7-2023

Effects of Low-Energy X-rays and UV Radiation on Fibroblast Cells

Michelle Gorodisky

Congyue Peng

Arianna Csiszer

Delphine Dean

Effects of Low-Energy X-rays and UV Radiation on Fibroblast Cells

Michelle Gorodisky[1], Congyue Peng[2], Arianna Csiszer[2], Delphine Dean [2]

[1]Halmos College of Arts and Science, Nova Southeastern University, Davie, FL USA

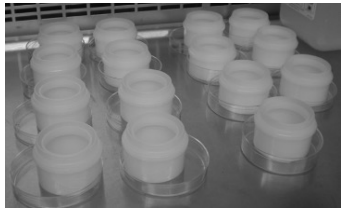
[2]Department of Bioengineering, Clemson University, Clemson, SC USA

Introduction

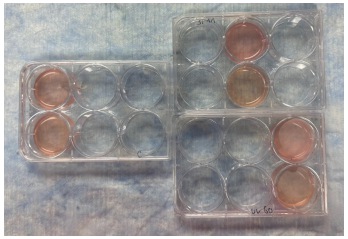
High-dose and high-energy radiation has proven to damage DNA and kill cells. High energy radiation has been studied in many contexts including environmental disasters and cancer radiotherapy (Grudzenski et al., 2010). In radiotherapy, high-dose x-rays are often used to kill cancer and effectively reduce cancer cell proliferation. Non-ionizing ultraviolet (UV) radiation can cause skin cancer and DNA damage from prolonged exposure (Rognoni et al., 2021). However, the mechanisms and effects of low-dose and low-energy radiation, such as those received during standard diagnostic imaging, are understudied in comparison. The application of low dose radiation to medical therapies and imaging technology encourages further research. The purpose of this study was to identify the effects of UV radiation and low dose (< 1mGy) x-ray radiation at varying dose rate on 3T3 fibroblast cells. The DNA damaged and apparent phenotypic changes were assessed.

Materials and Methods

3T3 fibroblast cells were plated on a Chemplex 6 µm thick Mylar® film. The Mylar® film does not significantly attenuate the low energy x-rays used in this study. One group of cells were then irradiated at a total dose of 0.2 mGy with a dose rate of 0.0424 mGy/min for 283 seconds, and another group of cells were irradiated at a total dose of 0.2 mGy with a dose rate of 0.0258 mGy/min for 466 seconds. The fibroblast cells were also plated on 6-well plates and exposed to UV light from above at 254 nm for 15 minute and 60 minute intervals. Immunofluorescence imaging, DNA damage assays, and proliferation assays were performed



High-density polyethylene cups assembled with 6 µm thick Mylar® film, exposed to x-ray irradiation.



6-well plates exposed to UV irradiation.

Results

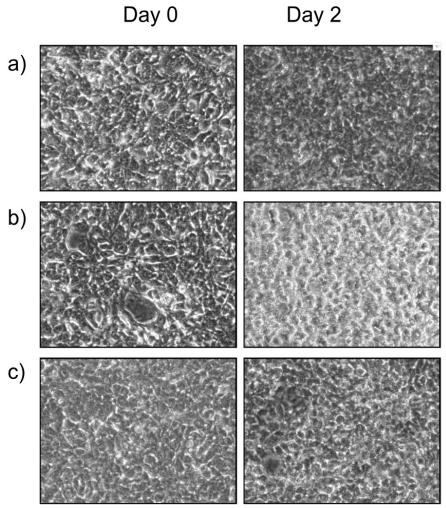


Figure 1. a) Control; b) UV 15 min.; c) UV 60 min.

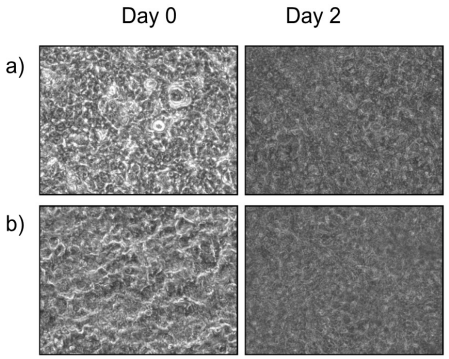


Figure 2. a) x-ray 0.0424 mGy/min. for 283 s.; b) x-ray 0.0258 mGy/min. for 466 s.

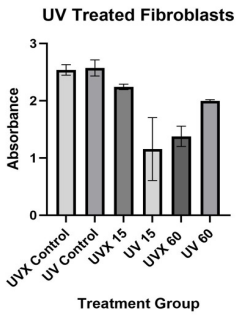


Figure 3. Proliferation assay of UV treated cells after 0 (Control), 15min, and 60min of exposure. X indicates the wells closer to the UV source.

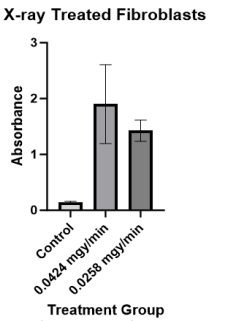


Figure 4. Proliferation assay of 0.2 mGy x-ray treated cells at two different dose rates (0.0424 mGy/min and 0.0258 mGy/min).

Discussion and Conclusion

After 2 days in culture post irradiation, there were no significant differences in cell morphology and cell density with UV or x-rays (Figures 1 and 2). However, differences were observed in the UV irradiation group cultures. The UV radiation seemed to affect the cell metabolism. These were even noticeable by visual inspection of the cell media. There is a visual difference between the wells proximal to the UV light source compared to the wells placed a few centimeters further from the UV. The wells further away from the UV were visibly paler in color. The cells closer maintained a darker color in the media, possibly indicating that those cells are less active at metabolizing nutrients from the media. In addition, cells exposed to UV showed significantly ($p < 0.01$) less proliferation than controls (Figure 3). Surprisingly, x-ray irradiated fibroblasts showed increased proliferation compared to controls (Figure 4).

One limitation of our current study is that the fibroblasts were extremely confluent (Figures 1 and 2), which may have caused a lack of adherence to the Mylar® film and 6-well plates. This limited the duration of our study to just 2 days after irradiation. In addition, the high cell density may have provided some radioprotective effects as cells in confluent monolayers decrease their proliferation rate and thus, have more time to repair damage from radiation sources. We are continuing this study with lower cell densities and longer culture times.

Acknowledgements

This research was supported by NSF and Clemson University, Clemson, SC. "Nature's machinery through the prism of physics, biology, chemistry and engineering" (NSF Award # 1757658)

References

Grudzenski, S., Raths, A., Conrad, S., Rübe, C.E., & Löbrich, M. (2010). Inducible response required for repair of low-dose radiation damage in human fibroblasts. PNAS, 107(32), 14205-14210. https://doi.org/10.1073/pnas.1002213107

Rognoni, E., Goss, G., Hiratsuka, T., Sipilä, K.H., Kirk, T., Kober, K.I., Lui, P.P., Tsang, V.S., Hawkshaw, N.J., Pilkington, S.M., Cho, I., Ali, N., Rhodes, L.E., & Watt, F.M. (2021). Role of distinct fibroblast lineages and immune cells in dermal repair following UV radiation-induced tissue damage. eLife, 10, e71052. https://doi.org/10.7554/eLife.71052