Stem cells long term preservation

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F. Chretien, P. Rocheteau (Pasteur Institute)

Interdisciplinary project between : Physics – Biology - Medicine

Funded by CNRS – MITI (FAIDORA Program) and Pasteur Institute

The genesis of the project

View from physicist:

Stem cells are stored in liquid nytrogen for tens of years

Stem cells are inert (no chemical activity)

Integrity of stem cells accumulating damages from natural radioactivity and cosmic-rays for tens of years ?

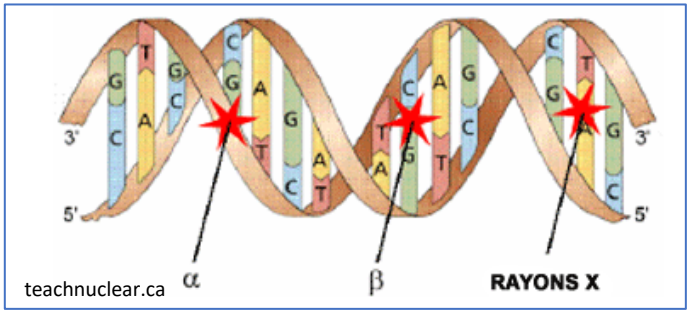
After several years , meeting with Fabrice Chretien Professor and Pierre Rocheteau biologist at Pasteur Institute

Interested to collaborate to make a first experiment with mouse muscle stem cell

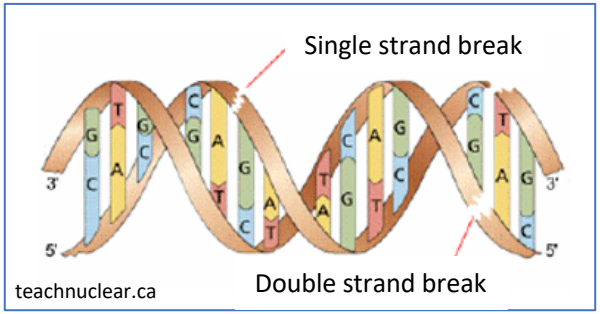
Support of MITI (Mission interdisciplinaire CNRS) and Pasteur Institute

Radiation effects

It is well know since long time that radiation can lead to DNA double strand break or ROS (Reactive Oxygen Species)

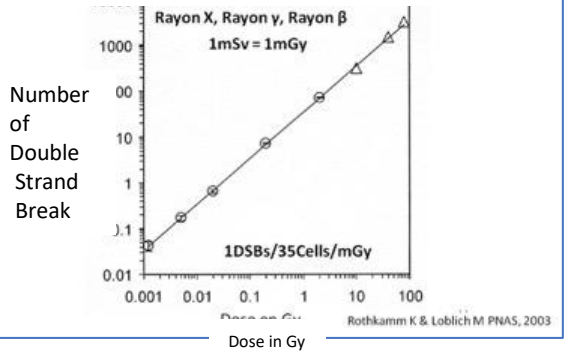


X



The DNA of a living cell is repaired several thousand times a day due to chemical effects and natural radiation.

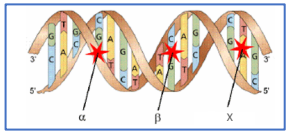
Double Strand Break caused by X-rays.



Radiation effects

Living cells

Radiations



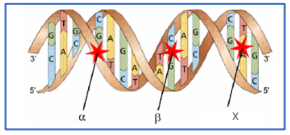
Repair

Immediate DNA repair

Frozen cells

DNA Damages

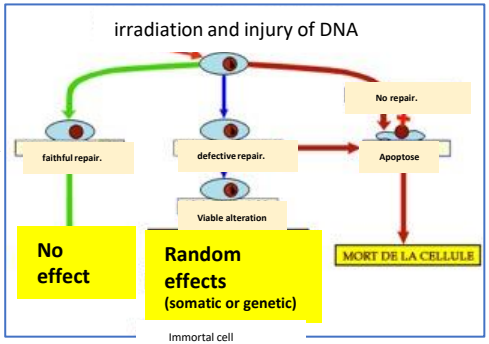
Radiations



Inert cell

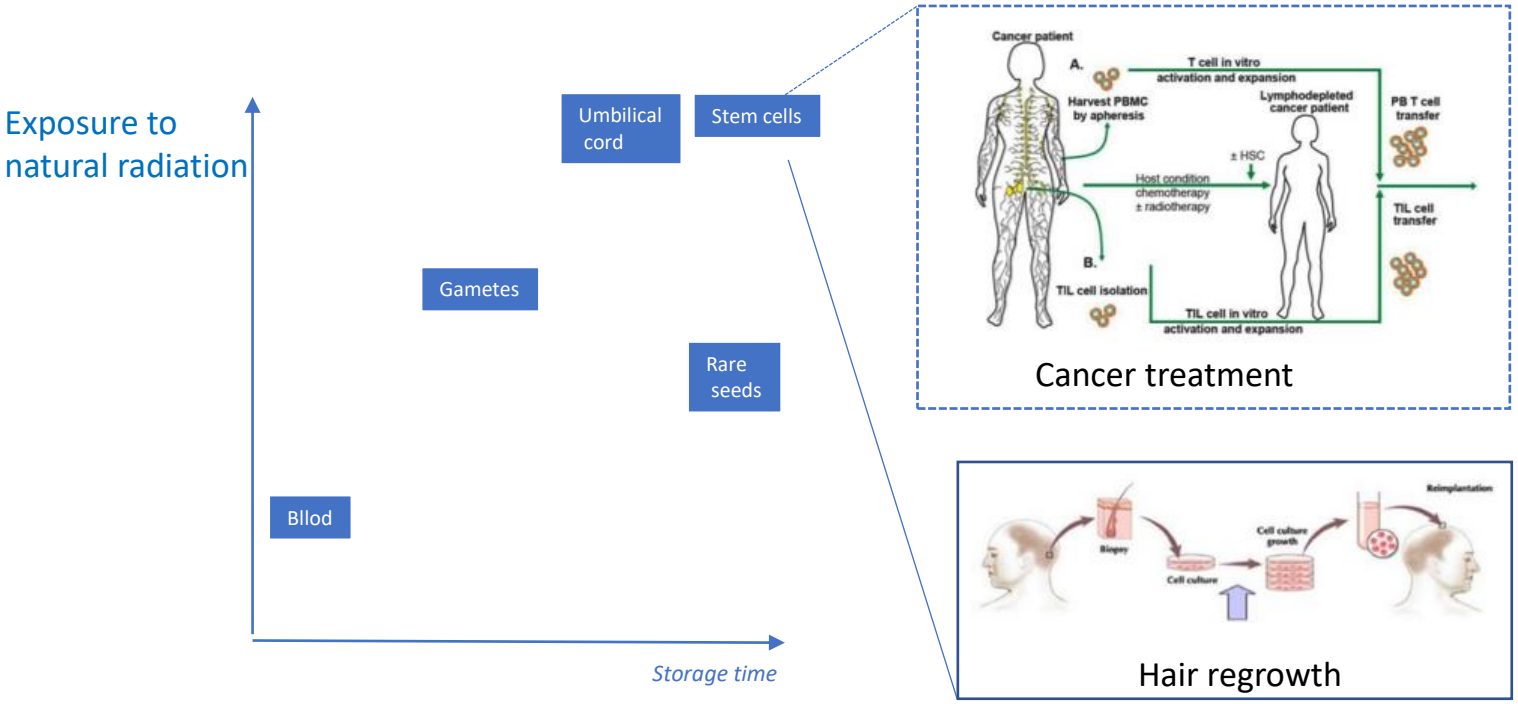
No DNA repair
Accumulation of damage over time

cells that have suffered too much damage can no longer repair themselves or repair themselves badly:
- Cell loss on thawing
- Potential mutations



Why the preservation of stem cells is im

By 2050, 2/3 of diseases treated by stem cells



Results of the studies with mouse stem

The objectives were to measure Double Strand Breaks of stem cells from mouse muscles in different conditions of natural radiation exposure and to do some in vivo tests.
We used 3 types of Stem Cells : Mesenchymal SC and Hematopoietic SC

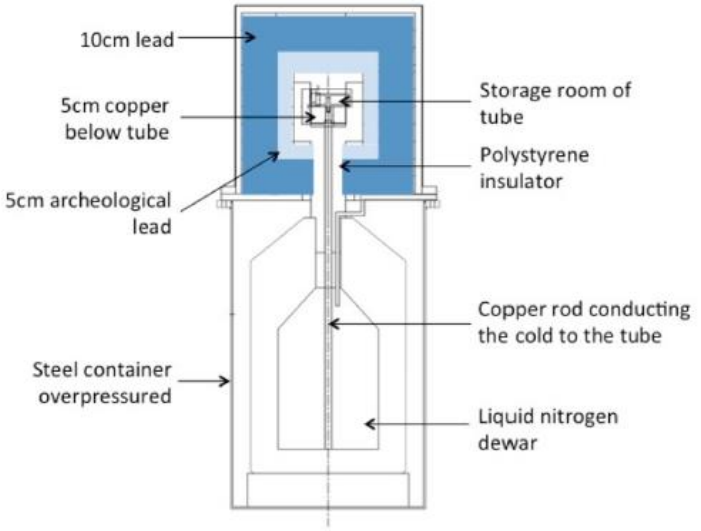
Condition		Depth (meter of water equivalent)	Cosmic ray dose (mSv/year)	Total dose (mSv/year)	Neutrons flux (neutron/cm^2.s)
I	Standard Pasteur Institute	10	0.04	0.52–1.77 (calculated)	3.10^{-3}
II	LSM surface + sand	0	1.17	76.65 (measured)	1.10^{-2}
III	LSM Underground	4,800	$<2.7 \times 10^{-6}$	$<2.7 \times 10^{-6}$(calculated)	3.10^{-6}
IV	LSM surface	0	1.17	1.65–2.91 (measured)	1.10^{-2}

Condition II: Increase exposure to CR flux and gamma rays by surrounding cells with sand (Thorium) corresponding to a daily dose of 0.21 mSv/day

Condition III : cells stored underground are shielded by lead

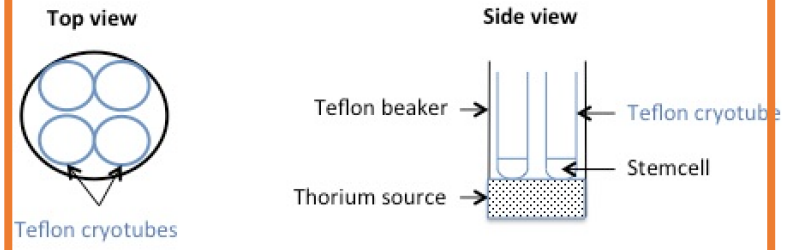
Experimental setup

Condition III



Underground condition

Condition II



LSM surface

Radiopurity measurements of all materials

Results

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Cryopreserved Stem Cells Incur Damages Due To Terrestrial Cosmic Rays Impairing Their Integrity Upon Long-Term Storage

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Abstract

Stem cells have the capacity to ensure the renewal of tissues and organs. They could be used in the future for a wide range of therapeutic purposes and are preserved at liquid nitrogen temperature to prevent any chemical or biological activity up to several decades before their use. We show that the cryogenized cells accumulate damages coming from natural radiations, potentially inducing DNA double-strand breaks (DSBs). Such DNA damage in stem cells could lead to either mortality of the cells upon thawing or a mutation diminishing the therapeutic potential of the treatment. Many studies show how stem cells react to different levels of radiation; the effect of terrestrial cosmic rays being key, it is thus also important to investigate the effect of the natural radiation on the cryopreserved stem cell behavior over time. Our study showed that the cryostored stem cells totally shielded from cosmic rays had less DSBs upon long-term storage. This could have important implications on the long-term cryostorage strategy and quality control of different cell banks.

Keywords

stem cell biology, radiation, mesenchymal stem cells, hematopoietic stem cells, muscle regeneration

Introduction

The long-term preservation of stem cells is a major challenge for therapeutic applications in the future. However, some studies have shown a decrease in cell quality or viability correlating with the time of cryostorage[1–4]. In the context of this study, we want to determine whether the cryoconservation of stem cells can lead to an accumulation of low radioactive doses (in the order of mGy) coming from the natural radioactive environment, leading to this decrease in cell quality. Indeed, at the surface of the earth, any biological sample is continuously exposed to radiations coming from telluric radioactivity (TR), radon radioactivity, and terrestrial cosmic rays (TCR).

The TR is defined as the radioactivity of natural radioisotopes coming from potassium, uranium, or thorium decay chains. TR (including radon) doses will depend on the location and the composition of the surrounding materials.

Terrestrial cosmic radiations correspond to particles produced in the earth atmosphere by primary cosmic rays (CR) continuously bombarding the earth atmosphere. These primary CR take their origin from astrophysical processes and are mainly high-energy ions (protons, helium), electrons, and photons. The primary CR interact in altitude with the atoms in the air, leading to the creation of a large number of secondary particles. The secondary particles reaching the earth's surface constitute the TCR flux. They consist of various particles (pions, muons, protons, neutrons, electrons) with a wide range of energy. The secondary particle flux will depend on the location on the earth and solar activity[5]. The only possibility to decrease the entire TCR flux is to protect

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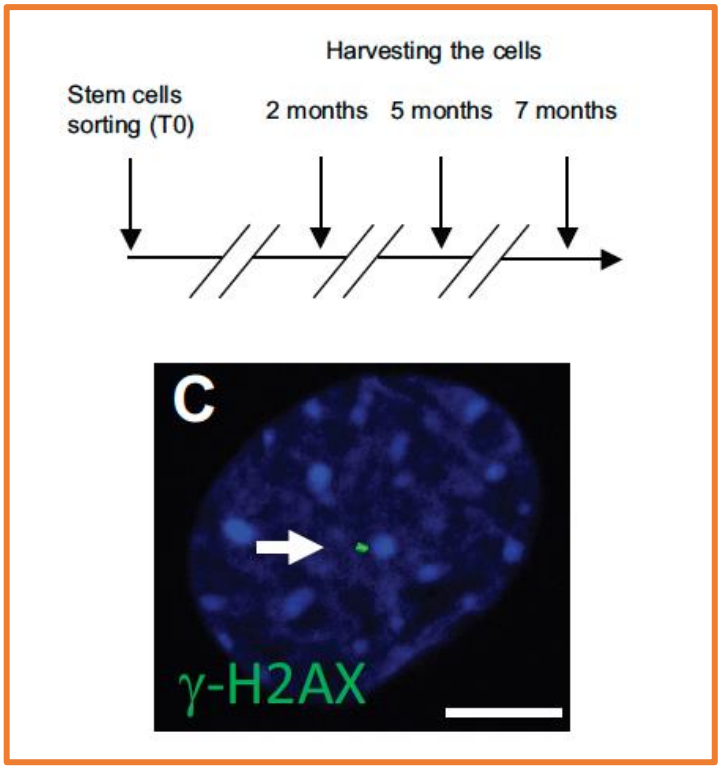
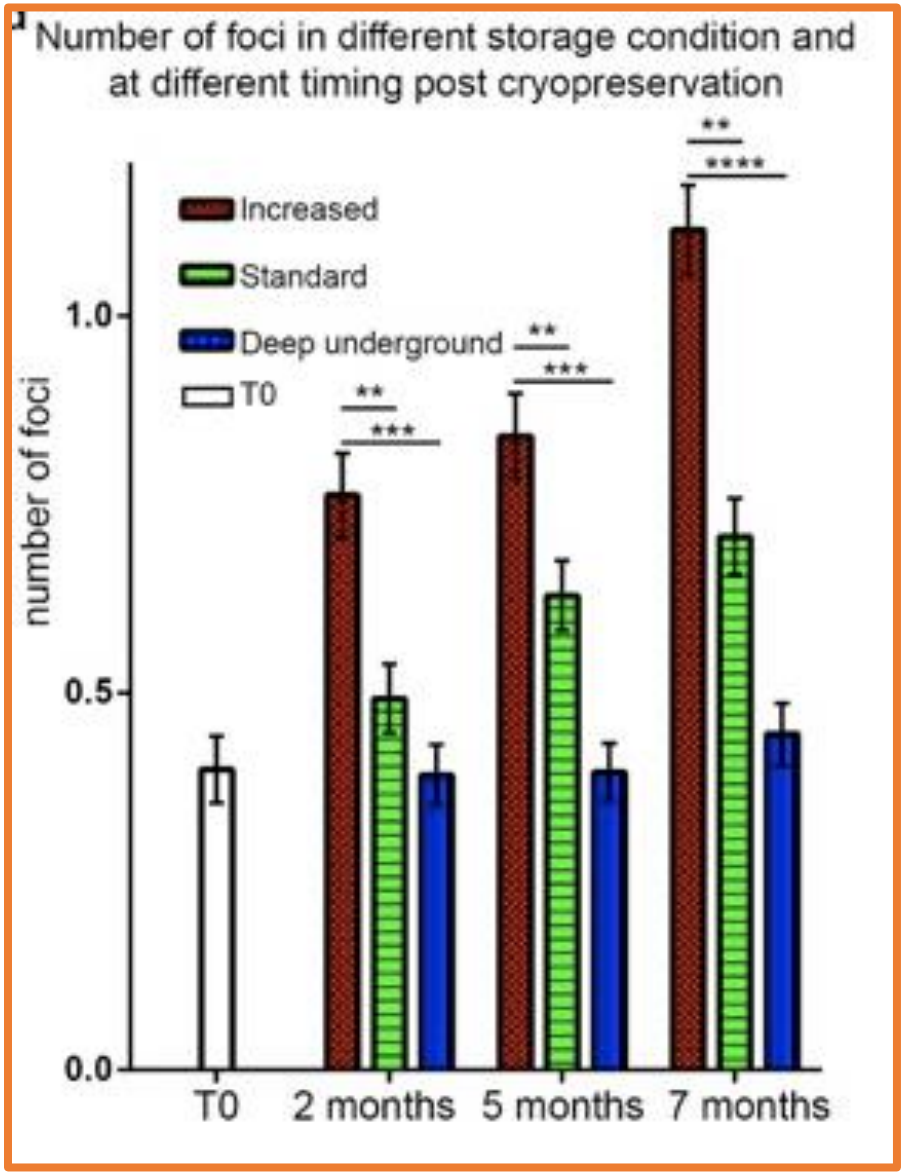
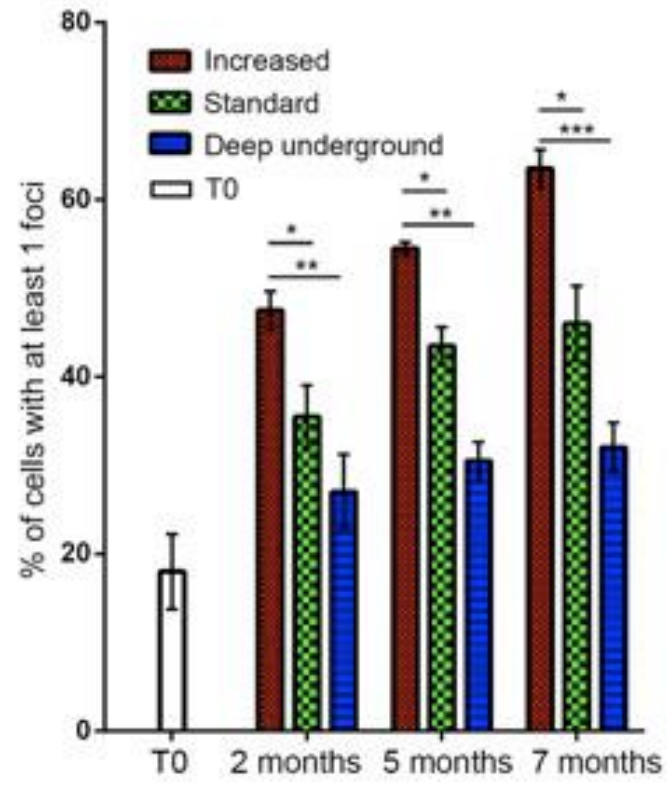
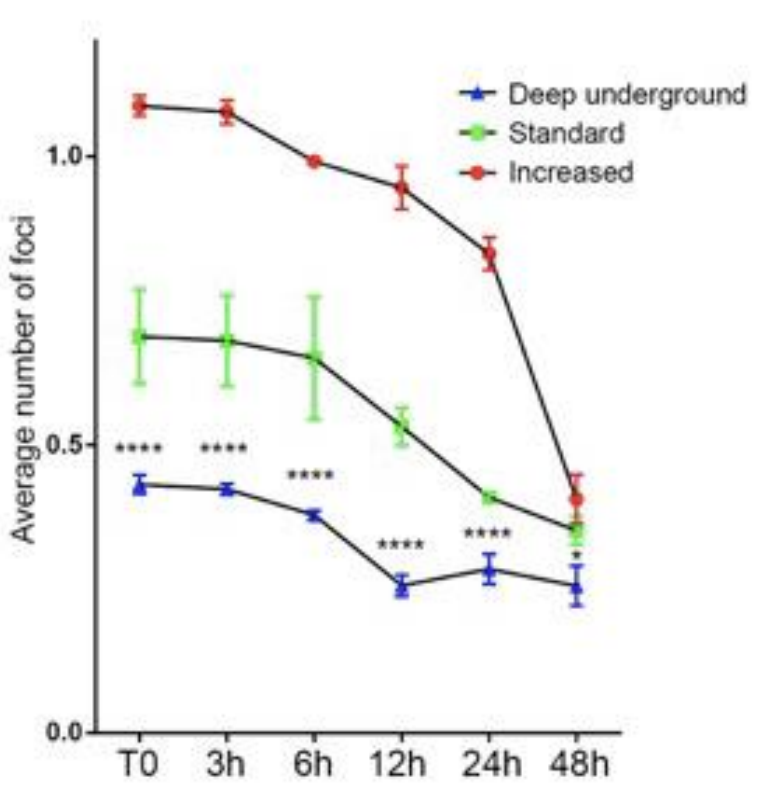
Results


Harvesting the cells
Stem cells sorting (T0)
2 months
5 months
7 months
C
γ-H2AX

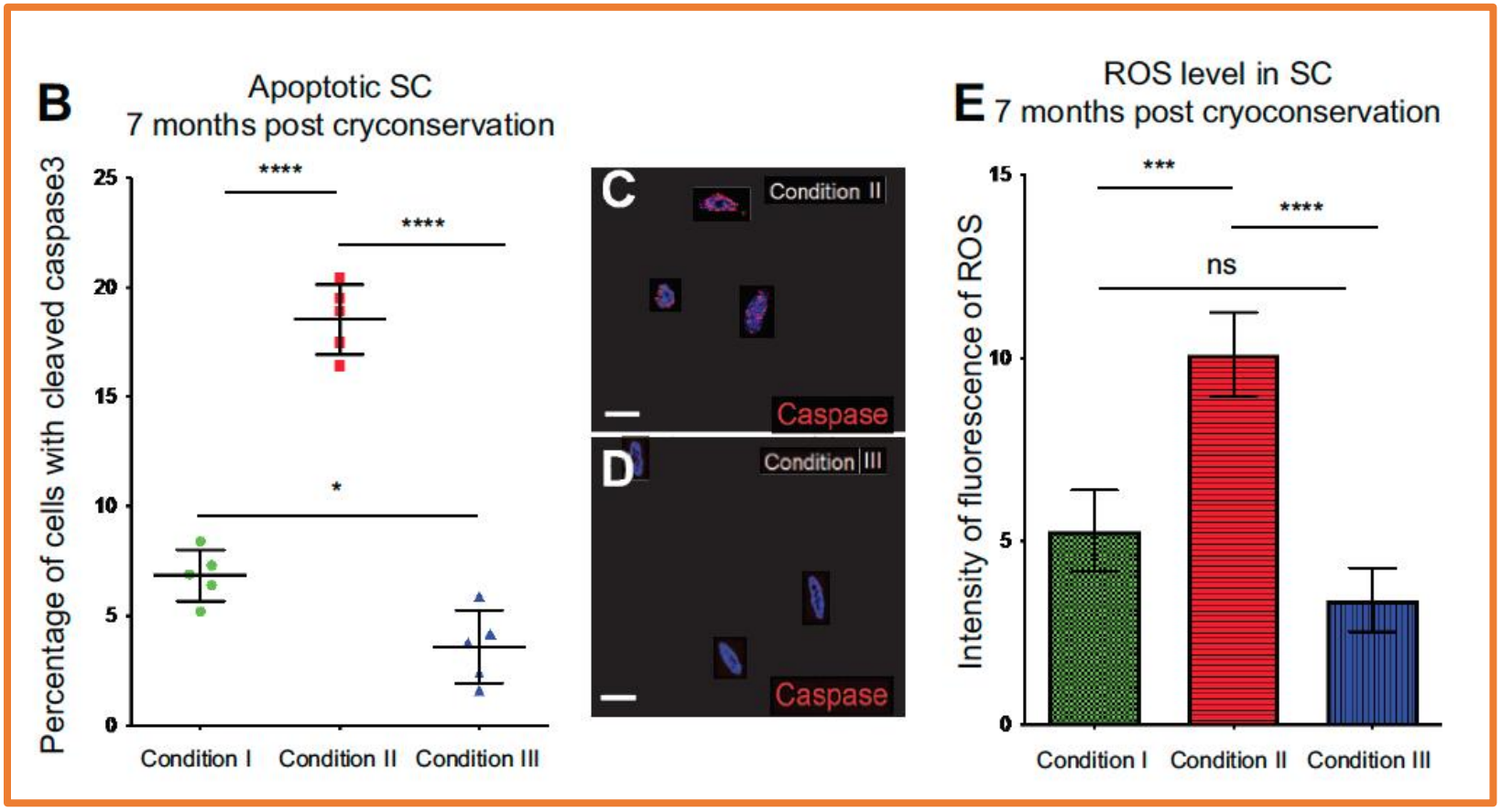

Number of foci in different storage condition and at different timing post cryopreservation
Increased
Standard
Deep underground
T0
number of foci
1.0
0.5
0.0
**
****
**
***
**
***
T0
2 months
5 months
7 months

Results

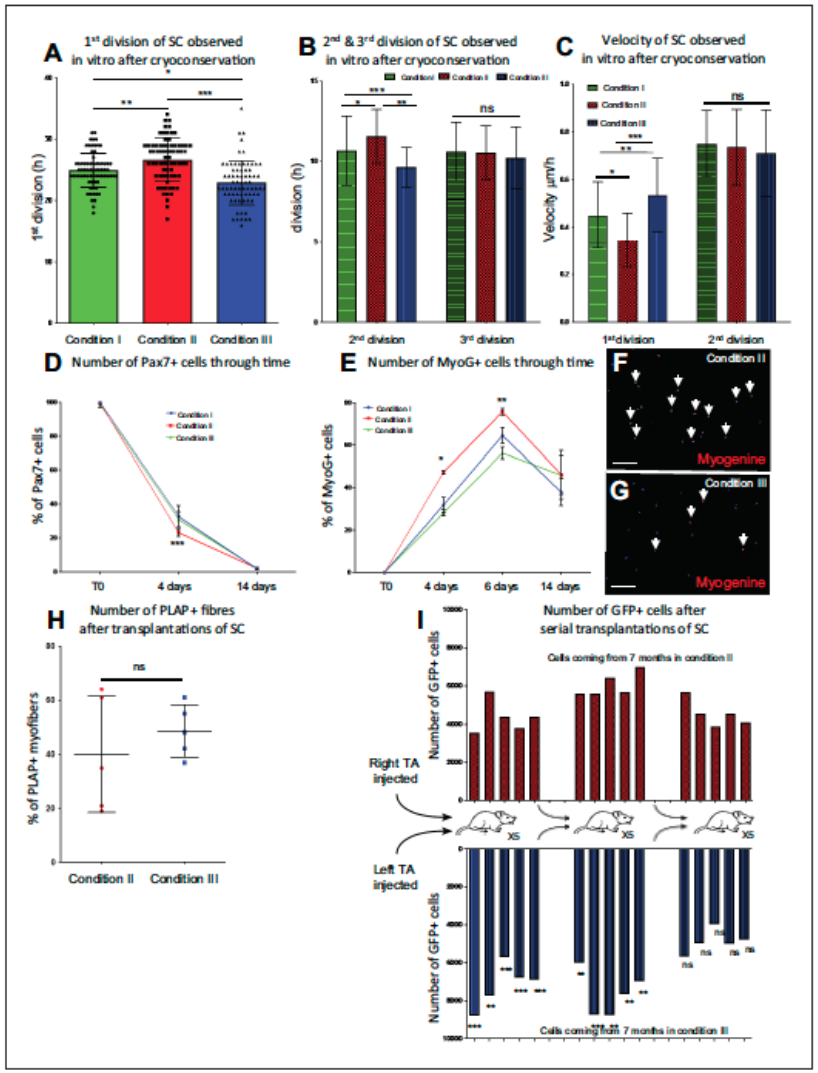

e Percentage of cells displaying at least one foci
% of cells with at least 1 foci
80
60
40
20
0
Increased
Standard
Deep underground
T0
*
**
*
**
*
***
T0
2 months
5 months
7 months


f Average number of foci in vitro through time
Average number of foci
1.0
0.5
0.0
Deep underground
Standard
Increased
****
****
****
****
****
*
T0
3h
6h
12h
24h
48h

Results

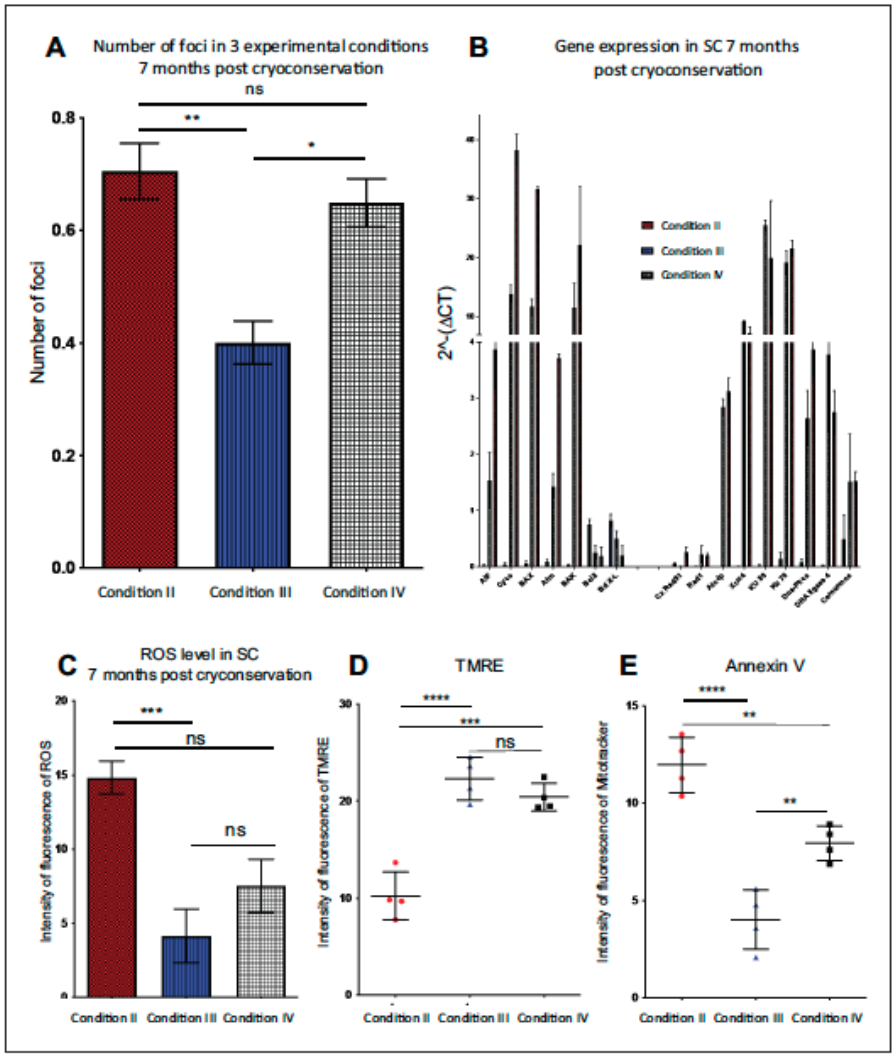

B
Apoptotic SC
7 months post cryconservation
Percentage of cells with cleaved caspase3
25
20
15
10
5
0
****
****
*
Condition I
Condition II
Condition III
C
Condition II
Caspase
D
Condition III
Caspase
E
ROS level in SC
7 months post cryoconservation
Intensity of fluorescence of ROS
15
10
5
0
***
****
ns
Condition I
Condition II
Condition III

Results



Quality of the muscle repair seems better after engraftment with stem cells protected from radiations

Results



Condition II : CR (neutrons) + Gamma

Condition III : Underground

Condition IV : CR (Neutrons)

Double strand Break seems to be related to CR

Outlook

- Still a lot of work to do to
- Long term preservation of biological materials is an issue for many applications : Fundamental research, Medical research, illness treatment, fertility, biodiversity,…
- European patent CNRS – Pasteur Institute :
WO2019077048 - HIGH-FIDELITY LONG-TERM STORAGE OF BIOLOGICAL MATERIAL
- US patent in progress
- A Startup has been created in particular for fund raising to continue research work
- For Pasteur Institute, we would anticipate a public health problem if treatment with stem cells are used in the future
- More studies with physicians and biologists in progress : preservation of gametes, seeds,….