National Aeronautics and Space Administration

Non-targeted Effects and LET: Considerations for Earth and Space Research

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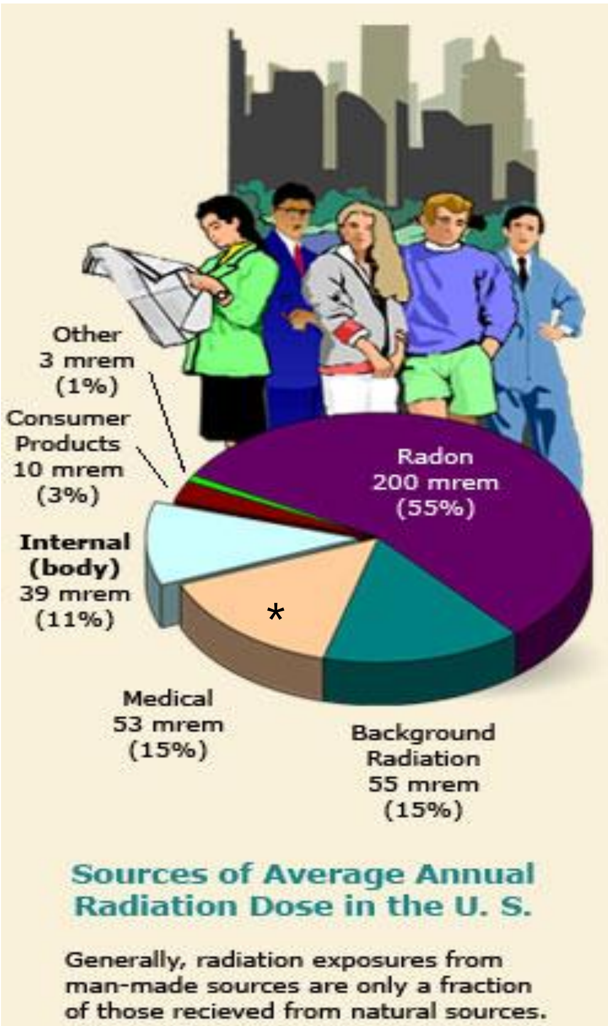
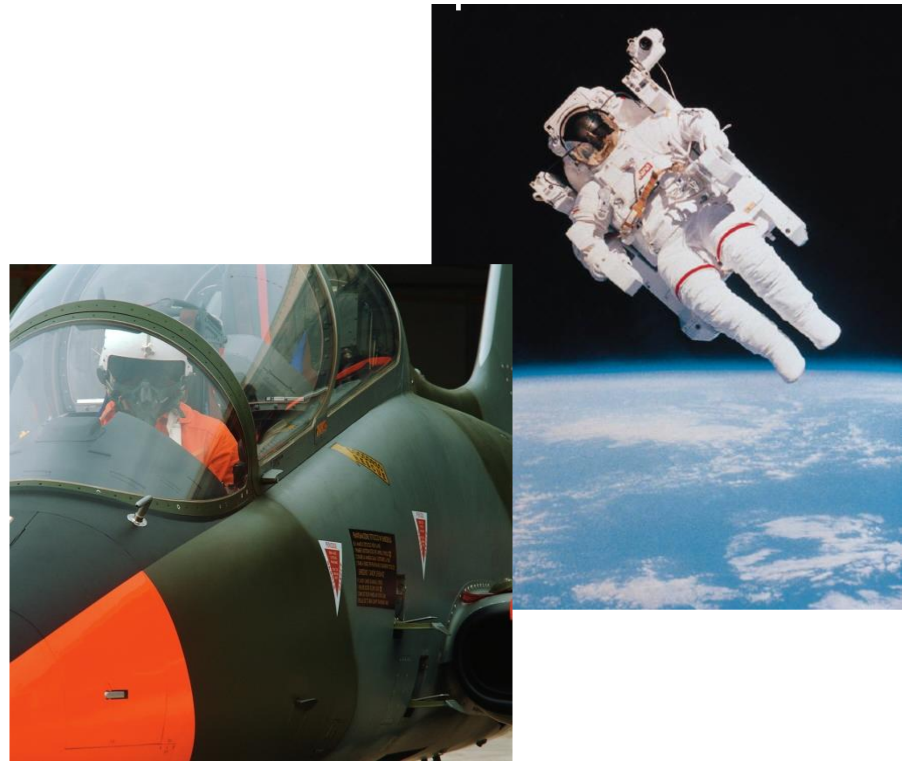
Radiation Exposure



http://www.ocrwm.doe.gov/factsheets/doeymp0337.shtml



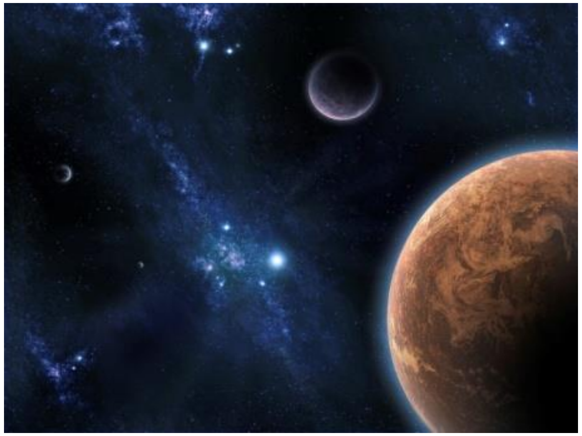
*Diagnostic radiology > 200 million procedures/year (USA). Two billion procedures world-wide. High dose/partial body.

Understanding Radiation Risk

Current radiation health effects assume:

1) the primary mode of action is linearly related to dose and
2) that the individual cell is the unit of risk.

Non-targeted effects and other low dose effects suggest responses occur non-uniformly over time at the multi-cellular scale

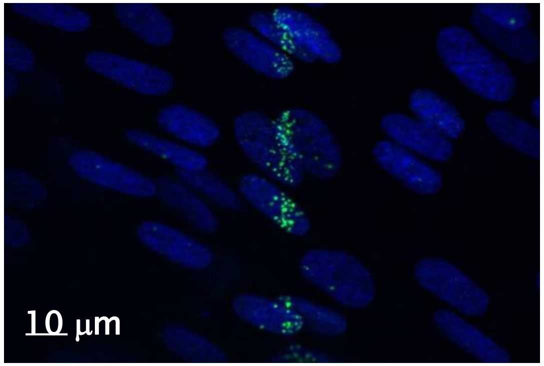


Not all radiation is equal: RBE

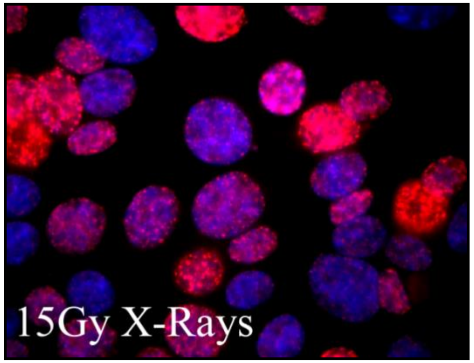
Space Radiation - High-LET

- Galactic cosmic rays (HZE)
- Solar Particle events
- Trapped radiation

Role of radiation quality and track structure?



High-LET



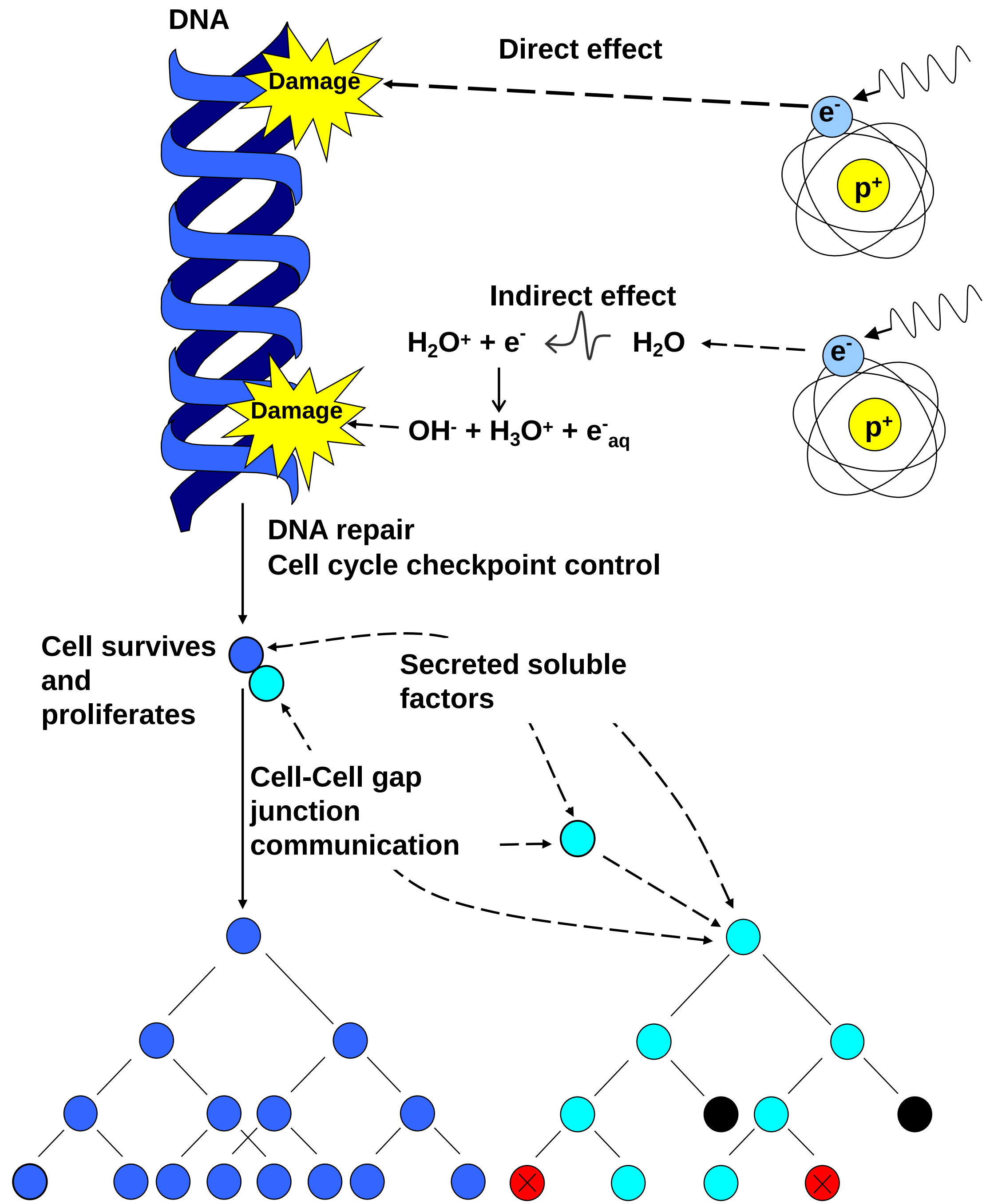
Low-LET

DNA damage is the result of direct and indirect effects of radiation

- All 4 bases subject to damage
- ~9eV sufficient to break DNA backbone
- SSB correlates poorly with lethality
- DSB most important lesion

Damage / Gy of X-rays:

- 40 DSBs
- 150 DNA crosslinks
- 1,000 SSB
- 2,500 base damages

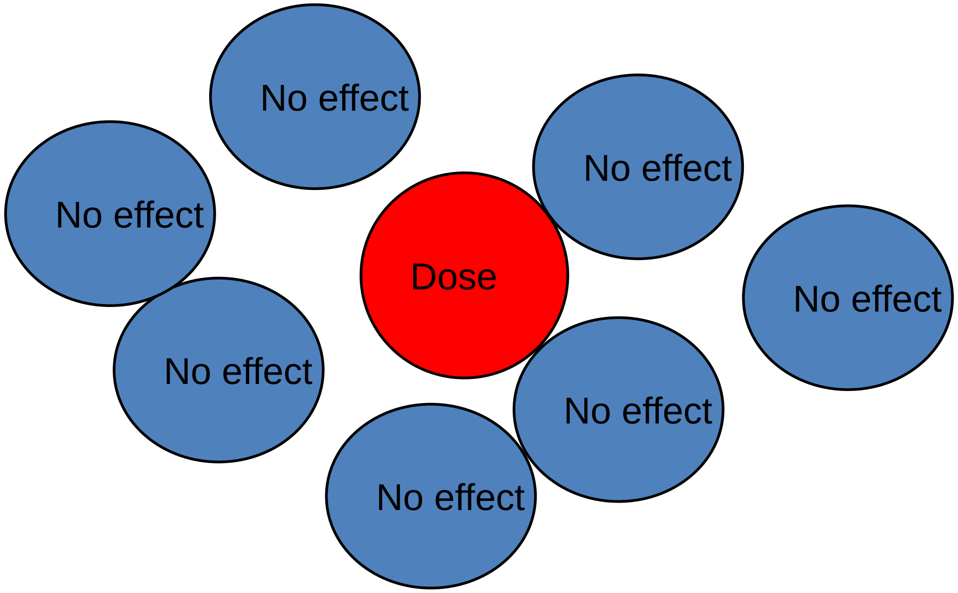


W. F. Morgan and M. B. Sowa, Effects of Ionizing Radiation in nonirradiated cells. Proc. Nat. Acad. Sci., 102, 14127-14128 (2005).

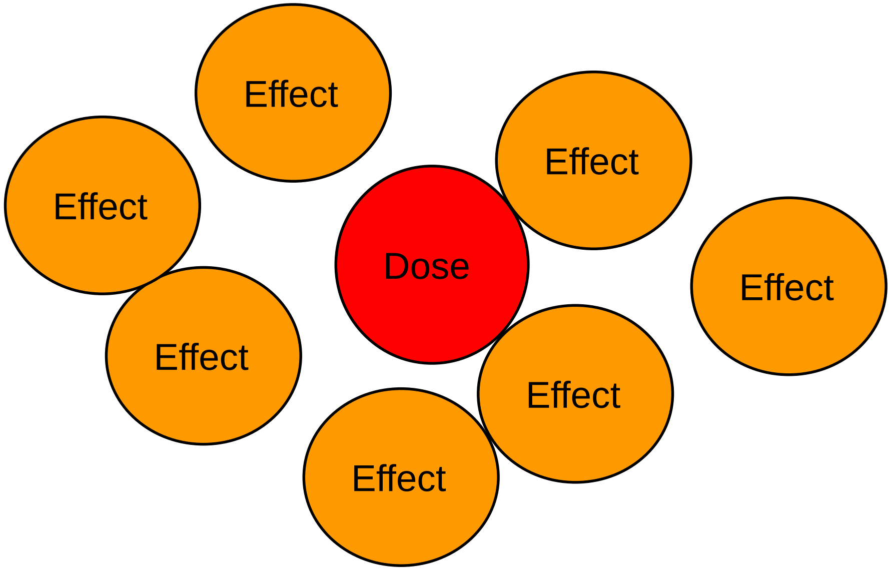
The Bystander Effect

Biological responses observed in cells that are not directly traversed by radiation

Historically:
One hit = one effect

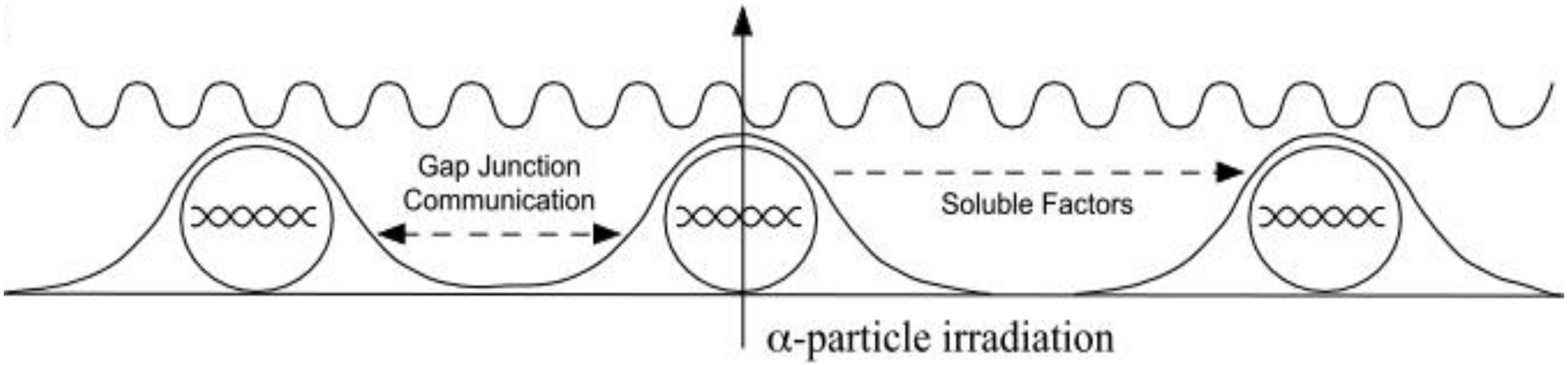


Bystander :
One hit = multiple effects



Endpoints include cytotoxicity, induced mutations, chromosome damage, gene expression, genomic instability and cell proliferation.

Mechanisms of Transmission of Bystander Effects



What is the signal transmitting information from irradiated cells to unirradiated cells?

secreted factor?
cell to cell gap junction communication?
dead / dying cells?

Methods for studying bystander effects:

Low fluences of α-particles

Single cell microbeam irradiation

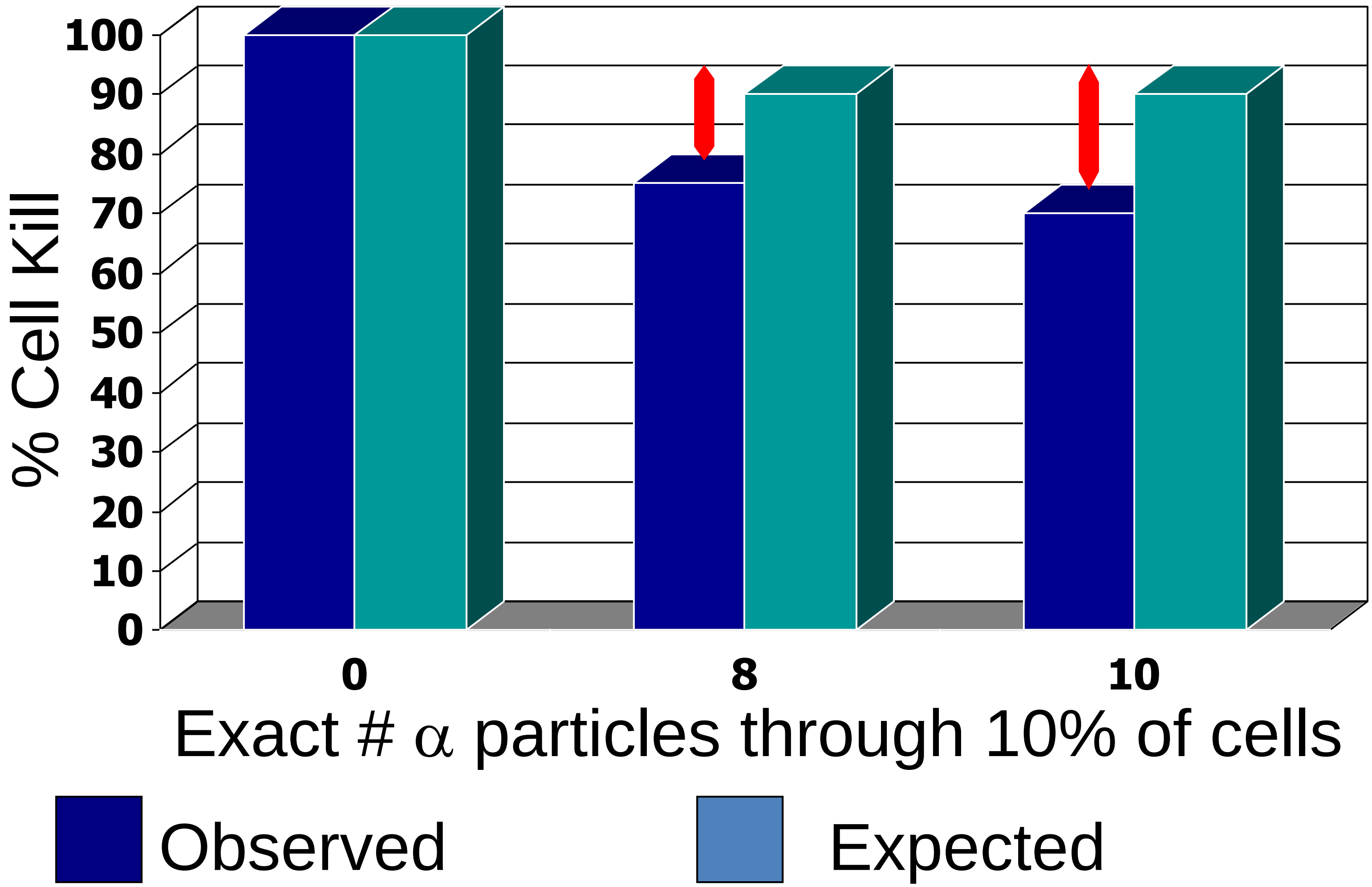
^{3}H-thymidine co-culture

Medium transfer experiments

Physically separated co-culture (dual membrane)

Sowa Resat, M. B., and Morgan, W. F., Radiation-Induced Genomic Instability: A Role for Secreted Soluble Factors in Communicating the Radiation Response to Non-Irradiated Cells, J. Cell. Biochem., **92**, 1013-1019 (2004).

Bystander effect for cell survival

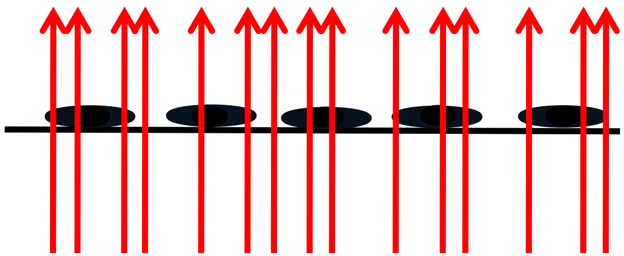


Cell kill due to bystander effect

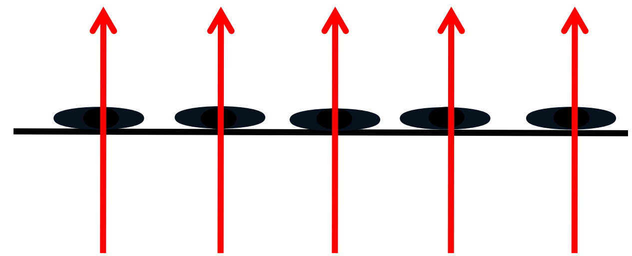
Sawant et al. ***Rad. Res.*** **156,** 177-180 (2001)

Microbeams in Radiation Biology

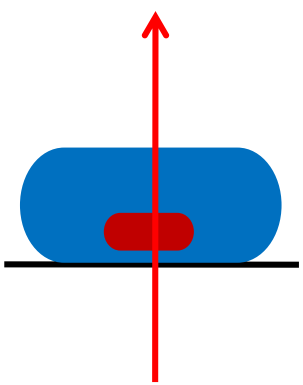
Conventional



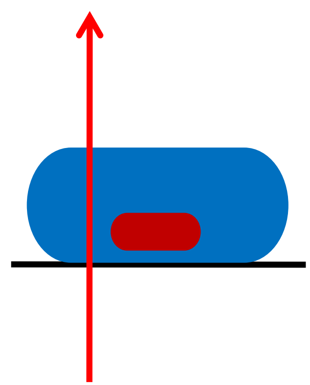
Microbeam



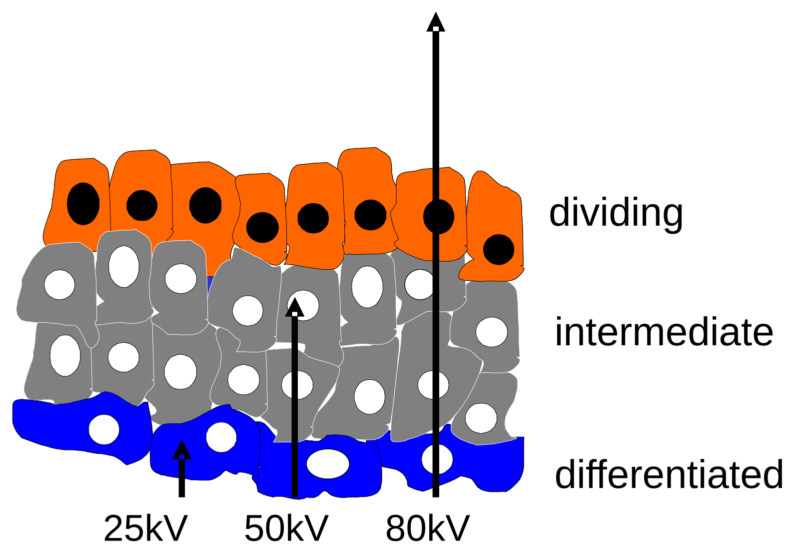
Possible exposure scenarios



Nuclear



Cytoplasmic



Tissue

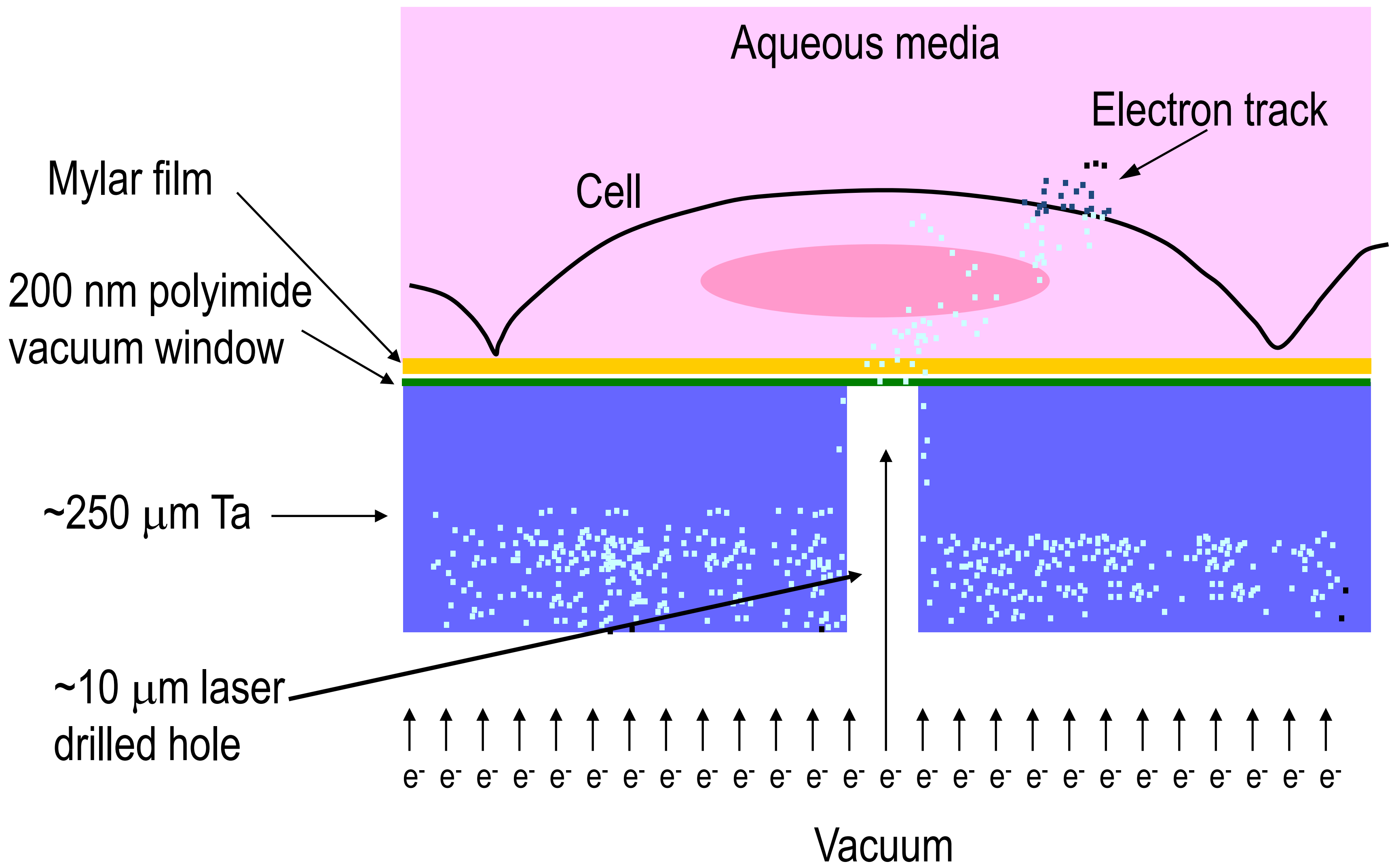
Low LET Electron Microbeam

- Variable Electron energy: 20 – 90 keV
- Built around a commercially available pulsed electron gun
- High spatial resolution – target individual cells
- Variable "Dose" – from one to 100's of electrons deposited in the target cell
- Variable "Dose Rate"
- Integrate with standard optical microscope
- Irradiate thin tissues and tissue analogs

Sowa, M. B., McDonald, J. C., Miller, J. H., Murphy, M. K., Strom, D. J., and Kimmel, G. A., *Rad. Res.* **164,** 677-679 (2005).

Sowa Resat, M. B., and Morgan, W. F., *Cancer and Metastasis Reviews*, **23**, 323-331 (2004).

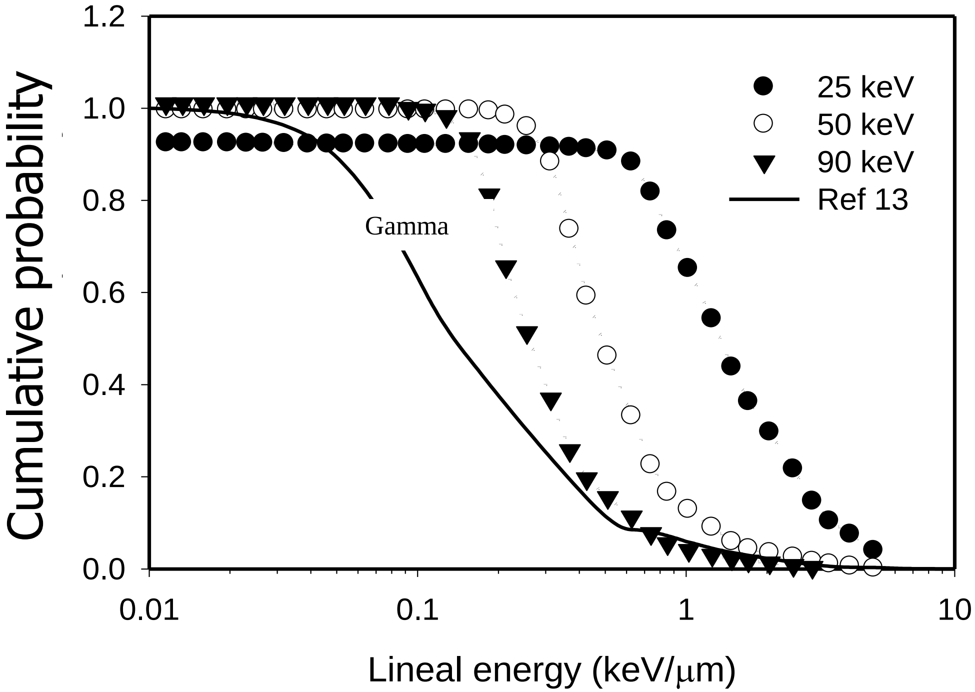
Electron Irradiator – Cell Interface



LET Spectra

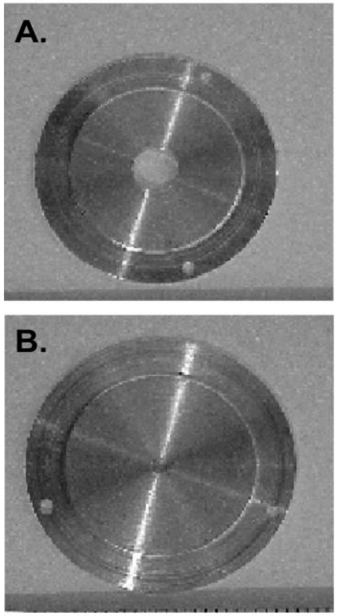
As the kinetic energy of the electron is increased, the lineal energy spectra shifts to lower values and approaches the average spectra for gamma-rays.

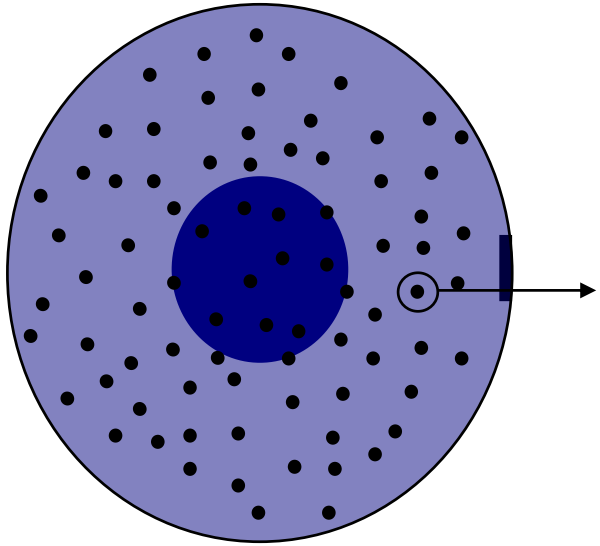
The electrons produced by the gun are monoenergetic and do not represent a heterogeneous energy distribution.



Miller et al., *Radiat. Environ. Biophys.* **39**, 173-177 (2000)

Localized irradiation





Cell Killing

Instability

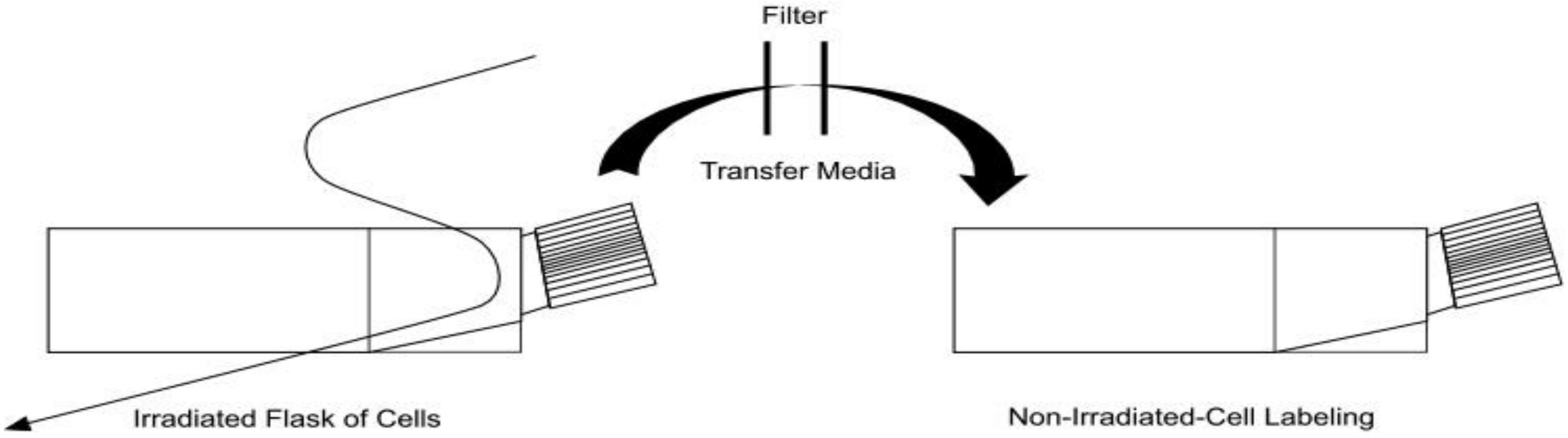
- Cytogenetics
- Micronuclei
- Mutation
- Microsatellites

When not targeting individual cells, aluminum shields are used for selectively irradiating a subset of cells.

Shields allows exposure of 10% or 1% of a dish.

Line scans through the center of the Gafchromic film made with the 10% and 1% apertures found a sharp fall off in dose at shield edges. Minimal scatter.

Bystander effect after medium transfer



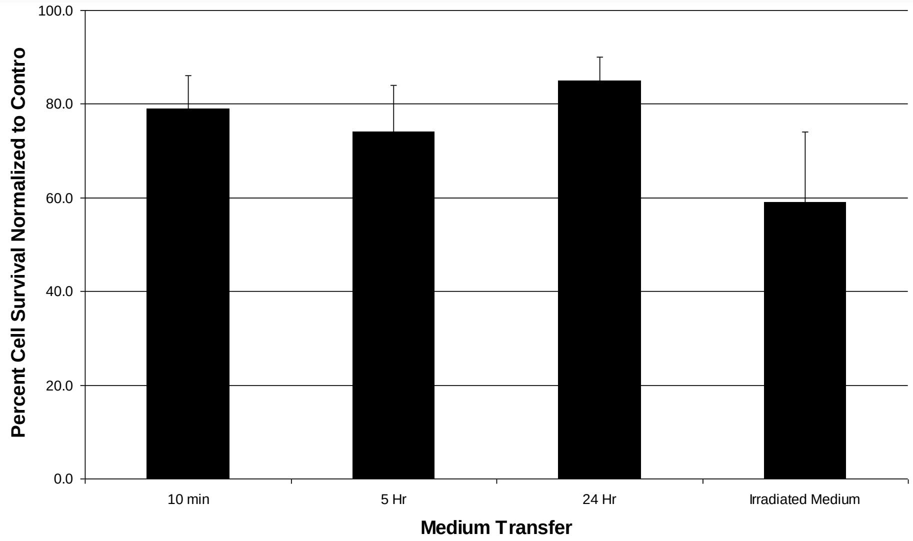
Mothersill and colleagues: Reduced plating efficiency in cells that have never been exposed to ionizing radiation

We measured survival and micronuclei in bi-nucleated cells.

Micronucleus frequency and clonogenic cell survival is unchanged relative to controls.

We have made direct comparisons between high and low LET media transfer experiments

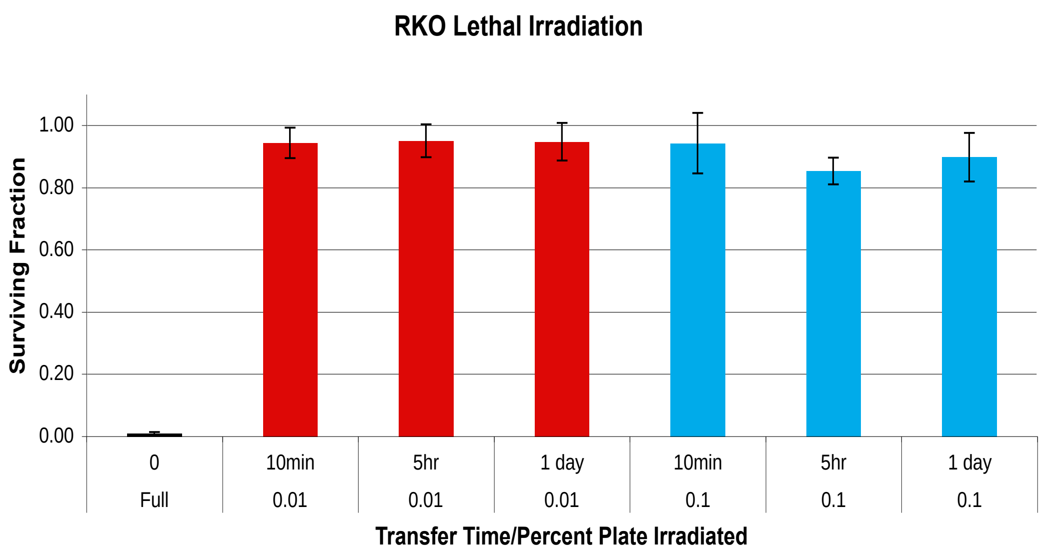
RKO36: Media transfer and direct irradiation with MB



50 keV electrons were used to irradiate all cells.

Media from irradiated cells was transferred to non irradiated cells.

No BSE observed.

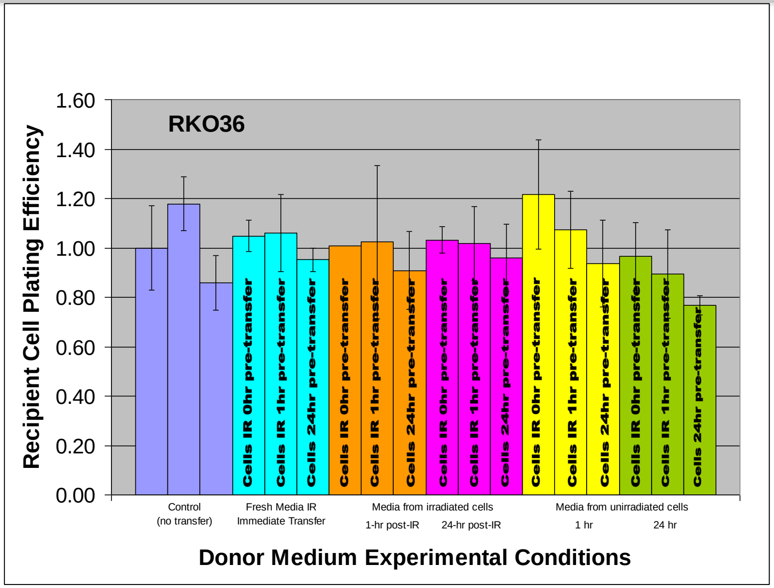


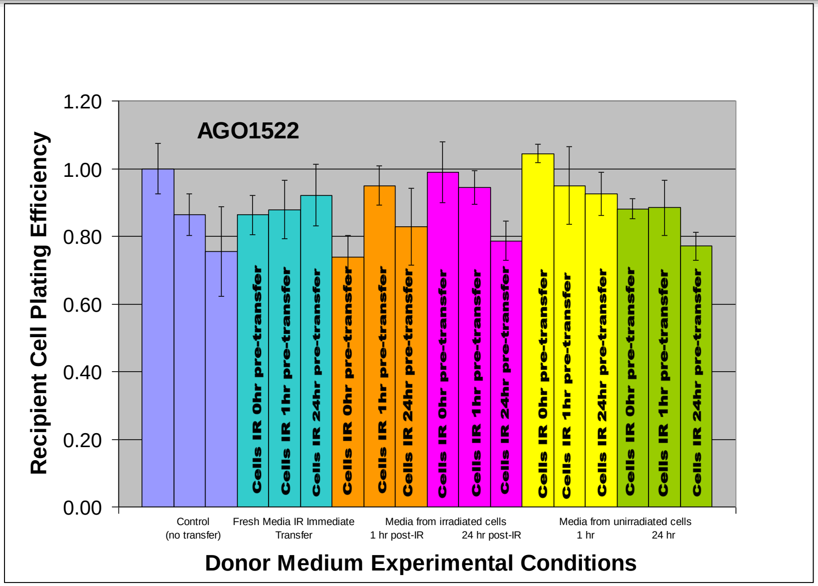
In a complementary experiment, 1, 10 or 100% of gap junction null RKO36 cells in a confluent monolayer were lethally irradiated (50 Gy) with 50 keV electrons.

Measured percent survival versus percent of cells directly exposed to electron radiation.

No significant effect observed

Media Transfer Data





AGO1522: Gap junction competent, exhibit high LET bystander effect

RKO36: Gap junction null cell line.

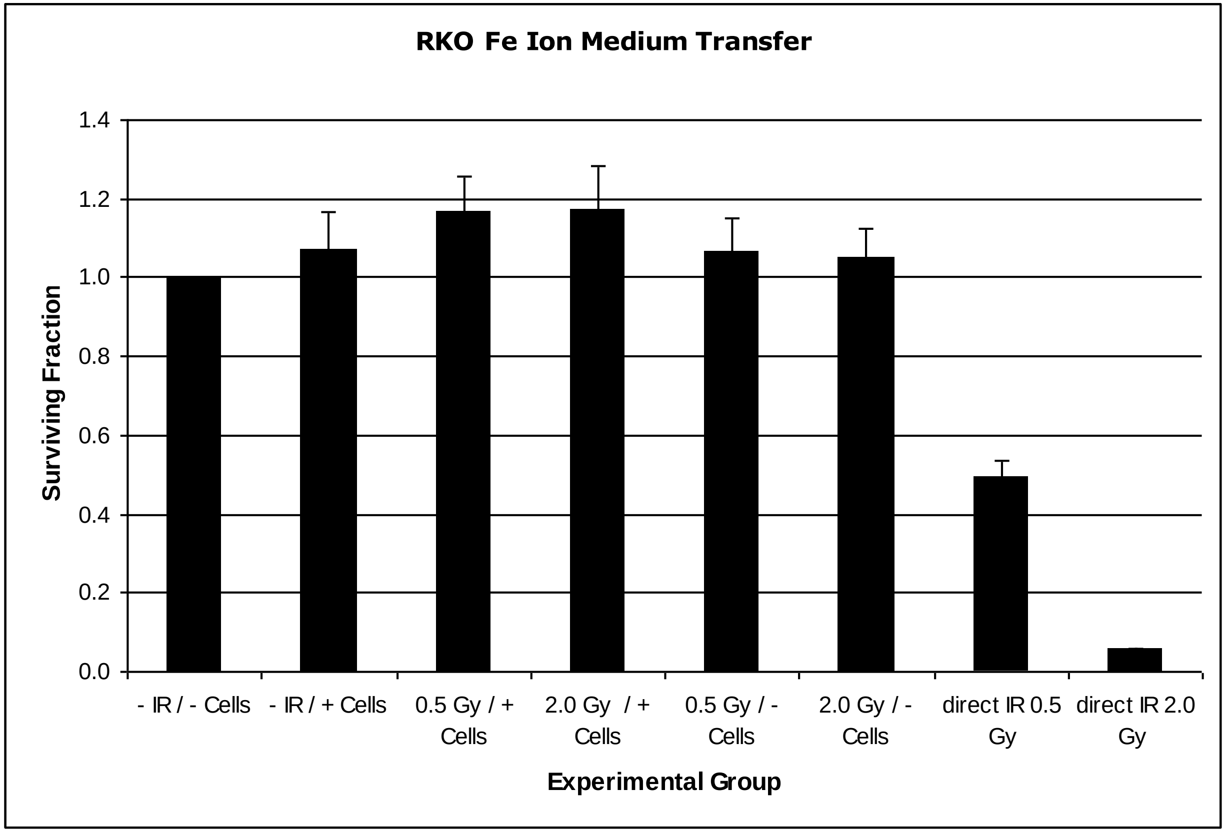
We see NO significant differences relative to control for either cell line

Possibilities:

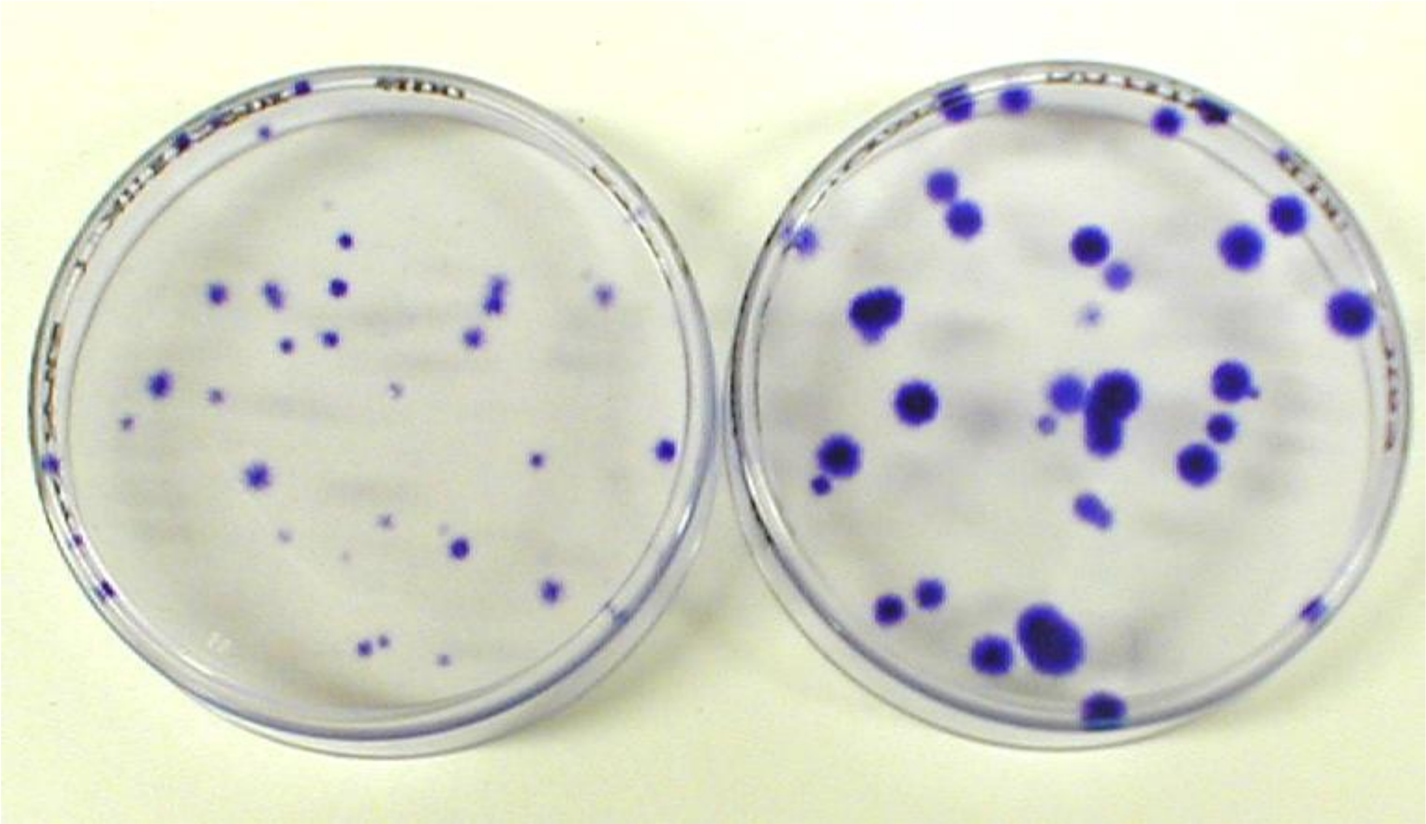
- No Low-LET bystander effect as measured by cell survival
- Cells incapable of producing or responding to the bystander factor
- There is no low-LET bystander effect

High LET Media Transfer

RKO cells did not show a high LET BSE for Media transfer.



No low LET bystander effect, rather a “conditioned media” effect



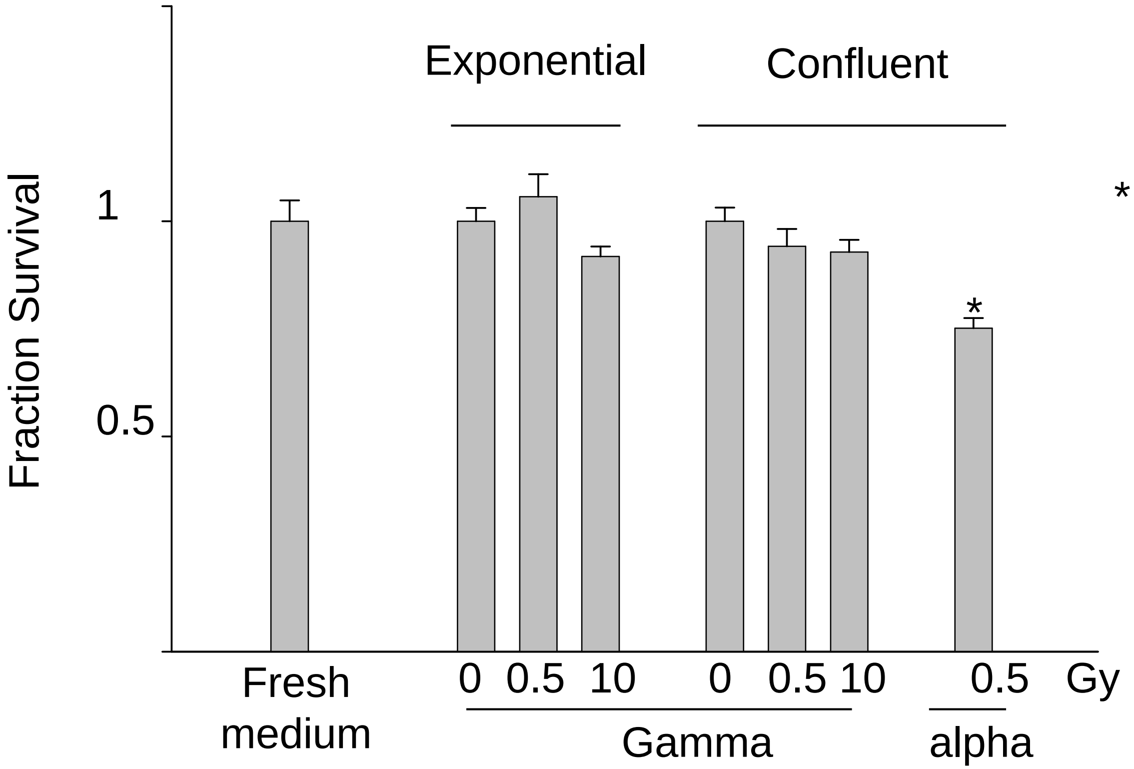
Direct comparison of low and high LET medium transfer BSE

Clonogenic survival of AG1522 normal human fibroblasts recipient of growth medium harvested from irradiated AG1522 cell cultures.

Growth medium was harvested at 1 h after exposure of confluent or actively growing AG1522 cultures to different doses of cesium-137 γ-rays or americium-241 α-particles.

Recipient cells were continuously incubated with the conditioned medium for 12 days when colonies were fixed, stained and counted.

A BSE was only observed following high LET exposure.



*, $p \leq 0.01$

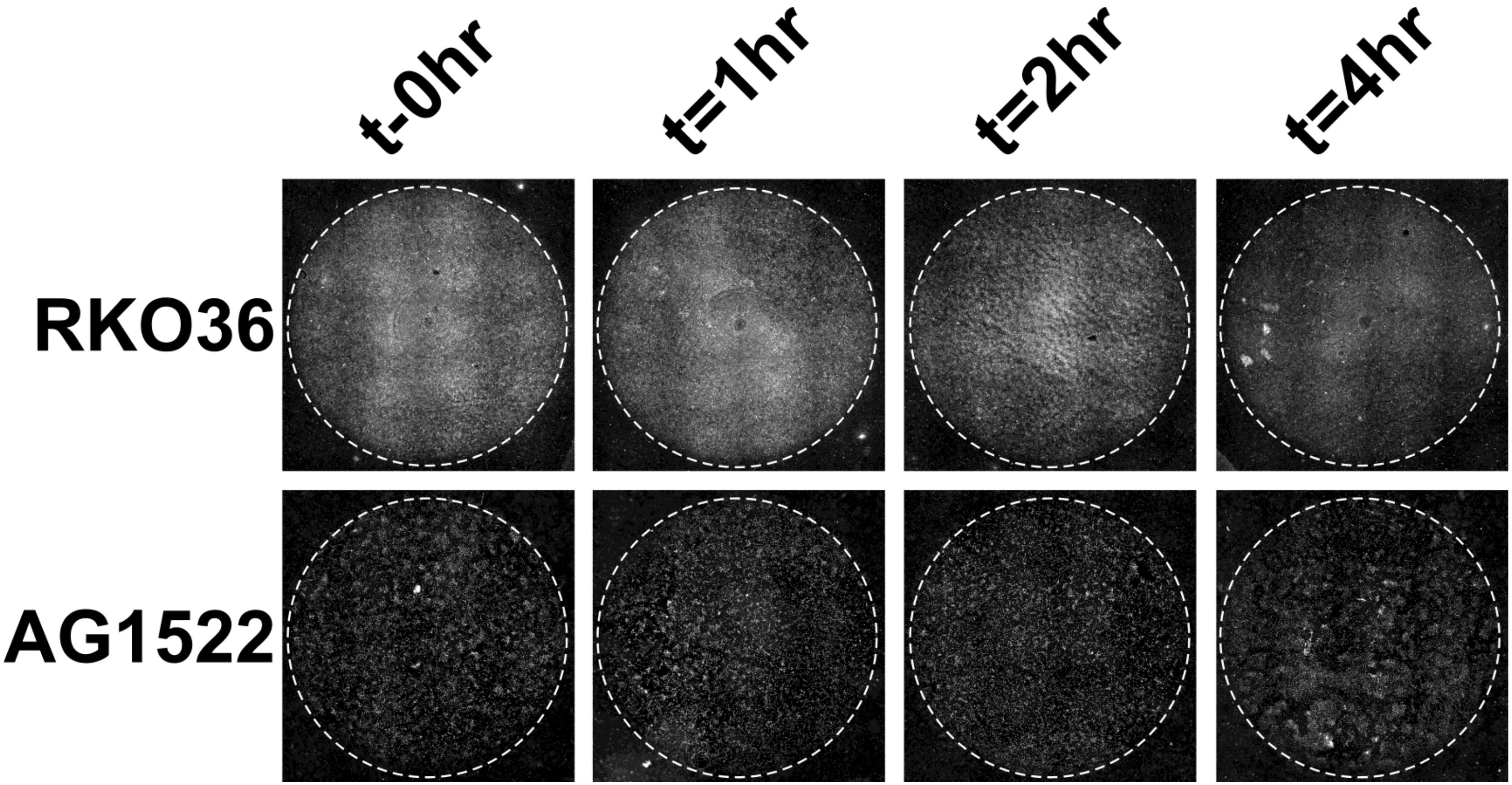
Sowa, M. B., W. Goetz, J. E. Baulch, D. N. Pyles, J. Dziegielewski, S. Yovino, A. R. Snyder, S. M. de Toledo, E.I. Azzam, W. F. Morgan, *International Journal of Radiation Biology* 86 (2010) 102-113.

Do cells need to be in the radiation environment?

Deliver a spatially localized dose to 10 % of cells

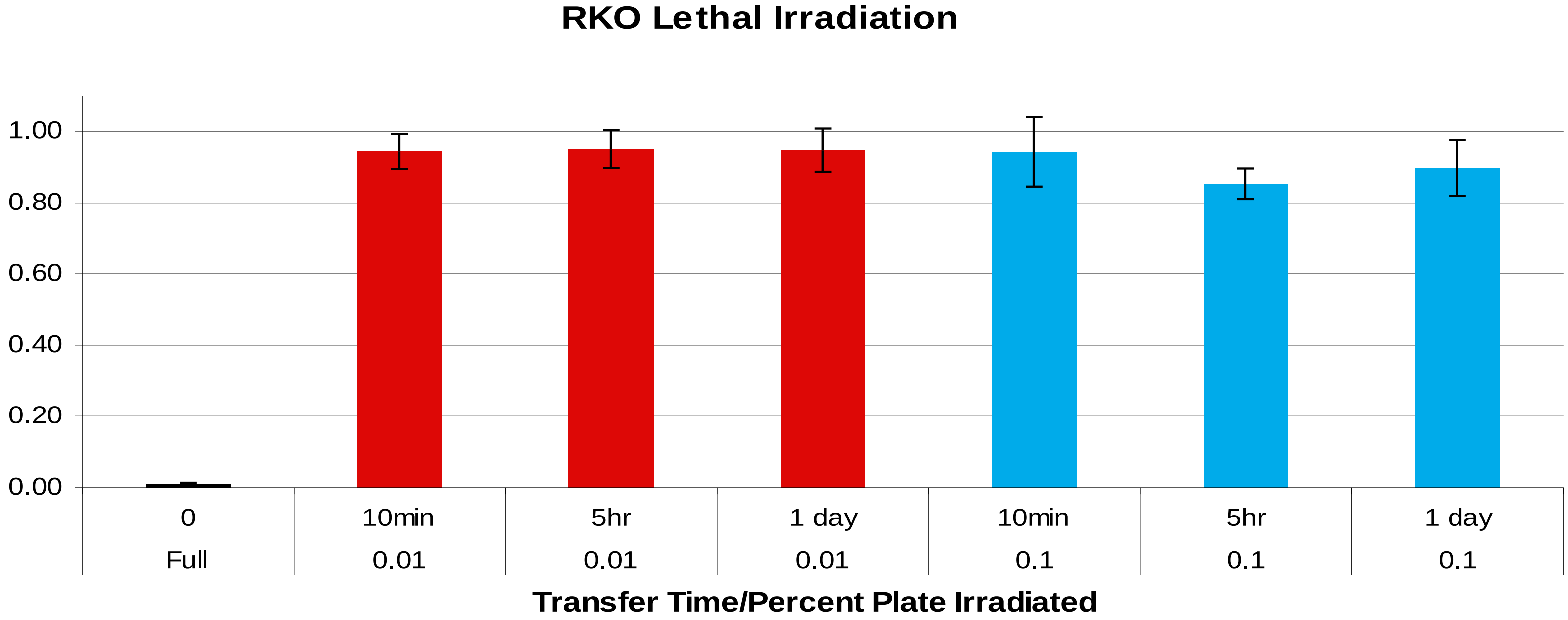
Cells were then stained at various times with γH2AX.

Foci formation was not observed outside the directly irradiated area.



Images are montage of multiple images.

Partial shielding



RKO36: effect of radiation environment

1, 10 or 100% of gap junction null RKO36 cells in a confluent monolayer were lethally irradiated (50 Gy) with 50 keV electrons.

Measured percent survival versus percent of cells directly exposed to electron radiation.

No significant effect observed

Conclusions

In our studies:

No bystander effect observed for media transfer or microbeam irradiation with X-rays, electrons, or Fe ions.

Gap junction positive and negative cells were evaluated

Endpoints: Cell survival and γH2AX, micronuclei

Possibilities:

- no Low-LET bystander effect for measured endpoints.
- these cells are incapable of producing or responding to bystander factor.
- The bystander effect is dependent on radiation quality.

This was the first chapter in an incredible journey….

No Regrets

Acknowledgments

Ruth Globus

Ludwig Feinendegen   Tony Brooks

Tom Hei

And all of our great friends from around the world for you constant friendship and support


DOSTAWCA DWORU
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PAŁAC
SPISKI
HAWEŁKA