The effects of sub-natural background radiation on CGL1 cell survival and growth on cells grown within SNOLAB, an underground research laboratory

Alyssa Labelle

MD Student (Y1), Northern Ontario School of Medicine
B.Sc. Biologie Biomédicale, Laurentian University

Thome Lab, NOSM

Friday, October 15th 2021

Northern Ontario School of Medicine
École de médecine du Nord de l'Ontario

NORTHERN HEALTH RESEARCH CONFERENCE

Disclosure of Affirmations, Financial & In-Kind Support

Affiliations

I have no relationships with for-profit or non-for-profit organizations.

Financial Support

This research has received financial support from the National Sciences and Engineering Research Council of Canada (Educational Award).

Northern Ontario School of Medicine
École de médecine du Nord de l'Ontario

Introduction : Radiation Exposure

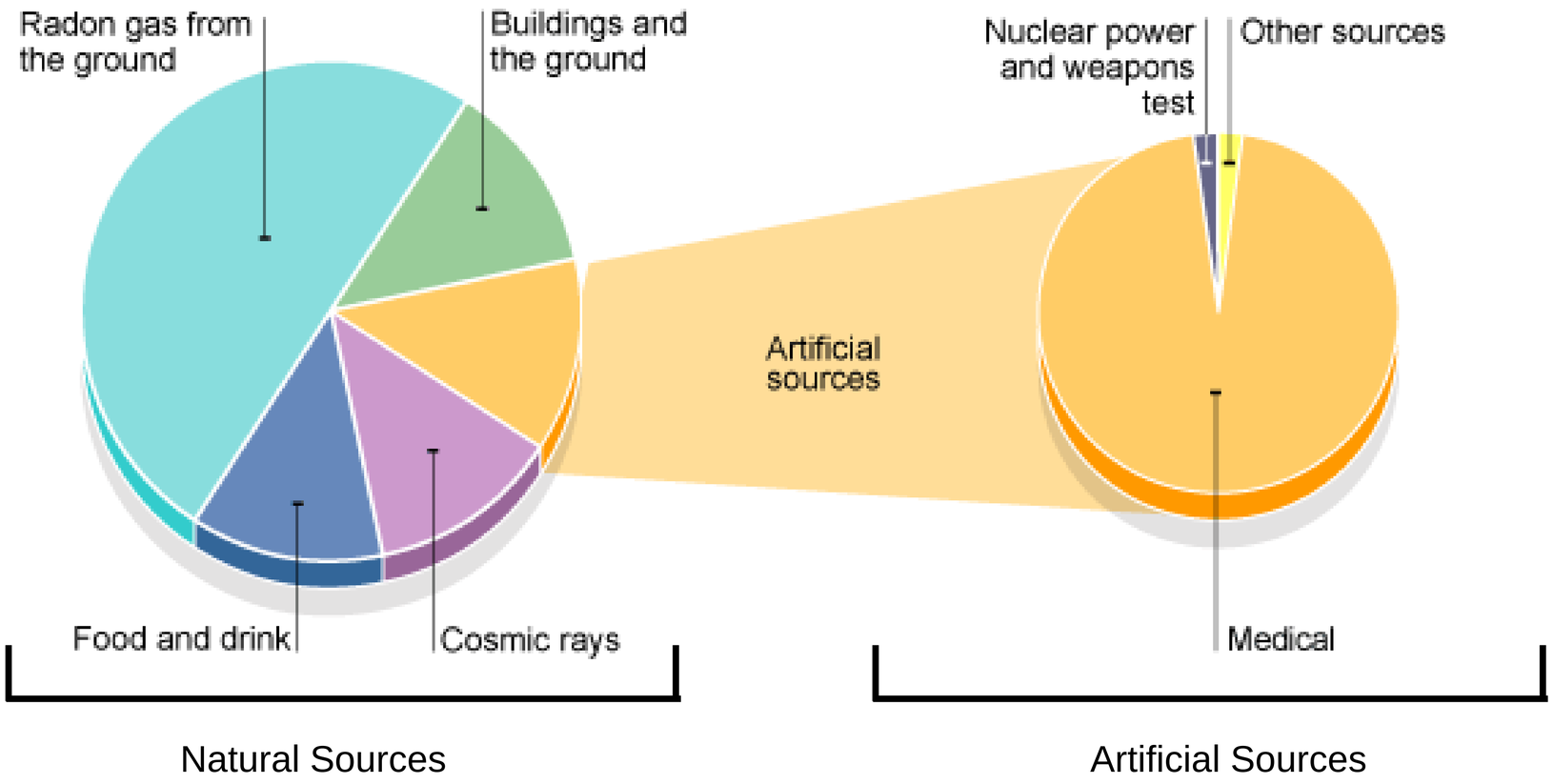


Image credit: https://www.thescientificstudent.com/understanding-radiation/

Introduction : Radiobiological models of risk

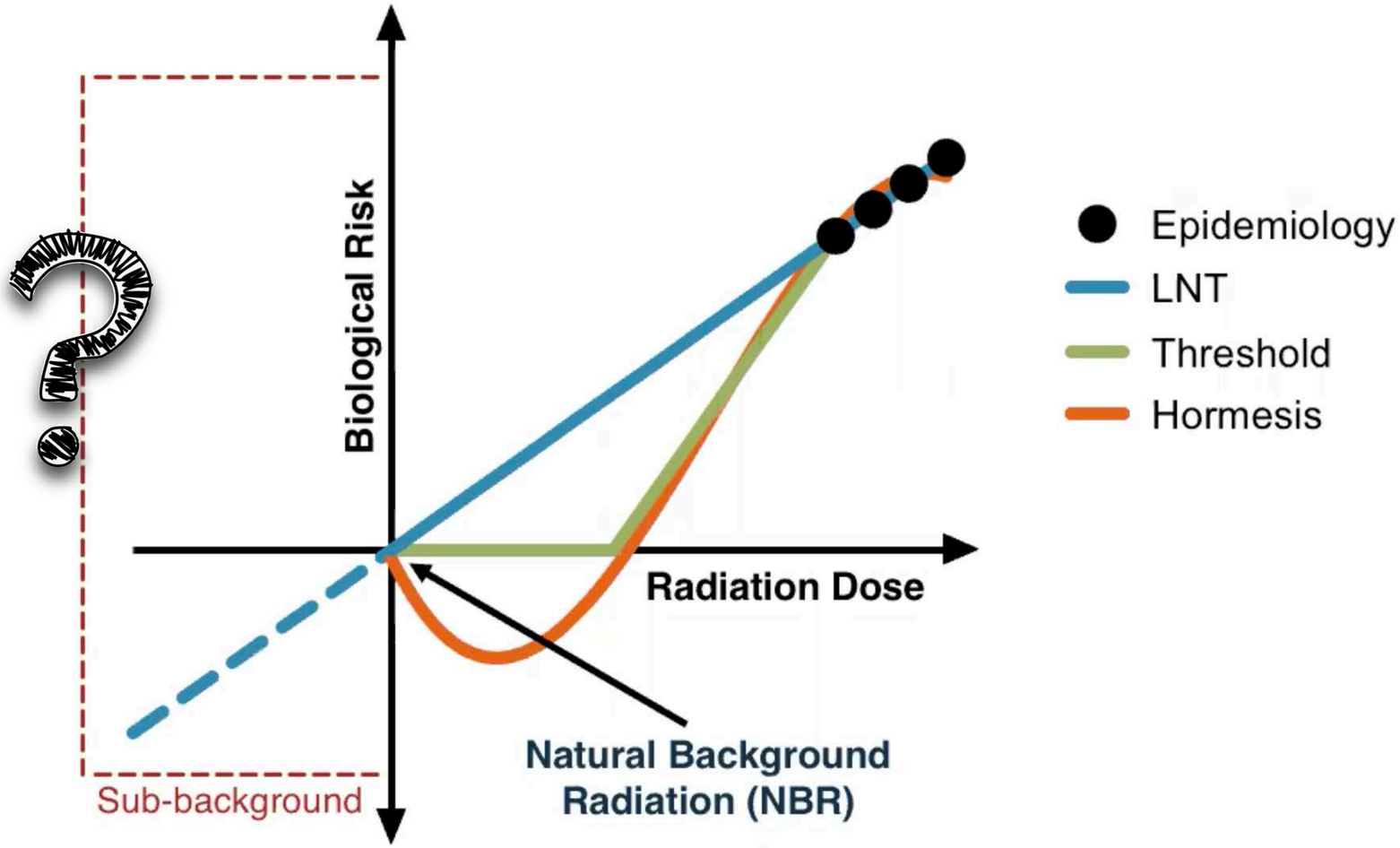


Image credits:

- J. Pirkkanen, 2020
- https://www.technologyreview.com/2009/03/25/31890/algae-move-by-singing-propulsion/
- https://theconversation.com/heres-what-that-house-proud-mouse-was-doing-plus-five-other-animals-who-take-cleaning-seriously-114040

Project Information

REPAIR Project:

"Researching the Effects of the Presence and Absence of Ionizing Radiation"

Goal:

Further understand the effects of natural background radiation on cells by limiting their exposure to it.

Experimental Locations

Northern Ontario School of Medicine
(NOSM)

Surface Control Environment

NBR Exposure (Cosmic Rays)

Sudbury Neutrino Laboratory
(SNOLAB)

Underground Control Environment

Radon Exposure

Specialized Tissue Culture Incubator
(STCI)

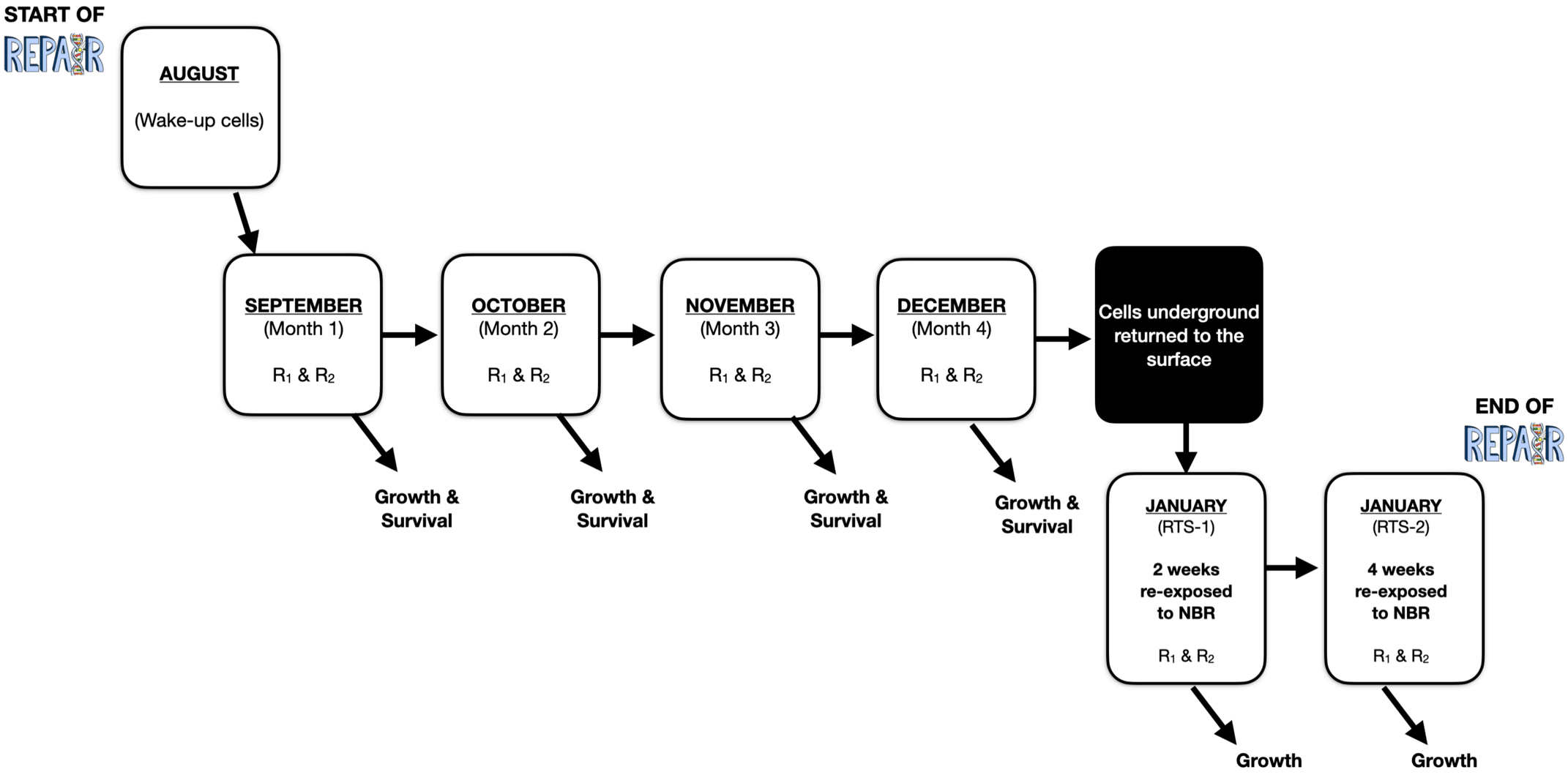
Underground Sub-Background
Experimental Environment

Completely Shielded

Image credits:

- https://www.sudbury.com/local-news/northern-ontario-school-of-medicine-training-military-physicians-2547038
- J. Pirkkanen, 2020

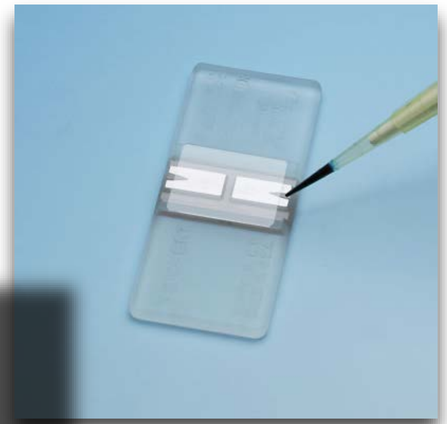
Project Timeline



Cell Growth

Methodology

- Seeded for 7 days in duplicates (14 tasks total per environment)
- 100 000 cells per flask
- Cells are counted every day for 7 days
- Media changes every 3 days



Data analysis:

- Cell doubling time
- 2-Way ANOVA

Image credits: http://www.davis.com/Product/Hemacytometer_Bright_line_phase_contrast_Pyrex_Includes_two_0_4_mm_cover_slips/DO-36219-10

Cell Growth (Month 1 - Month 4)

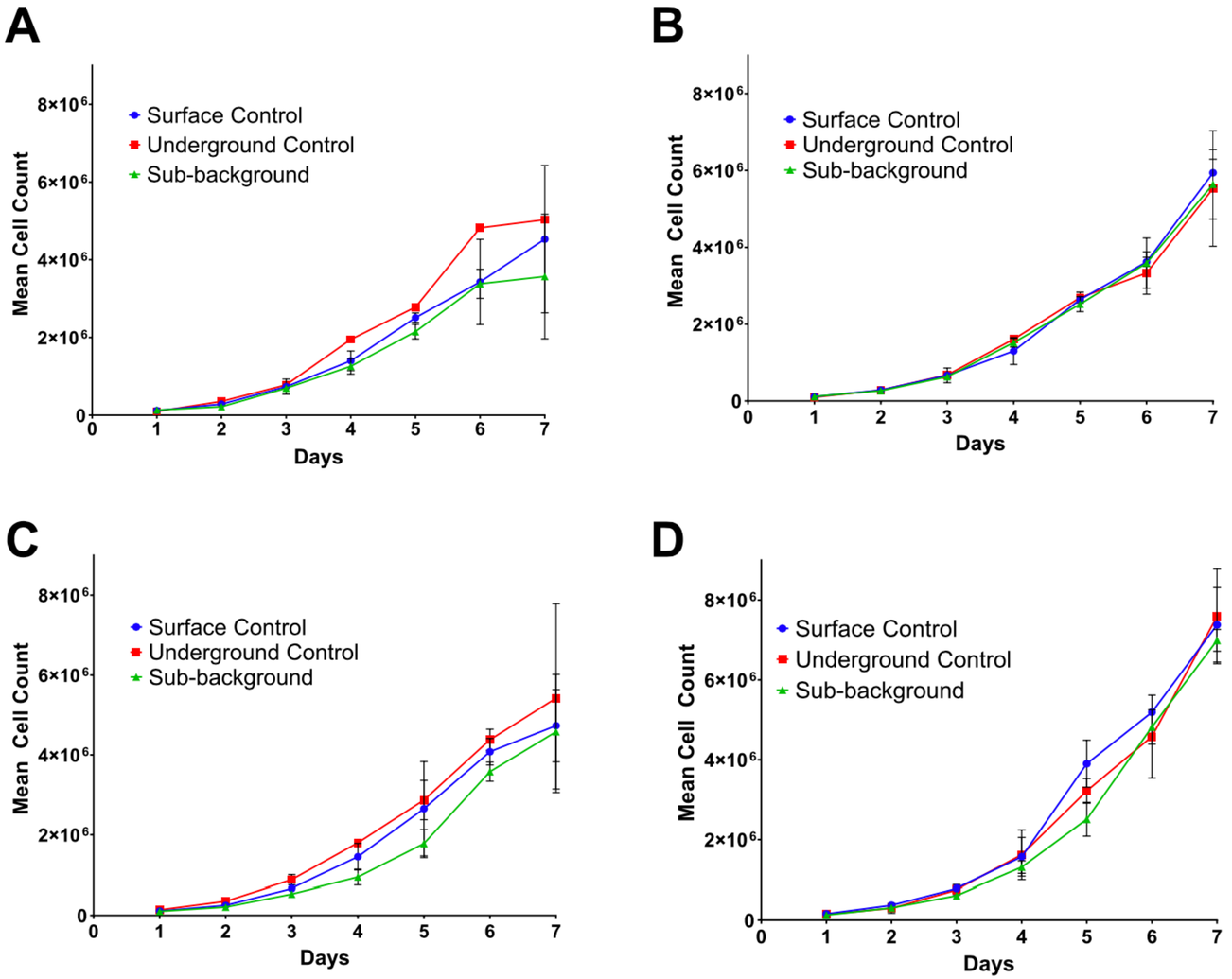


Figure 1: Growth curves for cells cultured over the course of four months in three different radiation environments; surface control (*blue circles*), underground control (*red squares*) and sub-background (*green triangles*).

Cell Growth (Re-exposure to NBR)

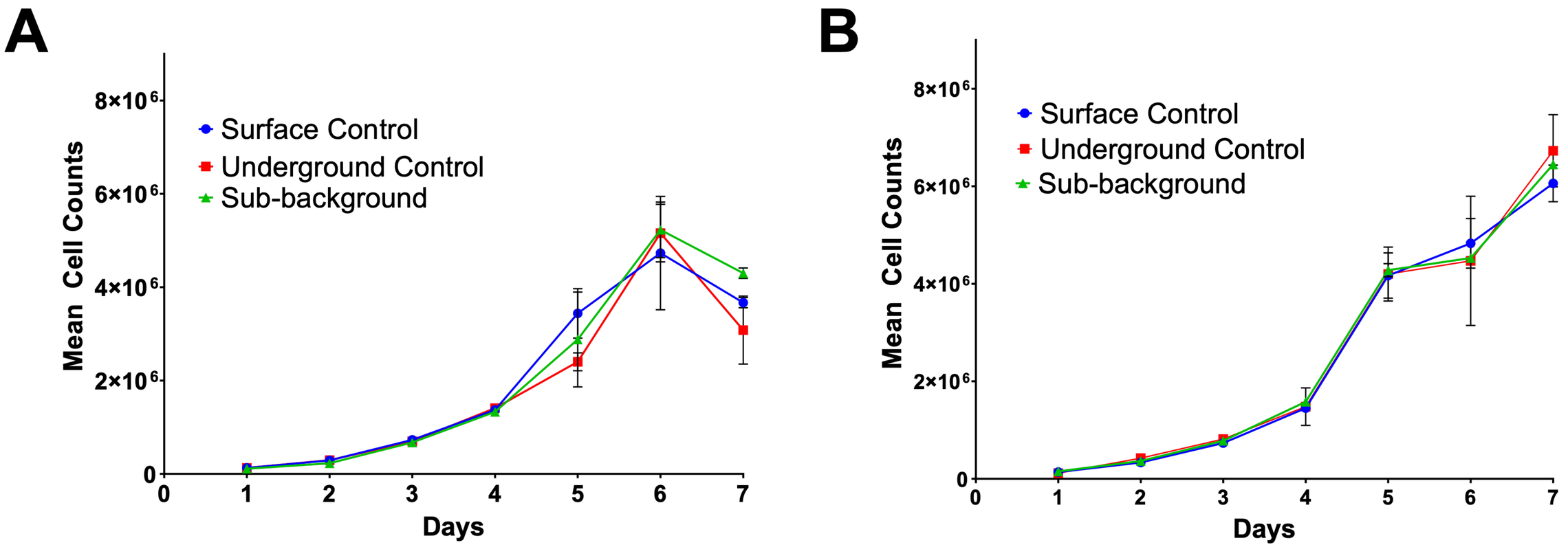


Figure 2: Growth curves for cells cultured following re-exposure to NBR in three different radiation environments; surface control (*blue circles*), within the underground control (*red squares*) and sub-background (*green triangles*). Growth curves were generated after re-exposure to NBR for **(A)** two weeks (RTS-1) and **(B)** four weeks (RTS-2). Data points represent the mean of 2 replicates ± standard deviation.

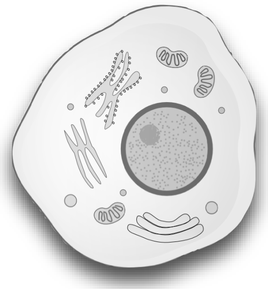
Cell Doubling Time

Table 1: Cell doubling times (DT) calculated based on the data from Figure 4 and 5. RTS-1 refers to the cells assayed after two-weeks following re-exposure to NBR, and RTS-2 refers to the cells assayed after four-weeks following re-exposure to NBR.

	Experimental Environment					
	Sub-background		Surface Control		Underground Control	
Experimental Time	DT (h ± SD)	N	DT (h ± SD)	N	DT (h ± SD)	N
Month 1	21.2±1.84	2	19.0±2.55	2	17.2±0.00	1
Month 2	19.5±0.11	2	20.4±1.18	2	17.8±0.27	2
Month 3	21.9±0.68	2	19.2±0.84	2	19.3±1.31	2
Month 4	21.6±2.07	2	22.1±1.54	2	20.6±1.75	2
RTS-1	19.5±0.62	2	20.7±178	2	20.6±1.99	2
RTS-2	21.2±1.88	2	21.3±0.38	2	20.4±3.25	2

Summary

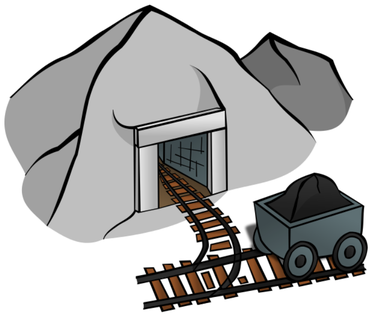
Overall, there were no changes in cell growth were detected between environments at any given time (p=0.0520) or within each environment over time (0.0856):



- Small sample sizes
- Cells were assayed at the surface.
- Experimental length (phenotypical vs. genetic changes)
- No pre-existing studies done with a human cell-line.

<u>Next Steps:</u>

- Culturing and assaying cells uniquely in their respective environments.
- Extending the length of the experiment to observe long-term effects on cell growth.



Cell Survival

Methodology

- Pre-determined volumes based on plating efficiency and dose rate.
- 6 doses: 0, 0.5, 1, 2, 4, 8 Gy
- Seeded in triplicates
- Irradiated 8 hours post-seeding
- Stained 9 days after, blinded & scored
- Media change 3 days after seeding

Data analysis:

- Calculated surviving fraction using plating efficiency
- Multiple linear regression analysis

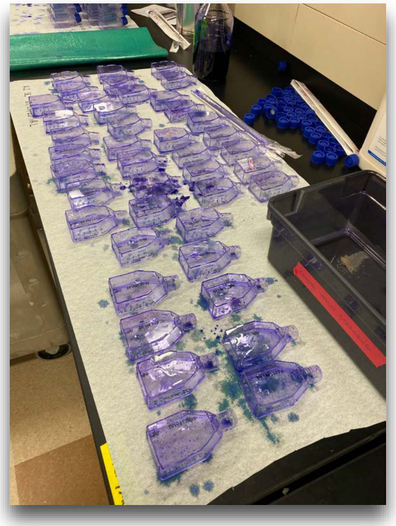
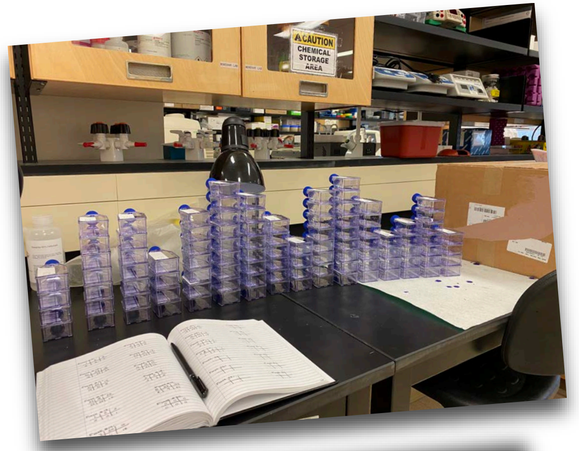


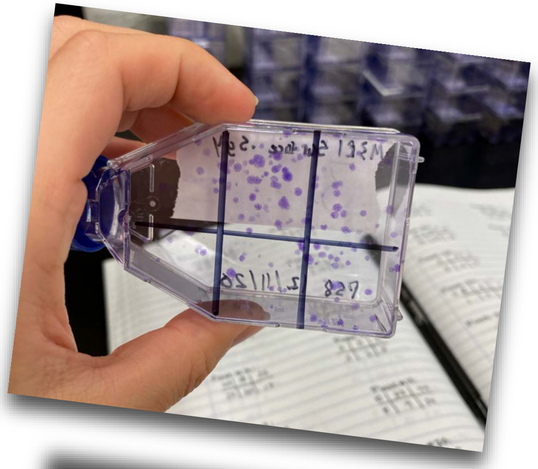
Image credits: https://gammagurus.com/products/cellrad-compact-cabinet-x-ray-cell-irradiator

Methodology

Staining

Scoring





UGC (0.5 Gy)

STCI (12 Gy)

Cell survival (Month 1 - Month 4)

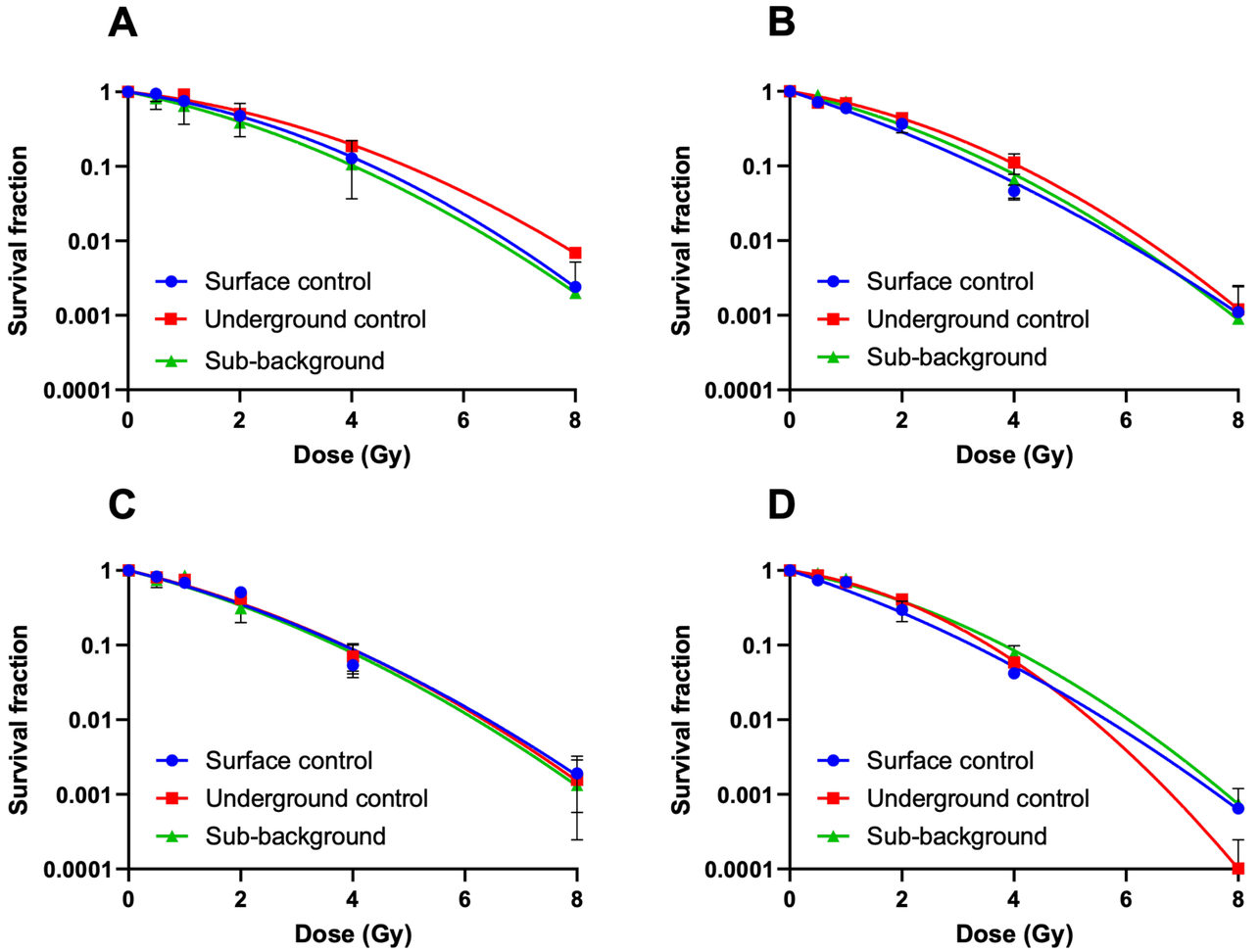
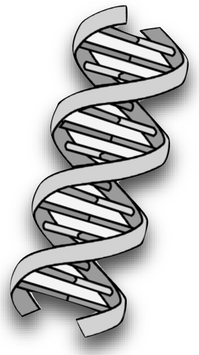


Figure 3: Cell survival curves for cells cultured over the course of four months in three different radiation environments; surface control (*blue circles*), underground control (*red squares*) and sub-background (*green triangles*). The data was fit with a linear quadratic relationship. Data points represent the mean of 2 replicates ± standard deviation.

Summary

- Time ($p<0.0001$) and dose ($p=0.0160$) had a significant impact on cellular survival (normal cell senescence)
- Environmental location did not have a significant impact on cellular survival ($p=0.7261$)($p=0.4989$).



- Cells were assayed at the surface.
- Experiment length (phenotypical vs. genetic changes)

Next Steps:

- Culturing and assaying cells uniquely in their respective environments.
- Extending the length of the experiment to observe long-term effects on cell survival

Image credits: https://precisionxray.com/x-rad/xrad-320/

Final Thoughts

- Research on this topic is scarce
- SNOLAB: One of the only facilities in which this type of research is possible

- Absence of NBR:
    - Cell regulation
    - Genomic stability
    - Disease progression
    - Low-dose radiation therapies
    - Cancer treatments and those working within underground environments (Sudbury mining population).

Image credits: Harvey, 2014

For Correspondence

Alyssa Labelle
Northern Ontario School of Medicine
allabelle@nosm.ca

Dr. Jake Pirkkanen
Laurentian University, Northern Ontario School of Medicine
jpirkkanen@laurentian.ca

Dr. Christopher Thome
Northern Ontario School of Medicine
cthome@nosm.ca

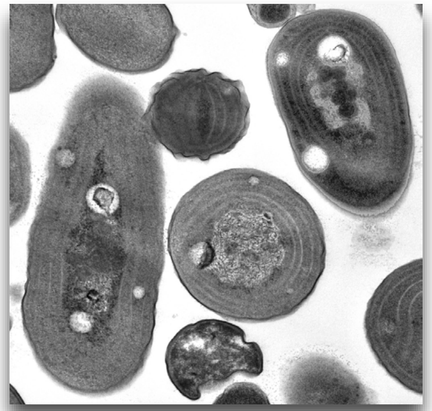
References

1. Brooks, A. L., Hoel, D. G., & Preston, R. J. (2016). The role of dose rate in radiation cancer risk: Evaluating the effect of dose rate at the molecular, cellular and tissue levels using key events in critical pathways following exposure to low LET radiation. International Journal of Radiation Biology, 92(8), 405–426. https://doi.org/10.1080/09553002.2016.1186301
2. Conter, A. (1987). Preirradiation of Medium Induces a Subsequent Stimulation or Inhibition of Growth According to the Physiological State in Synechococcus lividus in Culture. Radiation Research, 109(2), 342–346. https://doi.org/10.2307/3576959
3. Conter, A., Dupouy, D., & Planel, H. (1983). Demonstration of a biological effect of natural ionizing radiations. International Journal of Radiation Biology and Related Studies in Physics, Chemistry, and Medicine. https://doi.org/10.1080/09553008314550481
4. Cuttler, J. M. (2020). Application of Low Doses of Ionizing Radiation in Medical Therapies. Dose-Response, 18(1). https://doi.org/10.1177/1559325819895739
5. Donya, M., Radford, M., ElGuindy, A., Firmin, D., & Yacoub, M. H. (2014). Radiation in medicine: Origins, risks and aspirations. Global Cardiology Science & Practice, 2014(4), 437–448. https://doi.org/10.5339/gcsp.2014.57
6. Hall, E. J., & Giaccia, A. J. (2012). Radiobiology for the Radiobiologist. Lippincott Williams and Wilkins.
7. Huang, M., Miao, Z.-H., Zhu, H., Cai, Y.-J., Lu, W., & Ding, J. (2008). Chk1 and Chk2 are differentially involved in homologous recombination repair and cell cycle arrest in response to DNA double-strand breaks induced by camptothecins. Molecular Cancer Therapeutics, 7(6), 1440–1449. https://doi.org/10.1158/1535-7163.MCT-07-2116
8. Jones, J. (n.d.). Deterministic effects | Radiology Reference Article | Radiopaedia.org. Radiopaedia. Retrieved January 28, 2021, from https://radiopaedia.org/articles/deterministic-effects
9. Kawanishi, M., Okuyama, K., Shiraishi, K., Matsuda, Y., Taniguchi, R., Shiomi, N., Yonezawa, M., & Yagi, T. (2012). Growth Retardation of Paramecium and Mouse Cells by Shielding Them from Background Radiation. Journal of Radiation Research, 53, 404–410. https://doi.org/10.1269/jrr.11145
10. Li, Z., Zhang, W., Chen, Y., Guo, W., Zhang, J., Tang, H., Xu, Z., Zhang, H., Tao, Y., Wang, F., Jiang, Y., Sun, F. L., & Mao, Z. (2016). Impaired DNA double-strand break repair contributes to the age-associated rise of genomic instability in humans. Cell Death & Differentiation, 23(11), 1765–1777. https://doi.org/10.1038/cdd.2016.65
11. Pirkkanen, J., Laframboise, T., Liimatainen, P., Sonley, T., Stankiewicz, S., Hood, M., Obaid, M., Zarnke, A., Tai, T. C., Less, S. J., Boreham, D. R., & Thome, C. (2020). A Novel Specialized Tissu Culture Incubator Designed and Engineered for Radiobiology Experiments in a Sub-Natural Background Radiation Research Environment.
12. Pirkkanen, J. S., Boreham, D. R., & Mendonca, M. S. (2017). The CGL1 (HeLa × Normal Skin Fibroblast) Human Hybrid Cell Line: A History of Ionizing Radiation Induced Effects on Neoplastic Transformation and Novel Future Directions in SNOLAB. Radiation Research, 188(4.2), 512–524. https://doi.org/10.1667/RR14911.1
13. Reisz, J. A., Bansal, N., Qian, J., Zhao, W., & Furdui, C. M. (2014). Effects of Ionizing Radiation on Biological Molecules—Mechanisms of Damage and Emerging Methods of Detection. Antioxidants & Redox Signaling, 21(2), 260–292. https://doi.org/10.1089/ars.2013.5489
14. Scott, B. R. (2006). Stochastic Thresholds: A Novel Explanation of Nonlinear Dose-Response Relationships for Stochastic Radiobiological Effects. Dose-Response, 3(4), 547–567. https://doi.org/10.2203/dose-response.003.04.009
15. Sia, J., Szmyd, R., Hau, E., & Gee, H. E. (2020). Molecular Mechanisms of Radiation-Induced Cancer Cell Death: A Primer. Frontiers in Cell and Developmental Biology, 8. https://doi.org/10.3389/fcell.2020.00041
16. Stochastic effects | Radiology Reference Article | Radiopaedia.org. (n.d.). Retrieved January 28, 2021, from https://radiopaedia.org/articles/stochastic-effects
17. Thome, C., Tharmalingam, S., Pirkkanen, J., Zarnke, A., Laframboise, T., & Boreham, D. R. (2017). The REPAIR Project: Examining the Biological Impacts of Sub-Background Radiation Exposure within SNOLAB, a Deep Underground Laboratory. Radiation Research, 188(4.2), 470–474. https://doi.org/10.1667/RR14654.1
18. Vaiserman, A., Koliada, A., Zabuga, O., & Socol, Y. (2018). Health Impacts of Low-Dose Ionizing Radiation: Current Scientific Debates and Regulatory Issues. Dose-Response, 16(3). https://doi.org/10.1177/1559325818796331
19. Wang, J., Wang, H., & Qian, H. (2018). Biological effects of radiation on cancer cells. Military Medical Research, 5. https://doi.org/10.1186/s40779-018-0167-4

Additional Information

Background research

Conter & Planel, 1983



Growth of Synechoccus lividus
(Lead chamber)

Conter & Planel, 1987

Growth of Synechoccus lividus
(Cell Medium Irradiation)

Takizawa et al., 1992

Mouse Lymphoma L5178Y Cells
(Shielding Chamber)

Image credits:

- https://www.technologyreview.com/2009/03/25/31890/algae-move-by-singing-propulsion/
- https://theconversation.com/heres-what-that-house-proud-mouse-was-doing-plus-five-other-animals-who-take-cleaning-seriously-114040

Underground Laboratory Facilities within SNOLAB

Figure 4: The underground laboratory facilities within SNOLAB, housing the REPAIR project. The yellow arrow points to the incubator that houses the underground control cells.

Specialized Tissue Culture Incubator (STCI)

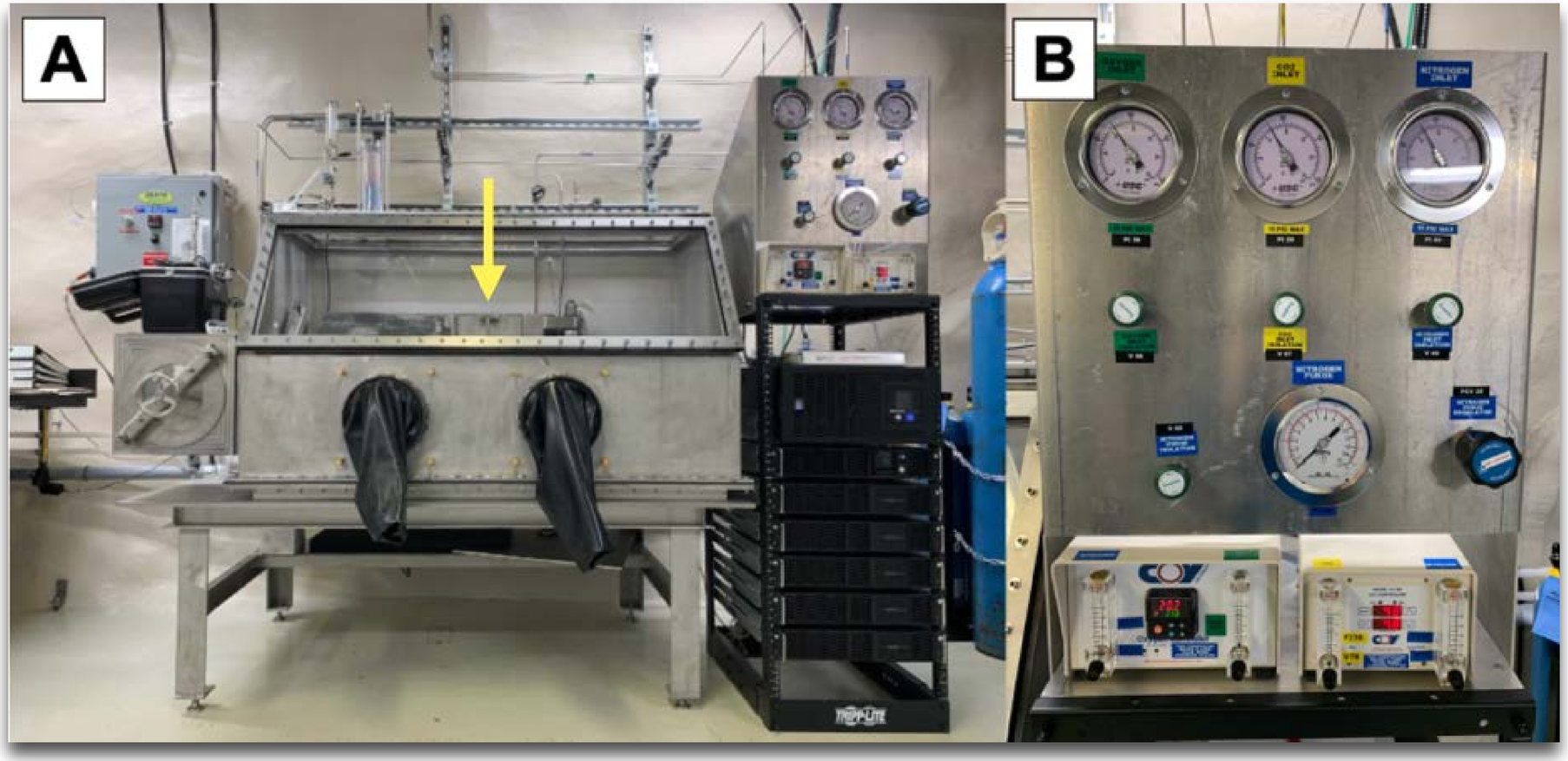


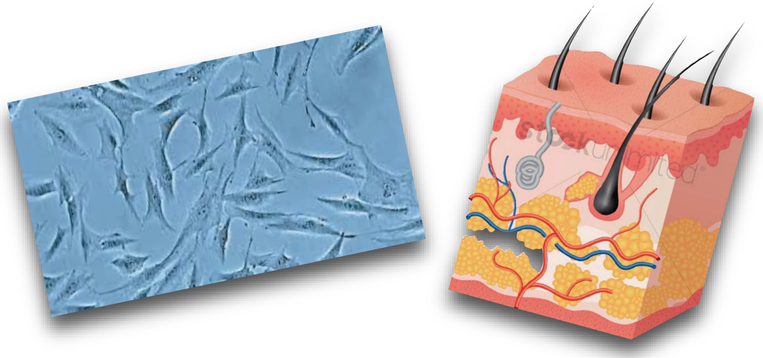
Figure 5: The Specialized Tissue Culture Incubator (STCI). **(A)** The STCI glovebox housing the lead castle incubator, where our sub-background cells are cultured. **(B)** Control panel on the STCI for N_2, O_2 and CO_2 flow rates, used by researchers to control the input rates of carbon dioxide, oxygen and nitrogen into the purge chamber and lead castle. The yellow arrow points to the lead castle incubator within the glovebox.

CGL1: Why this cell line?!

HeLa cell

X

Normal Skin Fibroblast



REVIEW ARTICLE | SEPTEMBER 05 2017

The CGL1 (HeLa × Normal Skin Fibroblast) Human Hybrid Cell Line: A History of Ionizing Radiation Induced Effects on Neoplastic Transformation and Novel Future Directions in SNOLAB

Jake S. Pirkkanen; Douglas R. Boreham; Marc S. Mendonca

Radiat Res (2017) 188 (4.2): 512–524.

https://doi.org/10.1667/RR14911.1 **Article history**

Share Tools

Growth Curve: Results (Weekly Growth Curve)

Table 2: Weekly cell doubling times (DT). RTS-1 refers to the cells assayed after two-weeks following re-exposure to NBR, and RTS-2 refers to the cells assayed after four-weeks following re-exposure to NBR.
* = A significant difference was detected between these two doubling times.

	Experimental Environment					
	Sub-background		Surface Control		Underground Control	
Experimental Time	DT (h ± SD)	N	DT (h ± SD)	N	DT (h ± SD)	N
Month 1	25.6±3.56	8	23.0±3.45	8	25.9±3.59	8
Month 2	24.5±1.93	8	22.0±1.13	8	23.0±2.44	7
Month 3	23.6±1.93	8	21.8±1.29	8	23.0±2.34	8
Month 4	26.5±2.89*	8	21.7±1.64*	8	24.0±2.14	8
RTS-1	21.0±2.06	4	22.3±0.91	4	21.2±0.60	4
RTS-2	21.1±0.49	2	21.4±1.59	2	21.2±0.69	2

Platting Efficiencies: Results

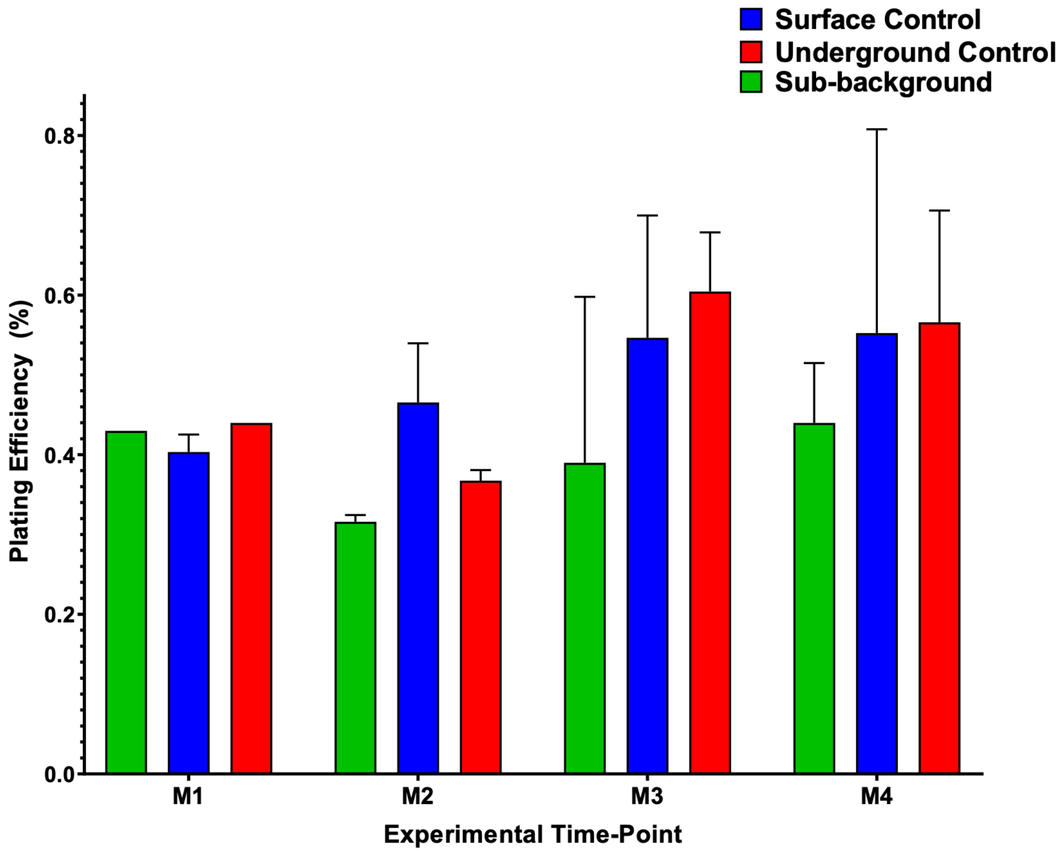


Figure 6: Plating efficiencies for cells cultured over the course of four months in three different radiation environments; surface control (*blue*), underground control (*red*) and sub-background (*green*). Plating efficiencies were recorded for cells cultured in each environment after one month (M1), two months (M2), three months (M3) and four months (M4). Bars represent the mean of 2 replicates ± standard deviation.