Validation of Radiobiological Monte Carlo Simulations Using the Dicentric Chromosome Assay Modified for Human Dermal Fibroblast Cells

Health Canada Santé Canada

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Biodosimetry Background

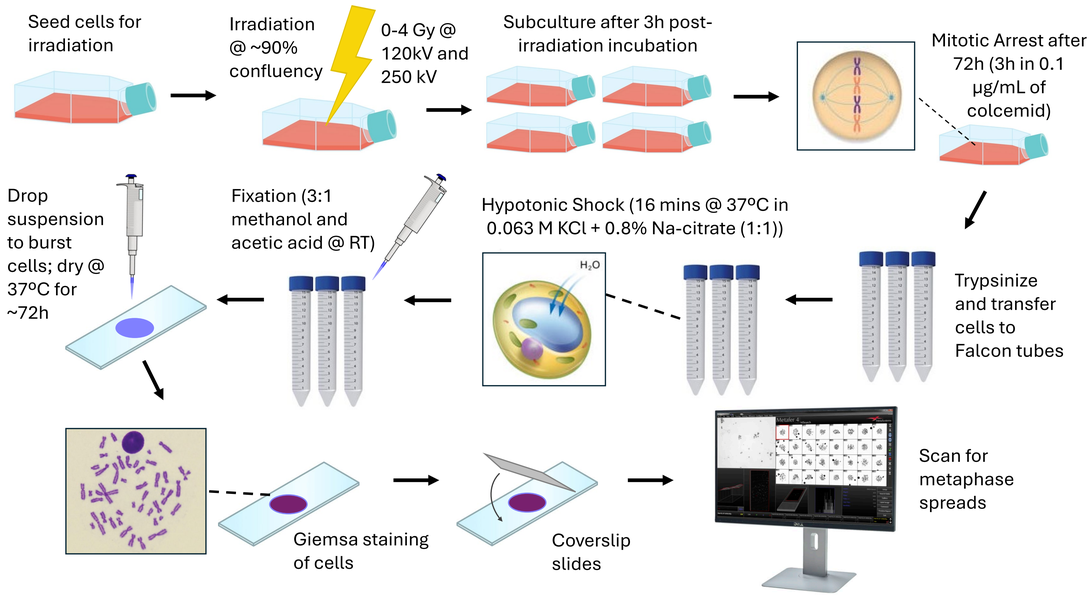
Accurate dose assessment in the event of exposure to ionizing radiation is critical to help mitigate potential health effects:

- **Biodosimetry (BD)** techniques correlate biological damage from ionizing radiation to absorbed dose. Health Canada (HC) produces in-house **dose response calibration curves** by exposing biological samples to a range of **known X-ray doses** and correlating the measured biological damage.
- Real-world radiation environments, such as **mixed fields produced from nuclear accidents** are too complex to be represented by a simple X-ray source.
- Computational models of fibroblast cells using Monte Carlo (MC) methods can address this by utilizing a more relevant source for calibration.
- BD at Health Canada is conducted with lymphocytes so the **BD methodology had to be modified** to work with fibroblasts before the computational simulations could be validated.

The purpose of this project is to modify existing biodosimetry methodology for fibroblast cells to produce an X-ray dose response curve. This curve can then be used to validate a computational simulation.

Fibroblast Dicentric Chromosome Assay Methodology

The key difference between fibroblasts and lymphocytes is the fact that **fibroblasts must have a surface to adhere to when growing** as opposed to being in suspension in a solution. Several aspects of the Dicentric Chromosome Assay (DCA) had to be changed to account for this difference. **A summary of the entire finalized DCA process is provided below:**



Experiment timing was determined using a growth curve to find the doubling time of the cells (**Figure 1**). For X-ray irradiation, the XRAD-320 was used as shown in **Figure 2**.

The following **key aspects of the protocol [1] were adjusted**:

- **Incubation time and colcemid concentration** for mitotic arrest was optimized to 3 hours at 0.1 µg/mL.
- **0.8% sodium citrate** was added to the hypotonic shock solution at a **1:1 ratio with 0.063 M KCl**.
- **BrdU**, (used to identify cells in second metaphase), was **removed from the process** as it changed the hypotonic shock conditions.

In turn, the **Cytokinesis-Block Micronucleus (CBMN)** assay was used to **quantify the number of cells in second mitosis** by arresting cells at cytokinesis, resulting in a single cell with two nuclei. Cells that have undergone additional mitoses will have three or more nuclei. **Figure 2** shows a tri-nucleated cell amongst several binucleated cells. After **48 hours, 1.5% of cells were found to have 3 or more nuclei**. Based on this observation, cells in second mitosis can be ignored.

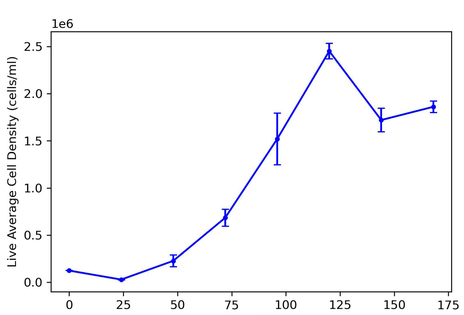


Figure 1: Growth curve for fibroblast cells.

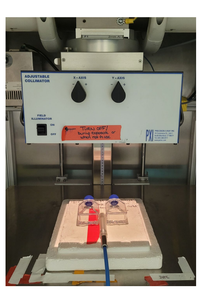


Figure 2: Irradiation setup for fibroblast flasks.

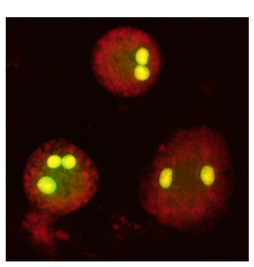


Figure 3: Differing number of nuclei indicate how many rounds of mitosis the cell has undergone.

Assessing Chromosome Damage

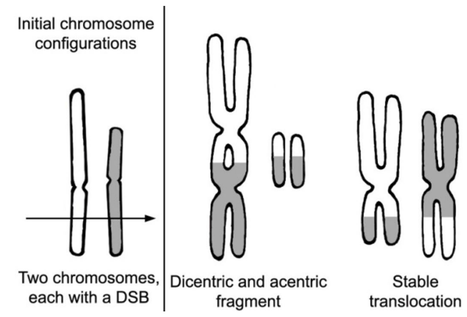
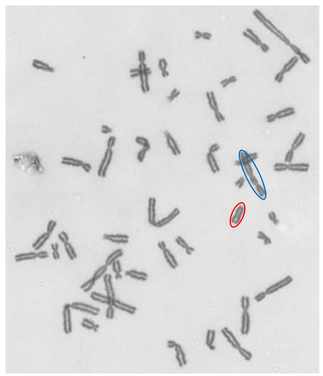


Figure 5: Chromosomes broken during irradiation can misrepair to produce dicentrics or translocations, and acentric fragments (above). An example of a dicentric chromosome (blue) and its associated fragment (red) can be seen on the microscope image (left).



For DCA, the main chromosome aberration of interest is the **dicentric chromosome, formed when two chromosomes incorrectly misrepair (Figure 5)**. The yield of dicentrics scales with radiation dose and can therefore be used to create the dose response curve to validate the MC simulation.

Simulated Fibroblast X-ray Radiation Response

- Simulation geometry consists of a **cellular model of a fibroblast cell in TOPAS-nBio**, developed by the Kildea Research Lab. [3]
  - **475 µm³ cubic nucleus** placed in a 2000 µm³ spherical volume.
  - Fibroblast cells are simulated for comparison to lymphocyte cells used in modern biodosimetry.
- Source is composed of **250 keV X-rays** distributed uniformly throughout the cell volume (secondary electrons simulated directly to improve efficiency).
- Using the **Mechanistic DNA Repair and Survival (MEDRAS)** model [4], chromosome misrepair was simulated and the frequency of dicentrics was calculated.
- By adjusting MEDRAS model parameters, namely the rejoining range, the model response can be adjusted to match data produced from the Health Canada lab.

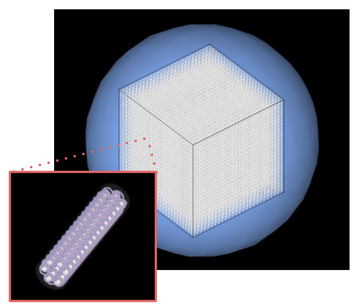


Figure 6: Simulation geometry in TOPAS-nBio replicating a fibroblast cell. Adapted from [3].

Simulation Validation and Adjustment

- The radiation response curves generated from the experiment, calculated by the MC simulation, and two curves from the literature can be seen in **Figure 7**.
- Overall, the **trends match between all curves**, showing an increase in dicentric yield with dose.
- The 120 kV and 250 kV **curves produced using the methodology presented here had a lower dicentric yield compared to literature**, but there exists a notable difference between the two curves from literature.
- Adjusting the rejoining range parameter σ in the simulation to 0.038, provided better agreement between the simulation and the experiment.

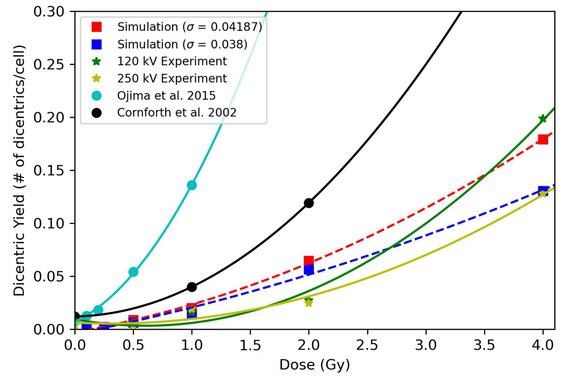


Figure 7: Yield of dicentric chromosomes from the fibroblast DCA experiment conducted, compared to the MC simulation calculations, along with historical experimental results with similar conditions.

Troubleshooting Low Dicentric Yield

- Due to the overall low dicentric yield results, some troubleshooting steps were taken to increase the yield.
- A small experiment (0.0, 1.0, 4.0 Gy) was run with the **incubation time post irradiation increased to 12 hours, from 3 hours**.
- **Yield was found to be significantly higher for the 1 Gy dose point**.
- The spreads for the 4 Gy slides were extremely low which caused the yield to be much smaller than the original experiment.

Dose (Gy)	Yield (dicentric/cell)	Change from original
0.0	0	↓ 0.004
1.0	0.042	↑ 0.026
4.0	0.108	↓ 0.091

Table 1: Results from troubleshooting experiment compared to original dicentric yield output.

Conclusions and Future Work

(1) The **fibroblast DCA methodology was successfully optimized** to produce a dose response curve.
(2) Experimental curve was found to have lower yields compared to similar studies, but as there is **significant interlaboratory difference** in literature, this result was not surprising.
(3) The rejoining range parameter in the repair simulation was adjusted to change the simulation output to better match the experimental output produced by our lab.
(4) A **120 kV simulation is underway** to validate the change to the rejoining range by comparing to the 120 kV experimental curve presented here.
(5) After validation, a lymphocyte model will be simulated to compare the cell lines.

References

[1] Binz et al., *STAR protocols* (2024)
[2] Montgomery et al., *Phys. Med. Biol.* (2021)
[3] McMahon & Prise, *Front. Oncol.* (2021)

Funding

Financial support for this work was provided through the CSA and through the NSERC Discovery Grant RGPIN-2022-0431.