

Radiation-Induced Functional Changes In The Lens Epithelial Cells and Implications for Cataract Development

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Disclosure of Affiliations, Financial and In-Kind Support

Affiliation:

- I have no relationships with for-profit or not-for-profit organisations.

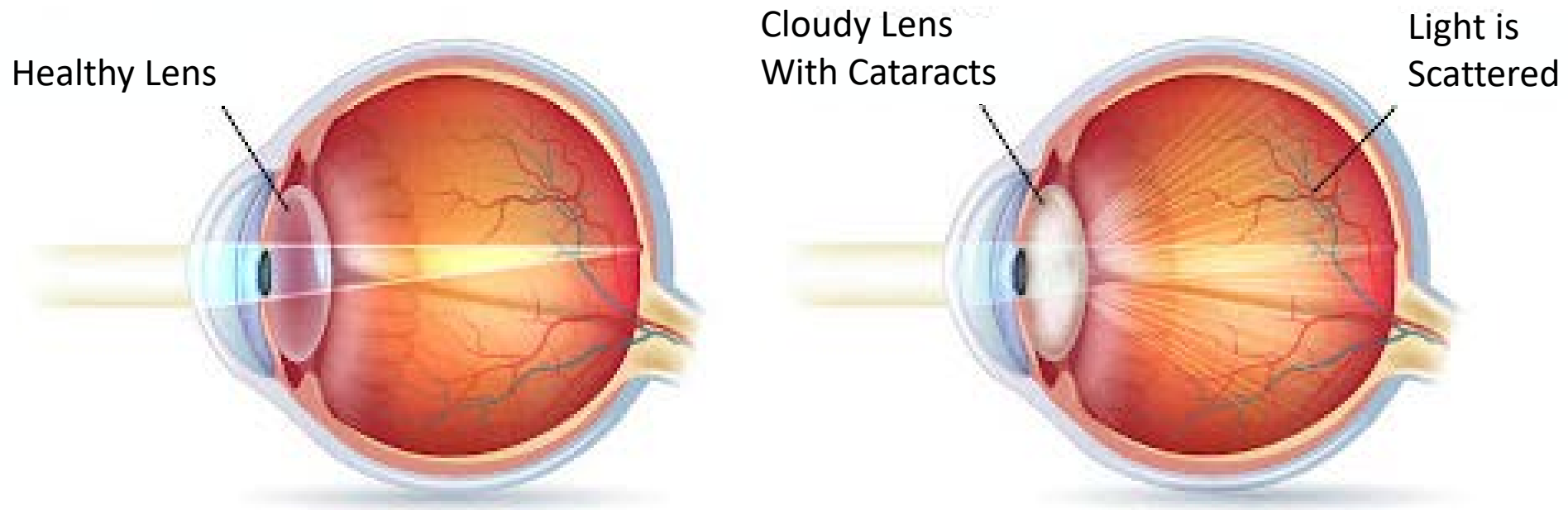
Financial support:

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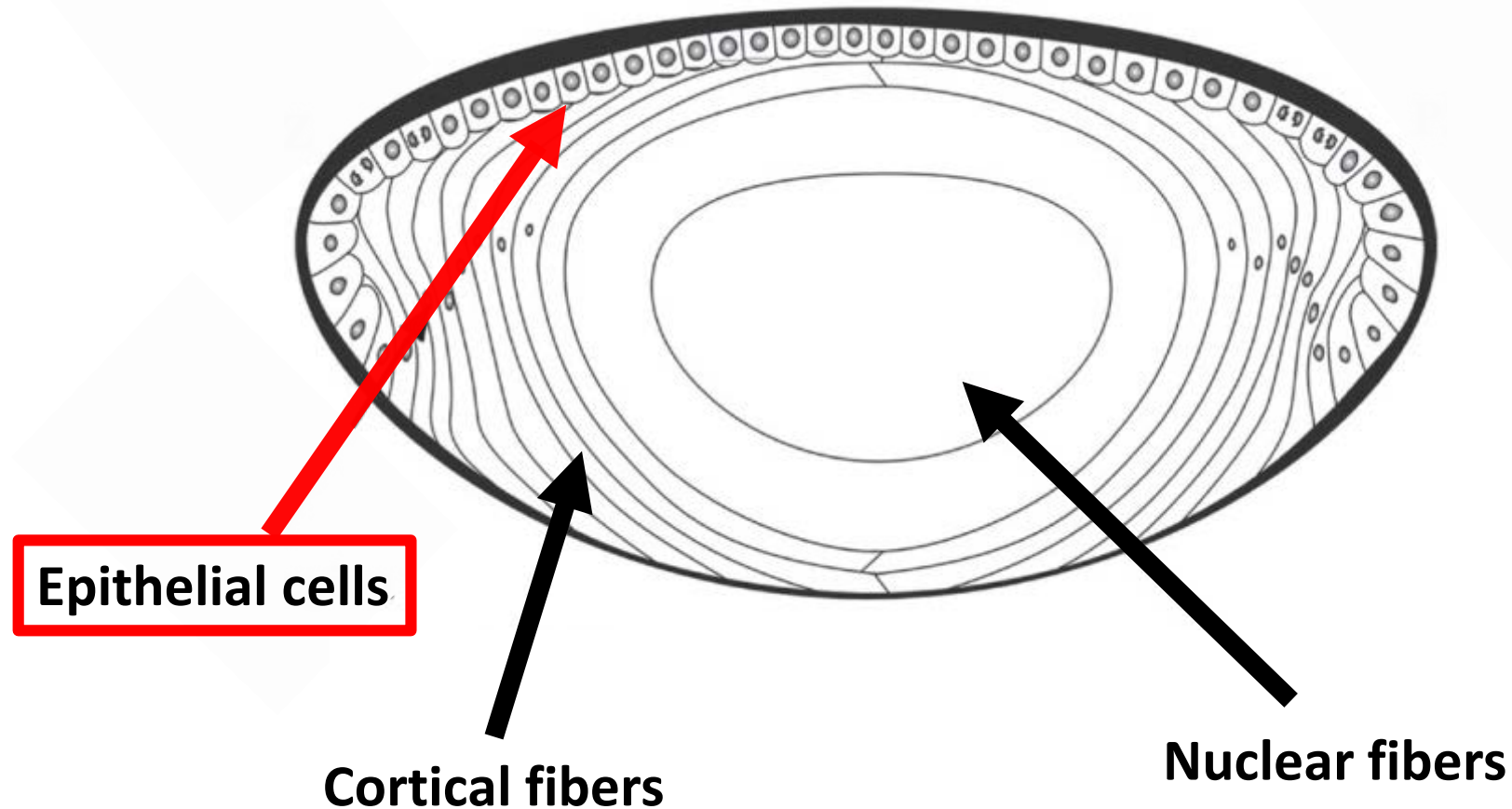
Cataracts

- One of the leading causes of blindness worldwide (51%).
- Over 2.5 million Canadians are living with this condition.
- 50% of people will have a cataract by age 65 years.
- Each year, 350 000 cataracts surgeries are performed in Canada.
- Three types of cataracts.
- Risk factors include age, ocular trauma, metabolic disorders, as well as exposure to ionizing radiation.

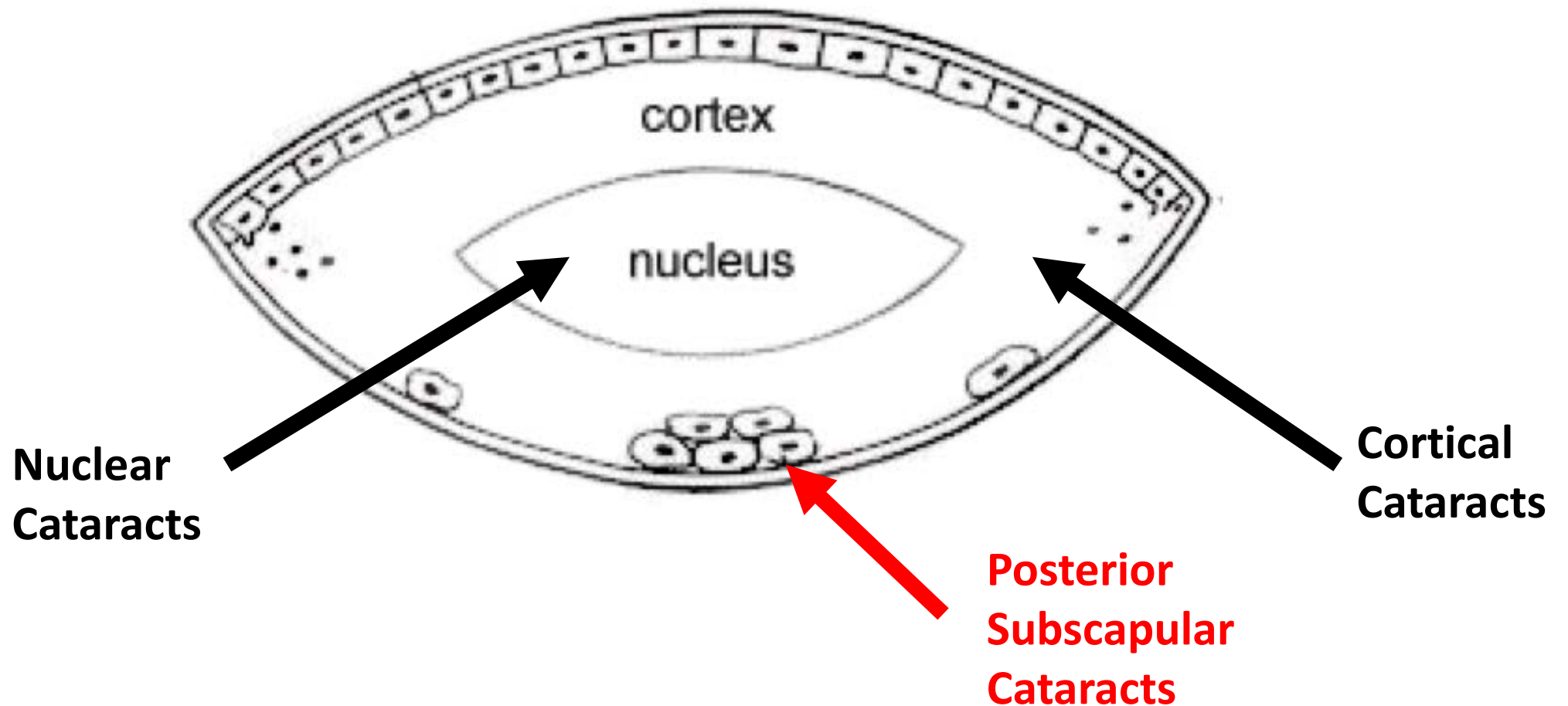
The lens of the eye refract light to be focused onto the retina



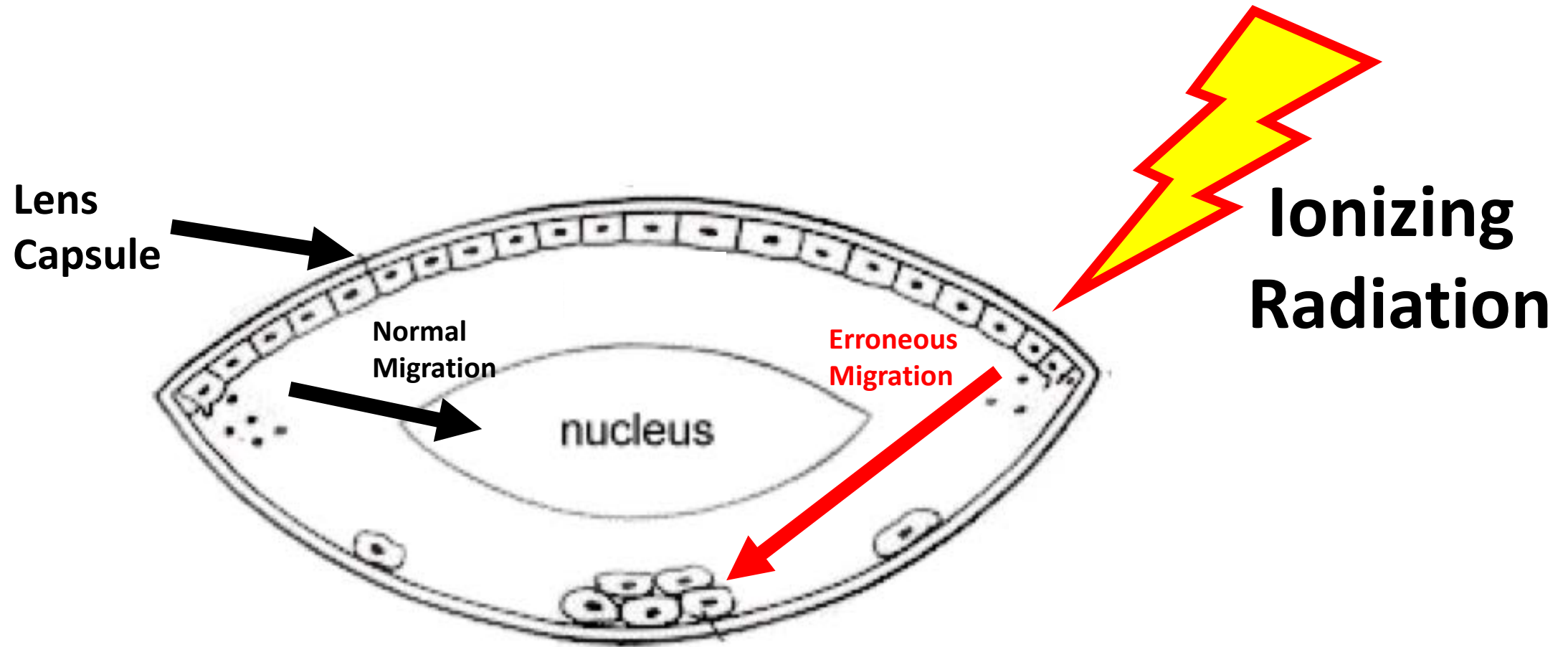
The lens are composed of two types of cells



Posterior subscapular cataracts are most common radiation induced cataracts



Radiation induced cataracts



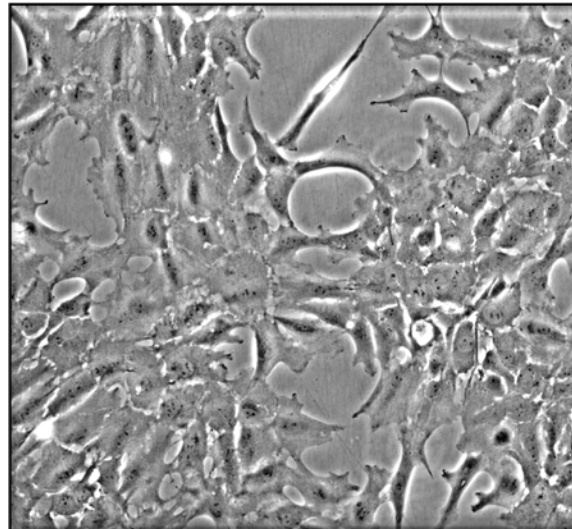
Objective

The objective of my research is to identify the downstream measurable changes in cell proliferation, migration and adhesion of the human lens epithelial cells caused by ionizing radiation.

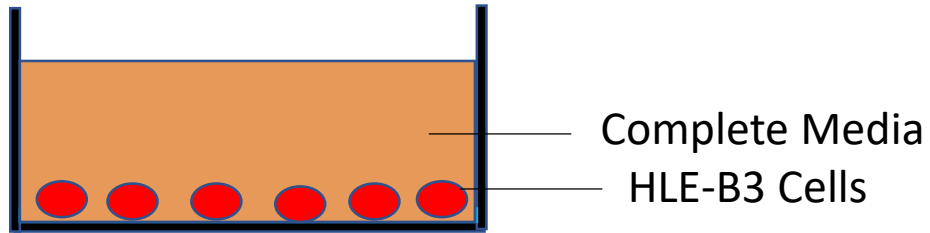
Methodology

Cell Culture and Irradiation

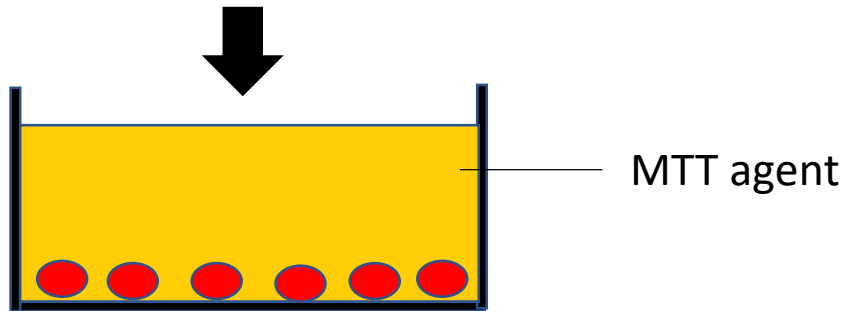
- Data was obtained using human lens epithelial cells (HLE-B3).
- The X-RAD 320 is the the X-ray system used to deliver radiation doses in all experiments.
- Cells were irradiated with single acute doses; 0 Gy, 0.1Gy 0.25 Gy, 0.5 Gy, 1 Gy, and 2 Gy.



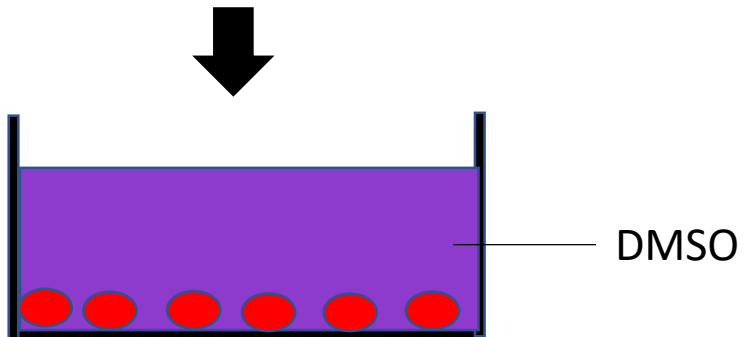
Proliferation



HLE-B3 cells are seeded at 7 500 cells inside a 96-well plate (incubate for 48H).

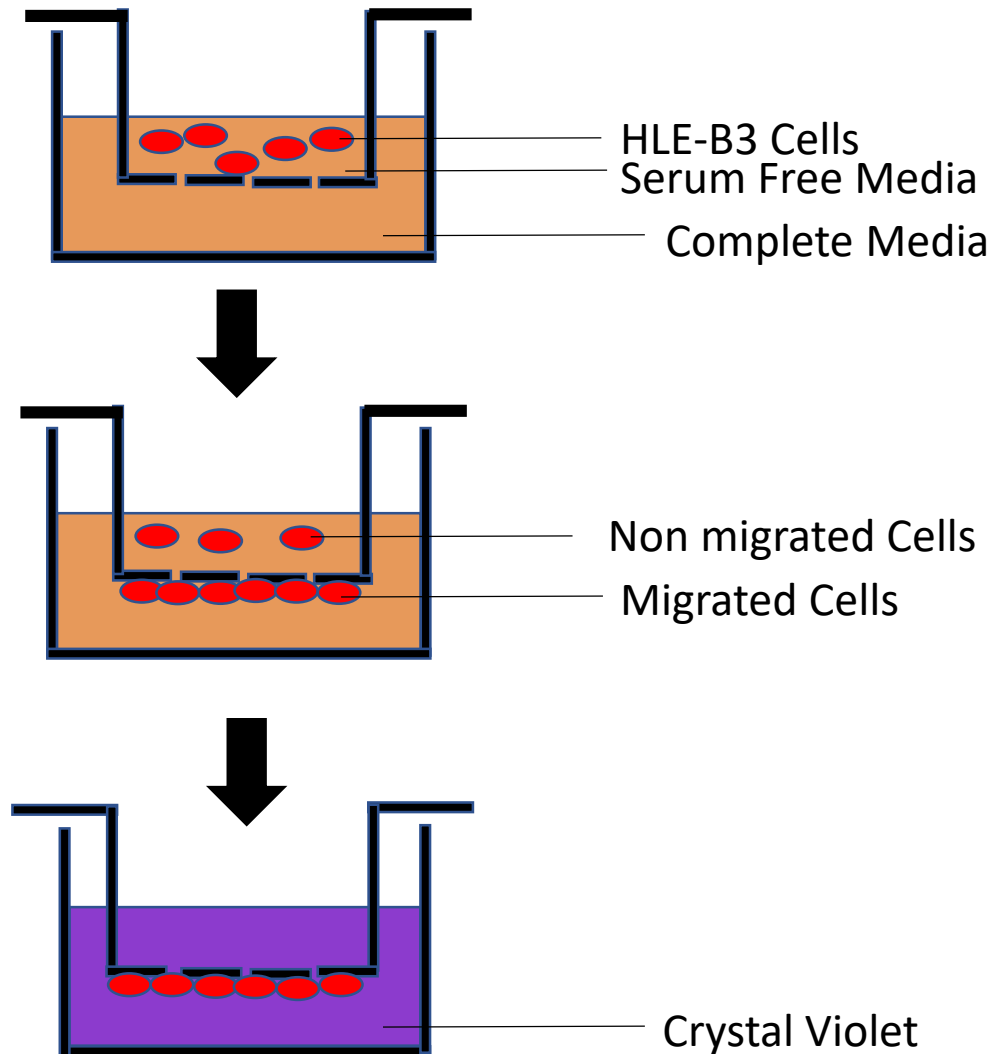


Add 100uL of (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT) to each well. Incubate for 3.5H.



Remove media, add DMSO and incubate for 15 minutes. Measure absorbance at 570nm.

Migration

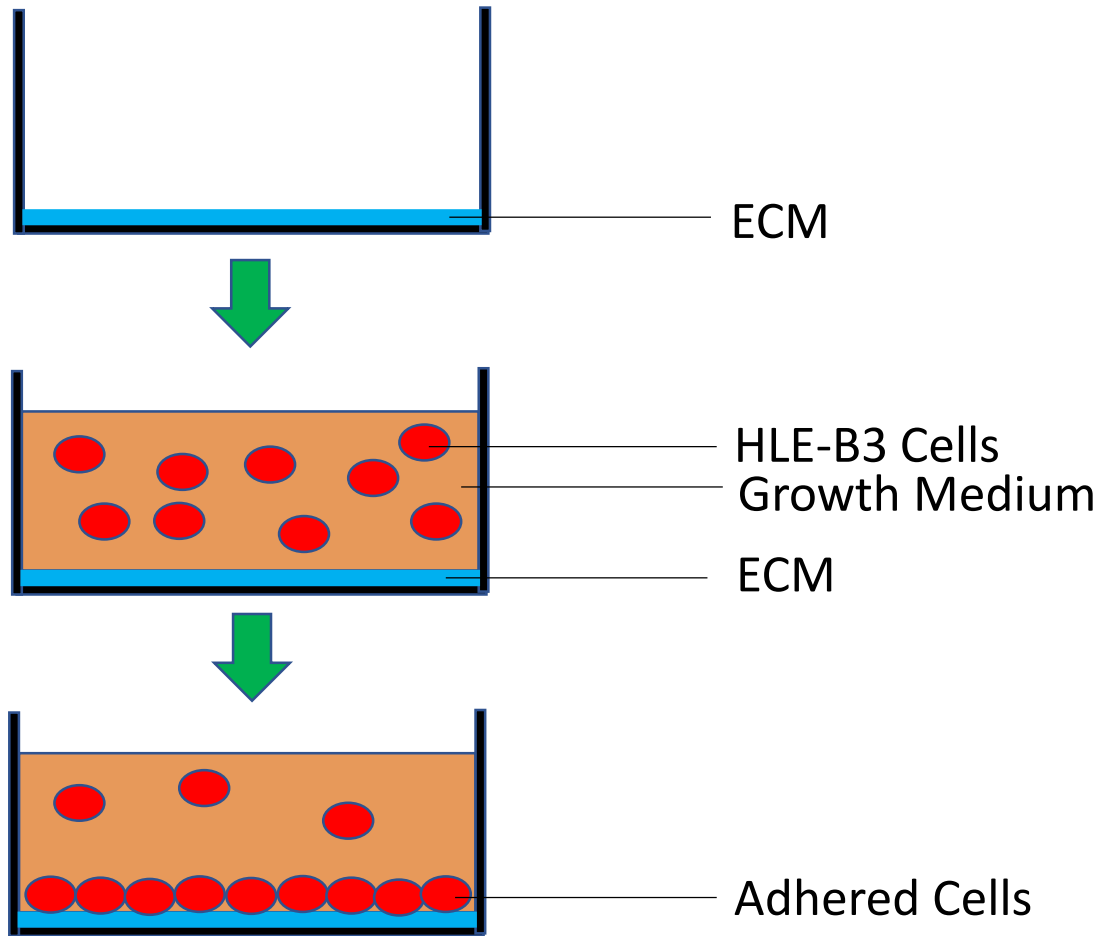


HLE-B3 cells are seeded at 100 000 cells in inside the insert of the 24-well plate.

Cells are given 8 hours to migrate before the staining step occurs.

HLE-B3 cells are then fixed with ice cold methanol and stained with crystal violet.

Adhesion



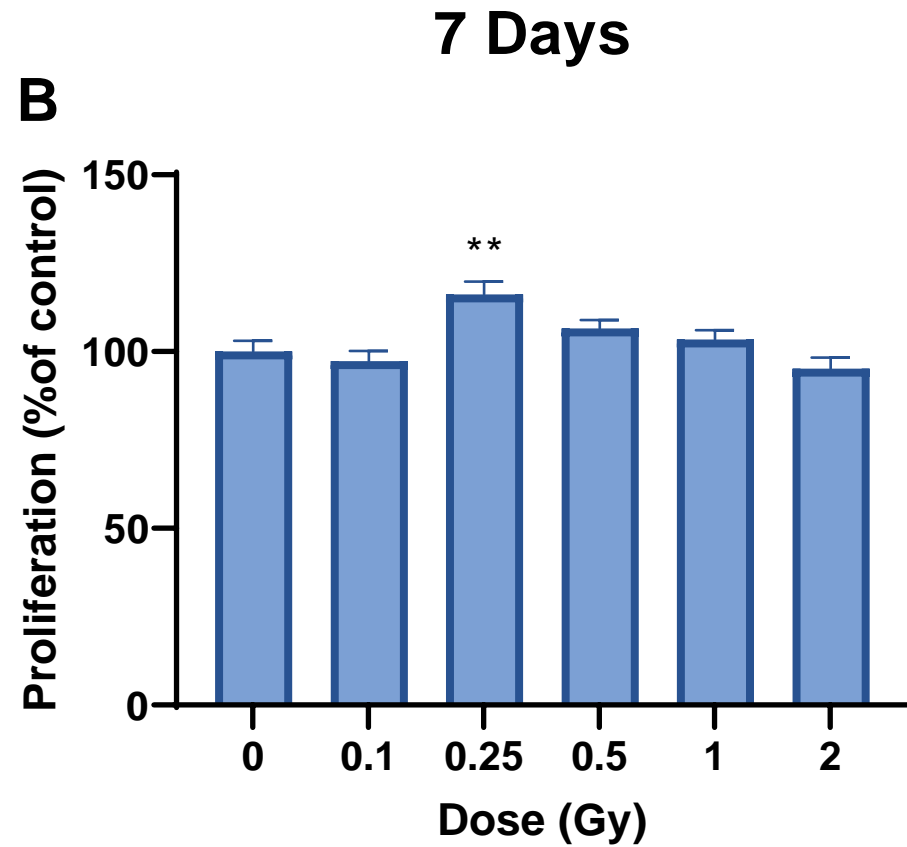
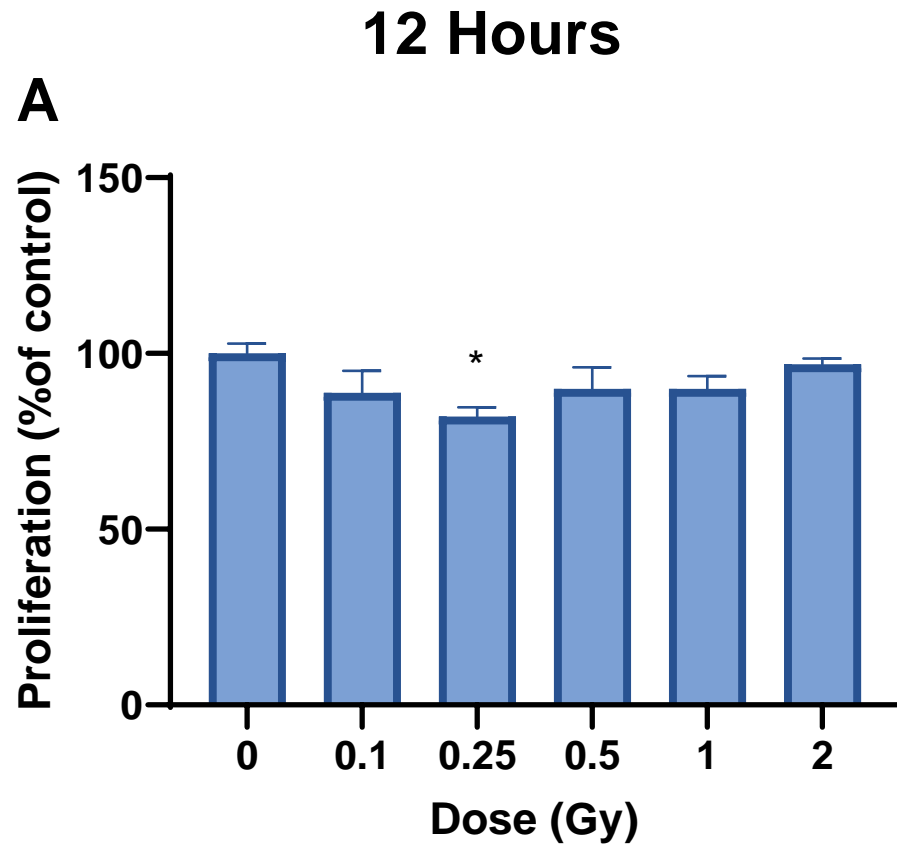
96-well plates and were coated with several kinds ECM (human type IV collagen, laminin and fibronectin). The plates were coated and were left to settle overnight.

Each well of the pre coated plate was seeded with 100000 cells.

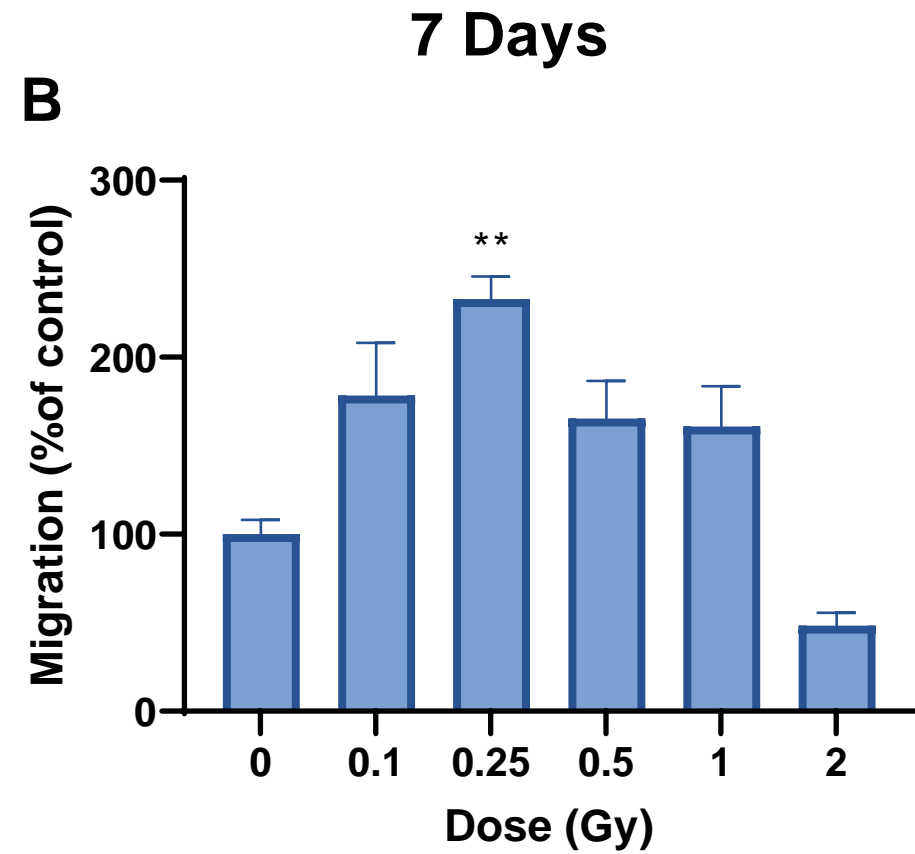
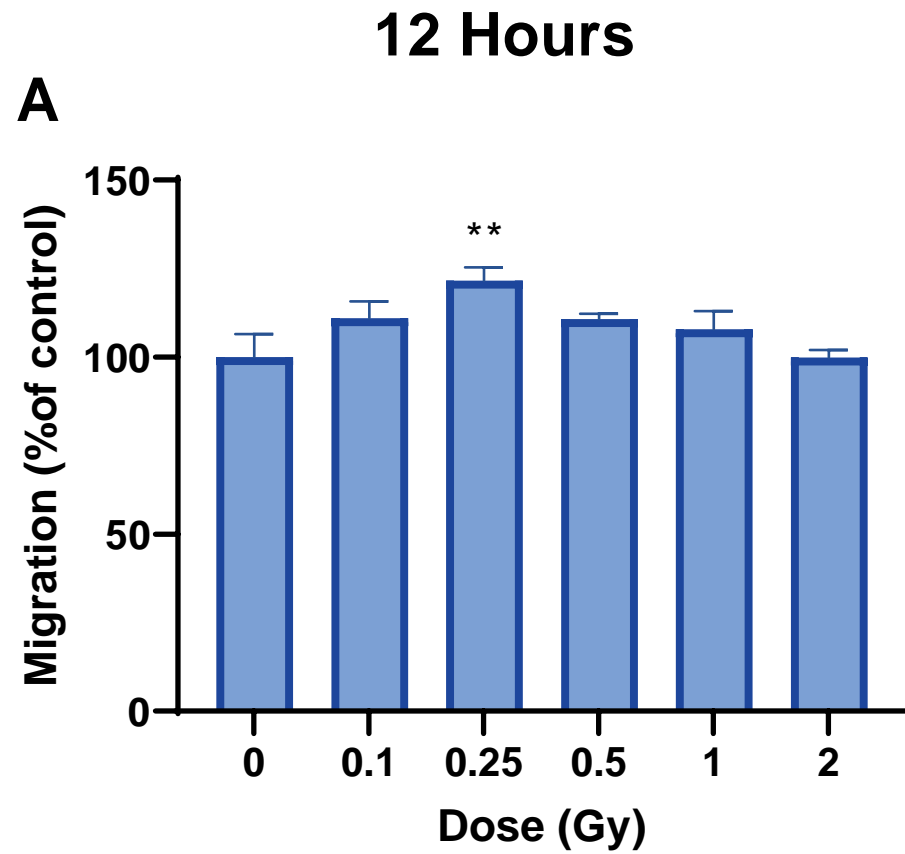
Unattached cells are then washed off while the adhered cells are stained and counted.

Results

Proliferation

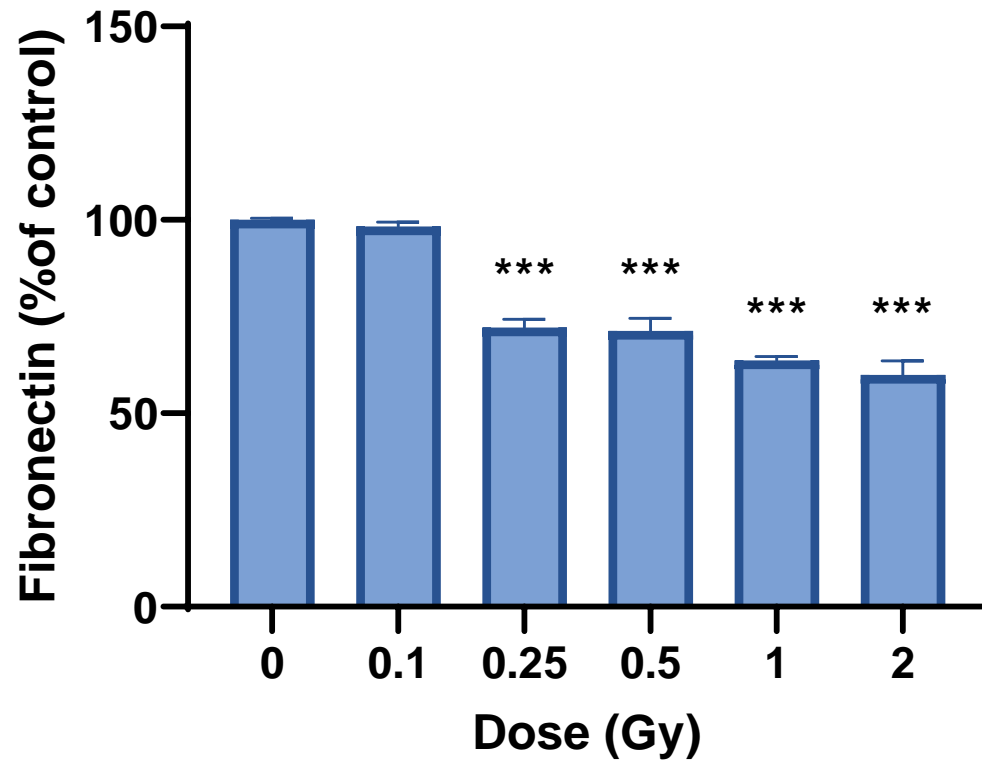


Migration

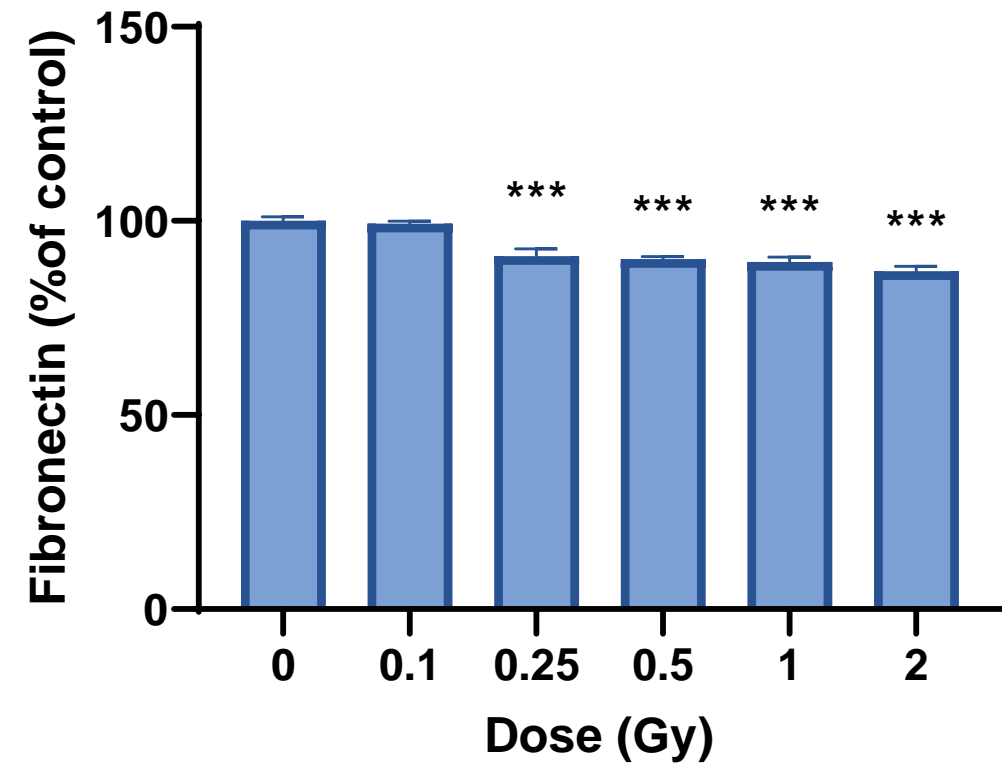


Adhesion (Fibronectin)

12 Hours

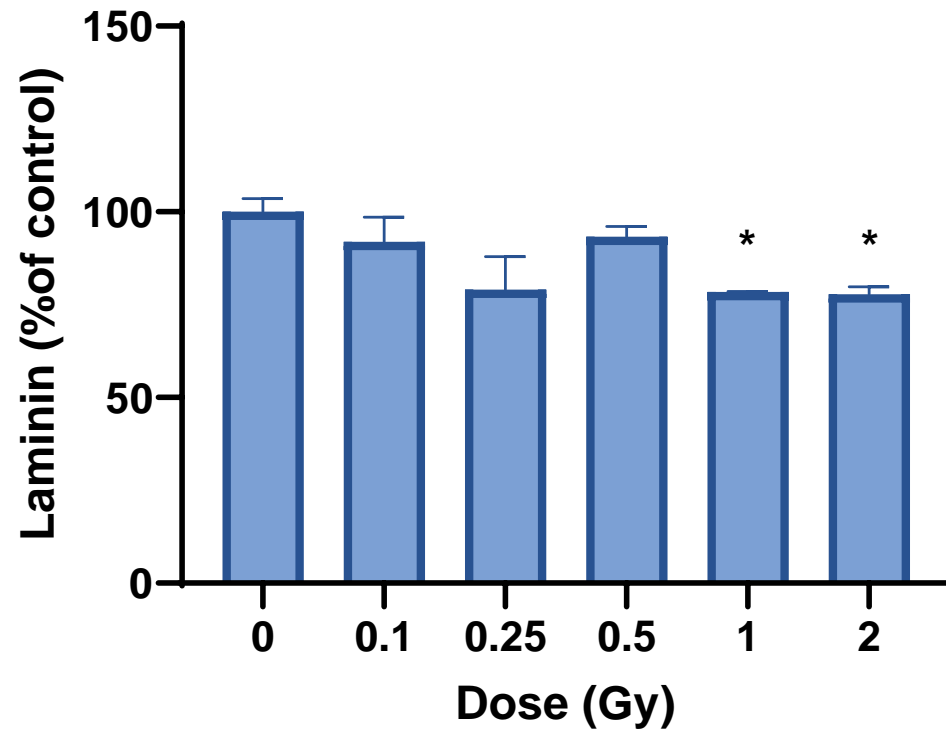


7 Days

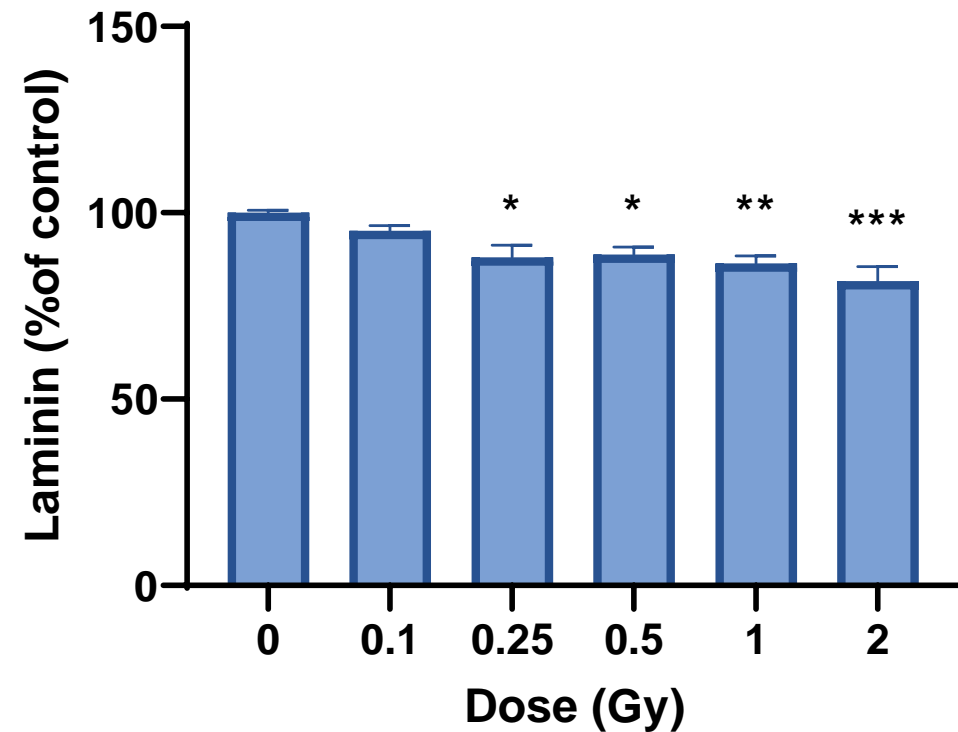


Adhesion (Laminin)

12 Hours

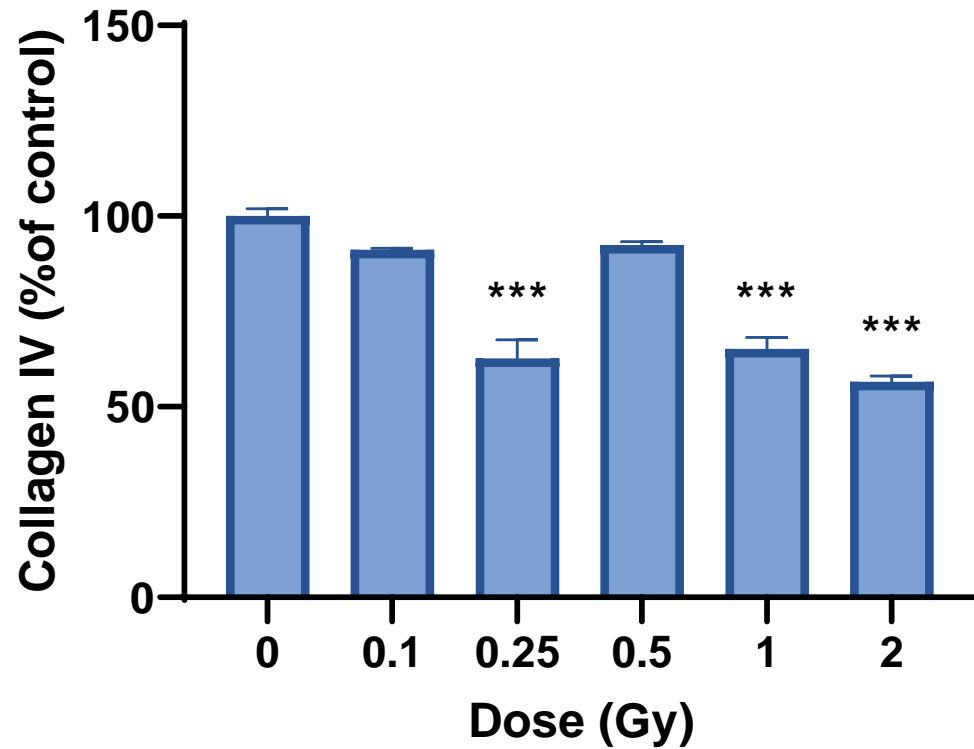


7 Days

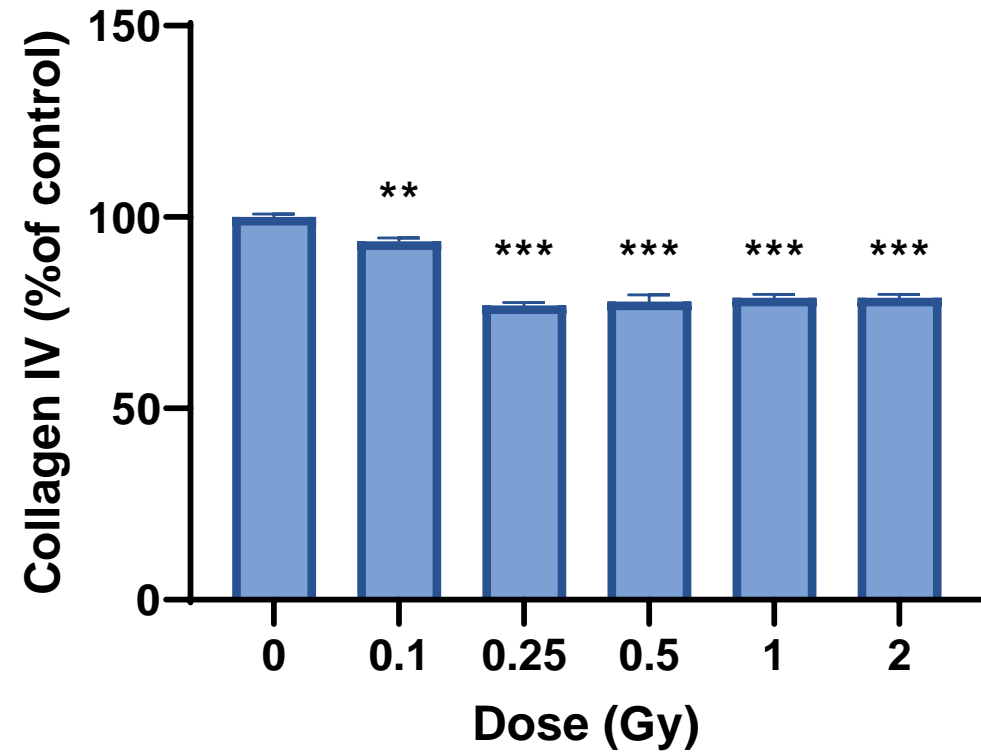


Adhesion (Collagen IV)

12 Hours



7 Days



Conclusion

- Significant changes in proliferation at 0.25Gy.
- Significant changes in migration at 0.25Gy.
- Higher acute radiation exposure leads to loss of adhesion.

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Thank you!
Any Questions?
