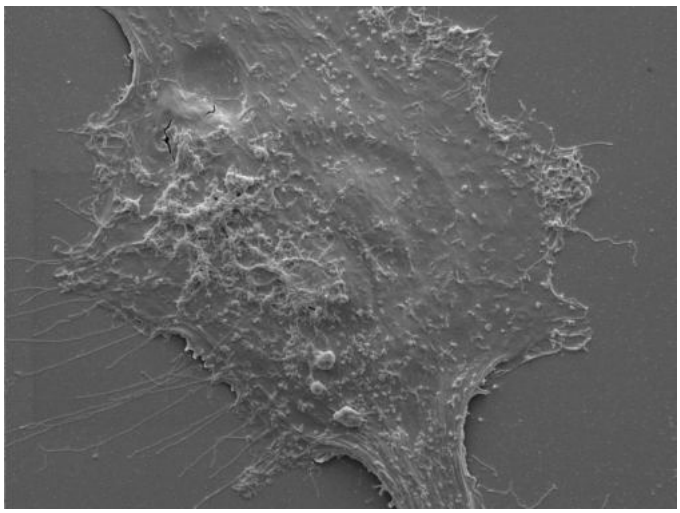
Fibroblast cell line



Cisterna et al., Int. J Mol. Sci. (2021)

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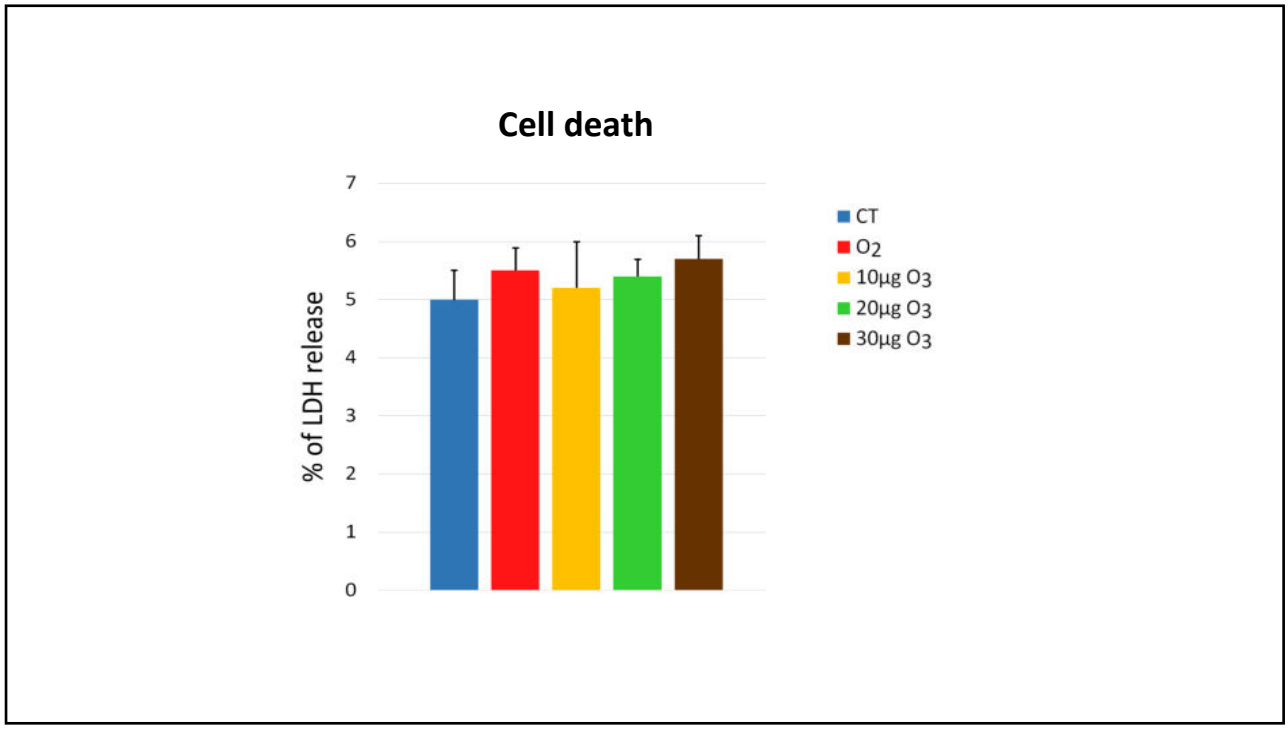
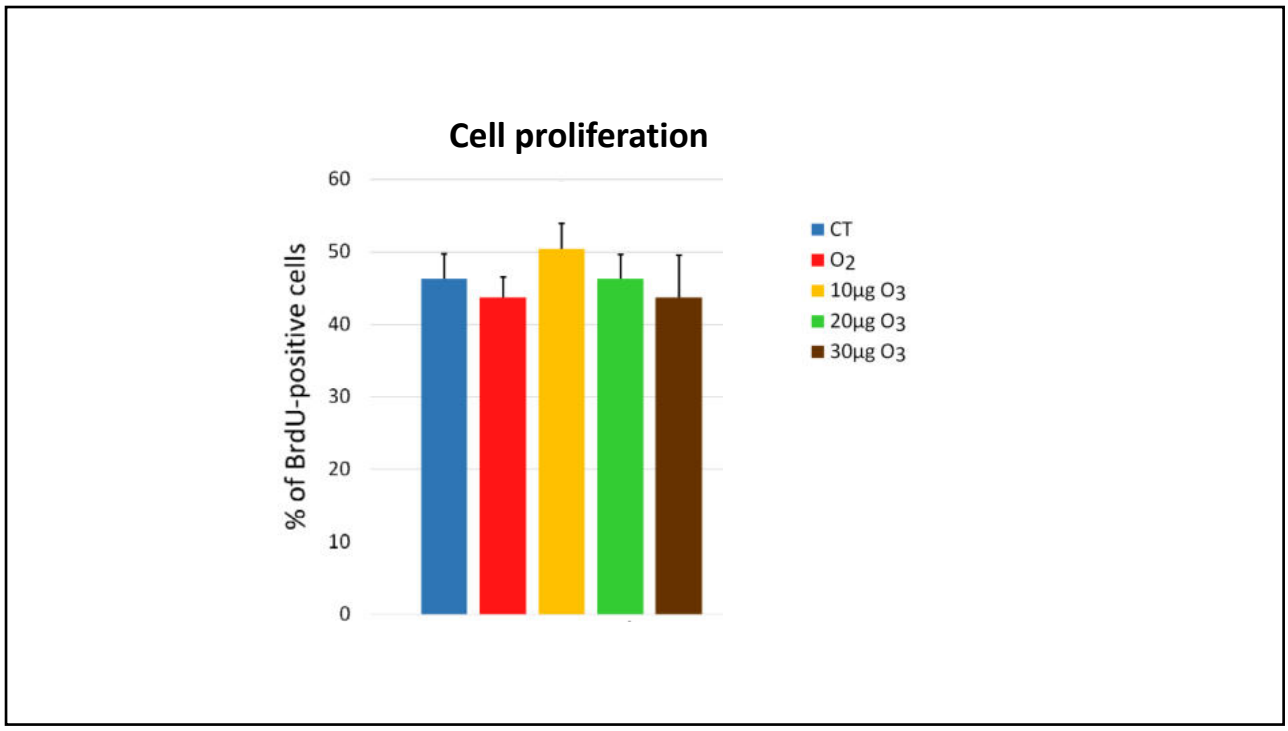
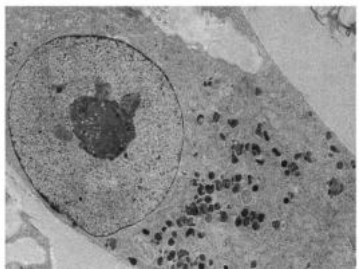

Cell death
% of LDH release
0
1
2
3
4
5
6
7
CT
O2
10µg O3
20µg O3
30µg O3

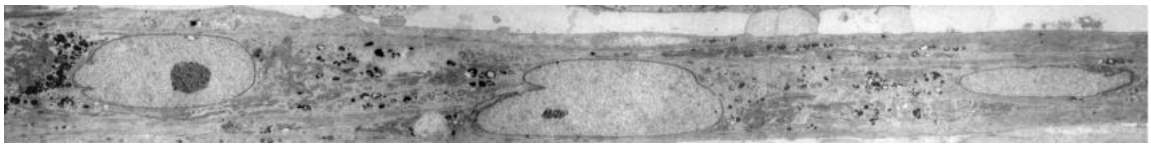
19


Cell proliferation
% of BrdU-positive cells
0
10
20
30
40
50
60
CT
O2
10µg O3
20µg O3
30µg O3

20

Skeletal muscle cell line





Inguscio et al., Int. J Mol. Sci. (2023)

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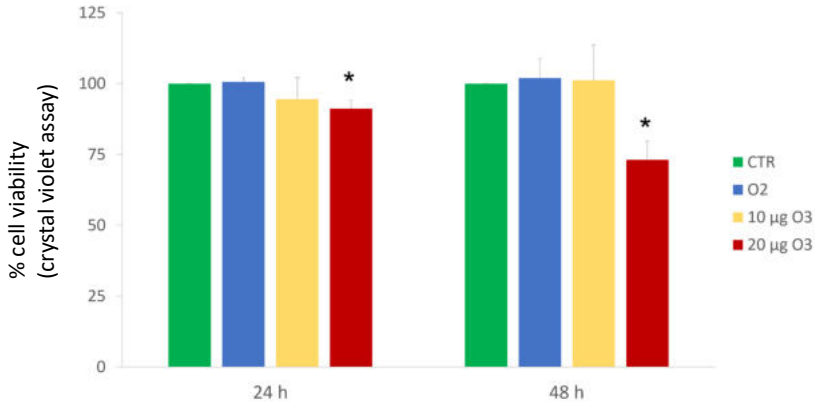

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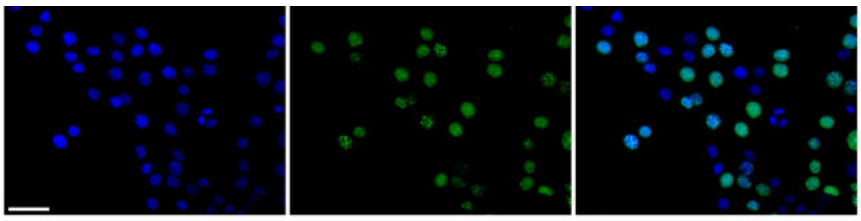
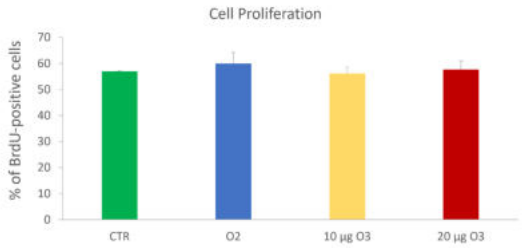
Cell proliferation





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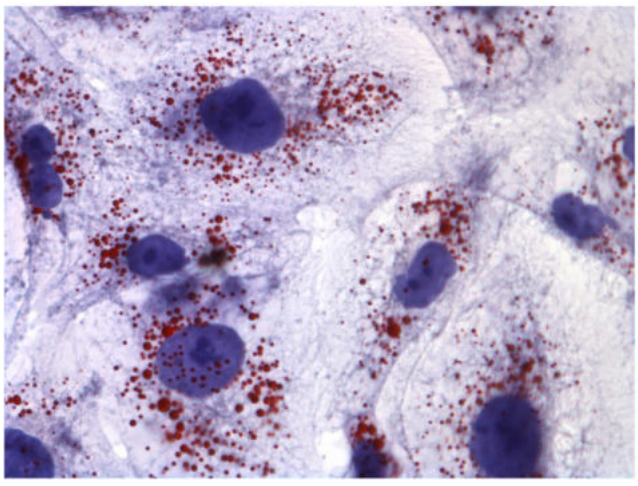
Adipose tissue-derived stem cells



Costanzo et al., Eur. J. Histochem. (2018)

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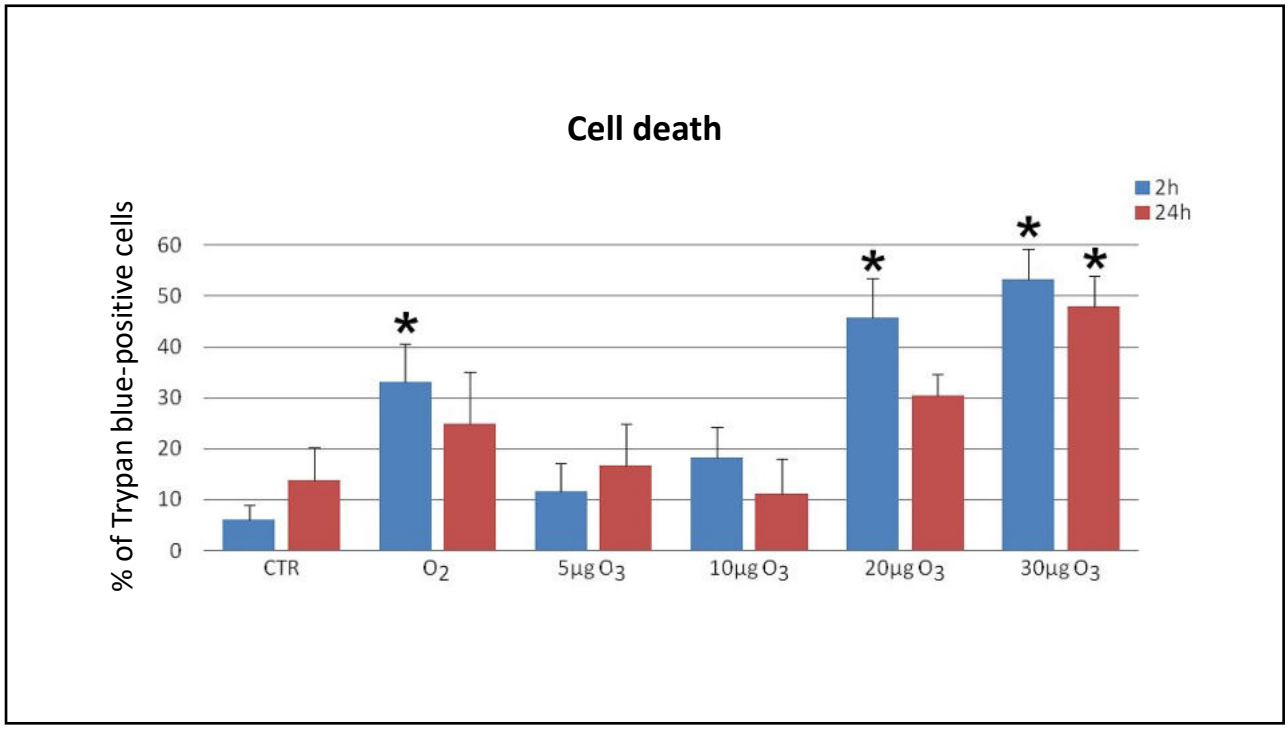
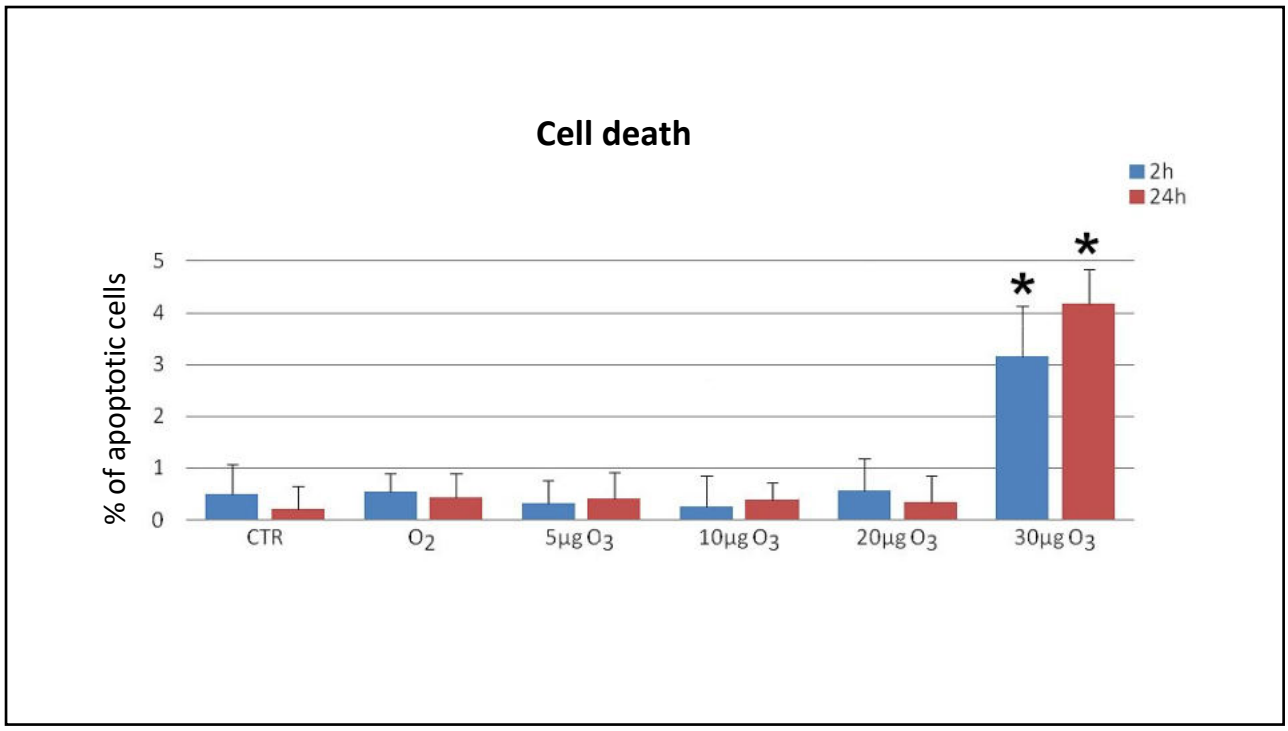

Cell death
% of Trypan blue-positive cells
2h
24h
60
50
40
30
20
10
0
CTR
O2
5µg O3
10µg O3
20µg O3
30µg O3
*
*
*
*

25


Cell death
% of apoptotic cells
2h
24h
5
4
3
2
1
0
CTR
O2
5µg O3
10µg O3
20µg O3
30µg O3
*
*

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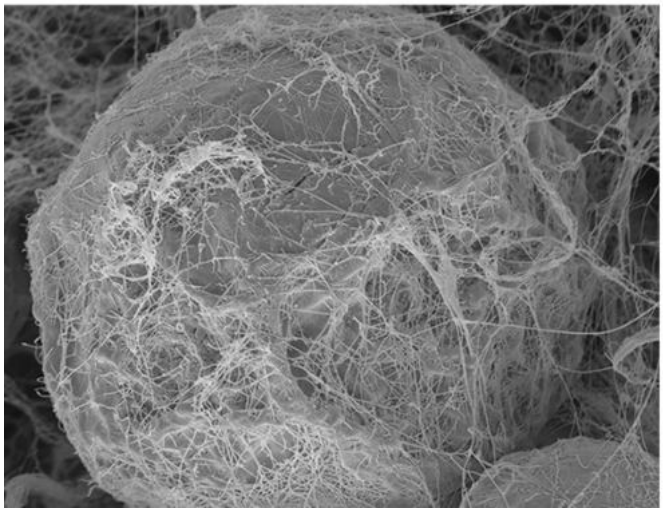
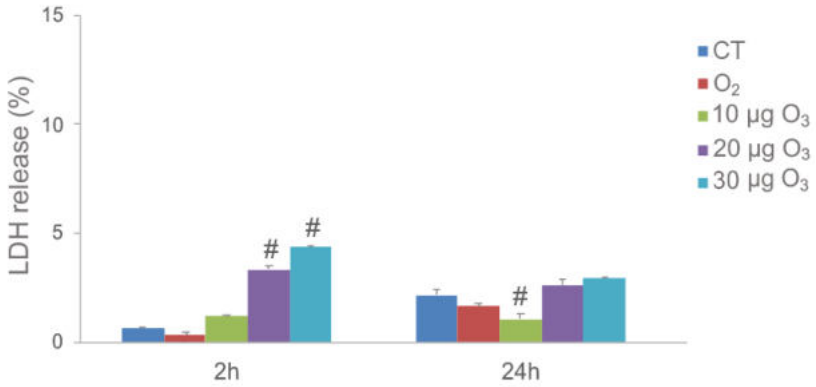
Mature adipocytes



Cisterna et al., Antioxidants (2020)

27

Cell death



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Our in vitro tests confirm that the low ozone concentrations usually applied in the clinical practice are generally safe because they do not alter markedly cell proliferation or viability.

However, different cell types may present different degrees of tolerance to ozone concentrations, which may induce death by necrosis and, less commonly, apoptosis in some cell types but not in others.

Different antioxidant response capability?

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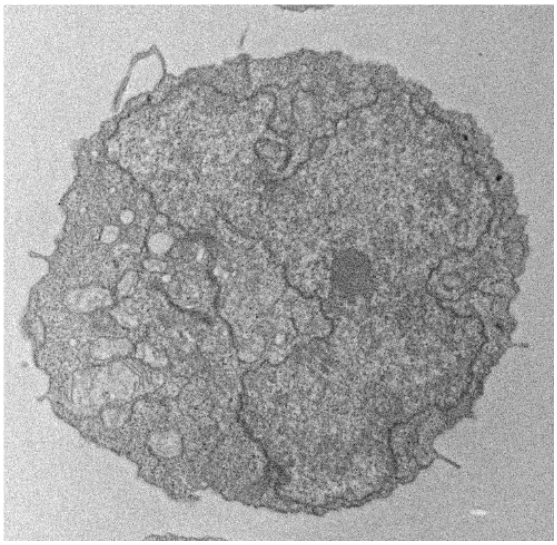
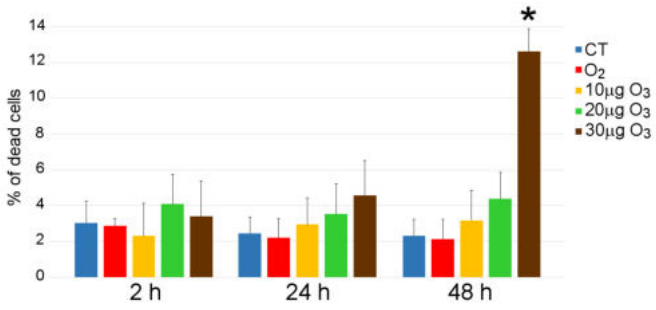
T Lymphocytes



Cappellozza et al., Processes (2021)

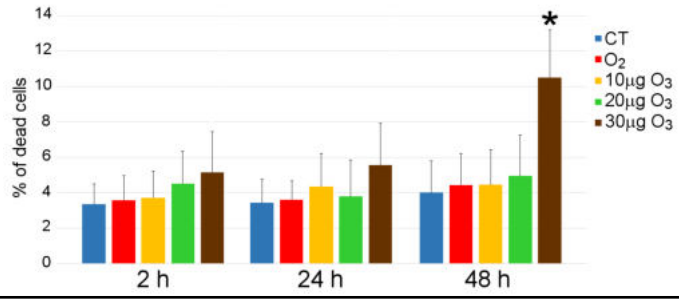
30

Cell death



Non-activated lymphocytes

LPS-activated lymphocytes



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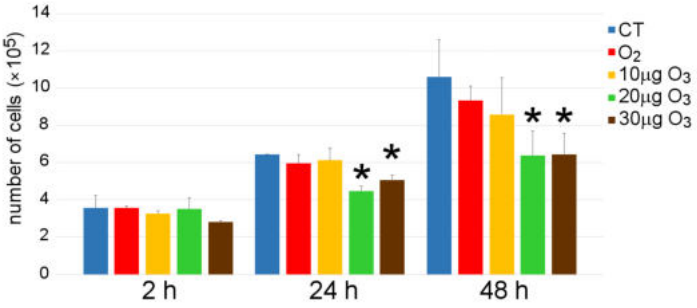
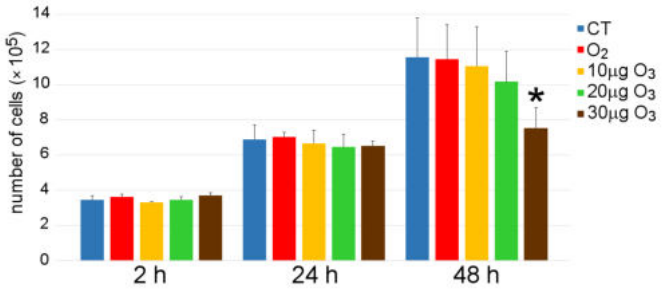
Cell proliferation



Non-activated lymphocytes

LPS-activated lymphocytes



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Non-activated T lymphocytes are more sensitive to ozone exposure that activated ones. This finding is consistent with previous observations on other activated immune cells, which show increased resistance to stressors probably due to the need to play their defensive role in environments rich of noxious and inflammatory molecules.

T lymphocytes are sensitive to relatively high concentration of ozone (30 μg), irrespective of their functional state. This suggests the use of lower concentrations in order to avoid damage of immune cells.

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Does ozone affect cancer cells' features?

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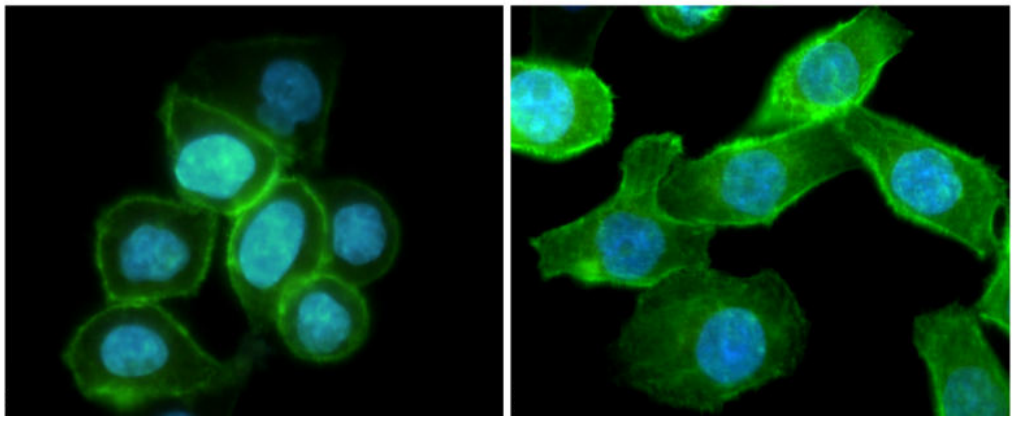
HeLa cells



Costanzo et al., Eur. J. Histochem. (2015, 2020)

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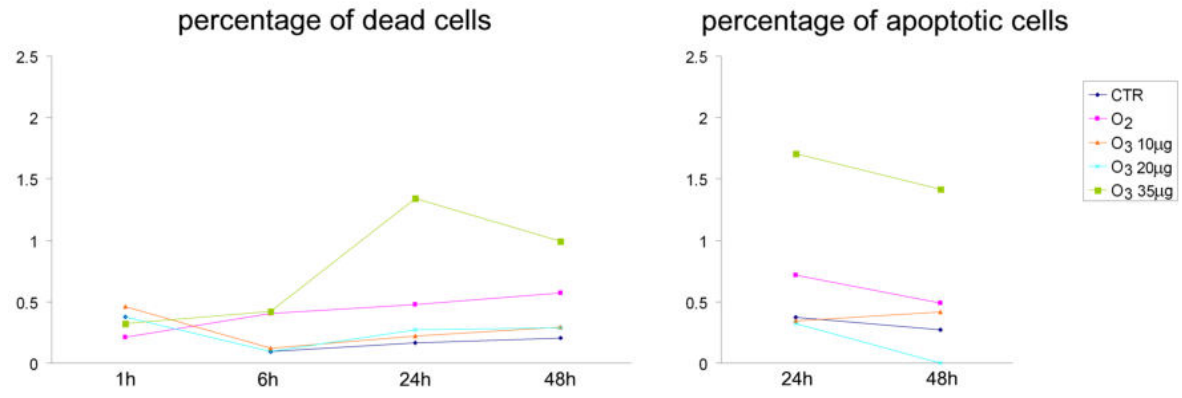
Cell death



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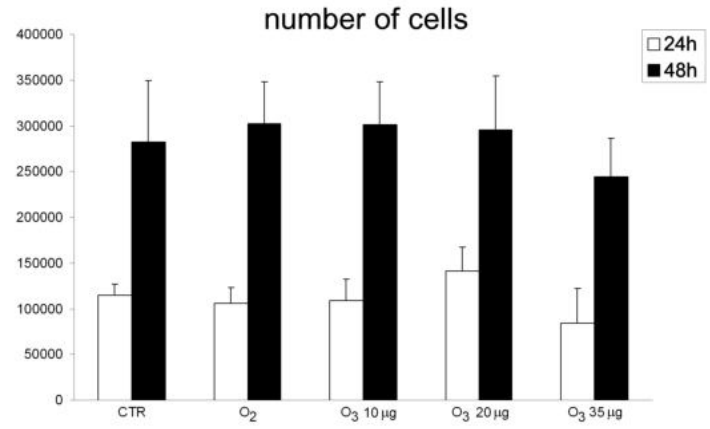
Cell proliferation



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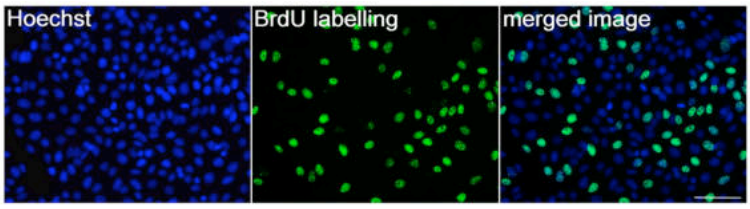
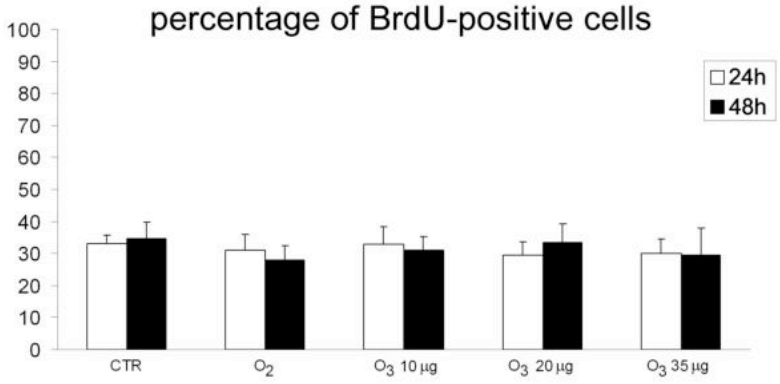
Cell proliferation





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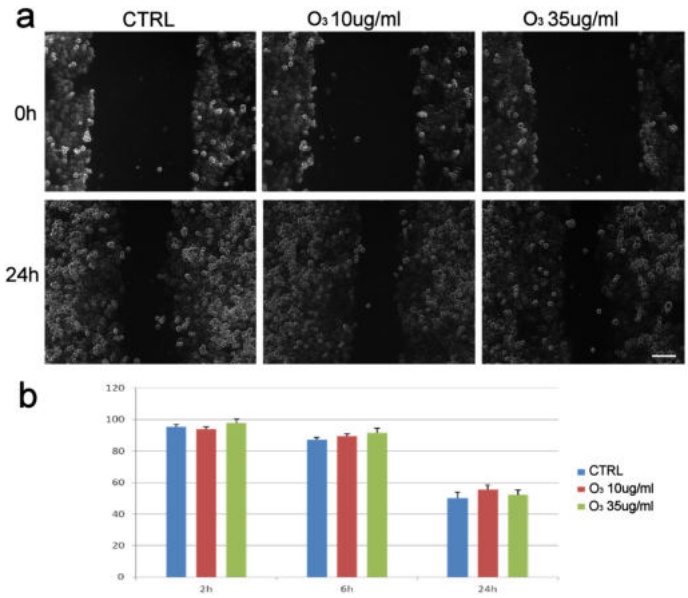
Wound healing test



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Under *in vitro* conditions, ozone does not alter death rate, proliferation or migration in cancer cells.

This supports the notion that ozone therapy should not promote tumour cells growth and metastasis.

What about ozone effects on cancer cells treated with anti-tumour drugs?

Work in progress…….

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References

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Cisterna B, Costanzo M, Lacavalla MA, Galiè M, Angelini O, Tabaracci G, Malatesta M. Low Ozone Concentrations Differentially Affect the Structural and Functional Features of Non-Activated and Activated Fibroblasts In Vitro. Int J Mol Sci. 2021;22(18):10133. doi: 10.3390/ijms221810133.

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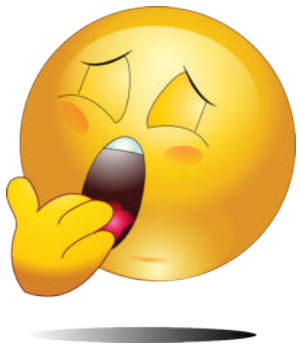
Thanks for your attention



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