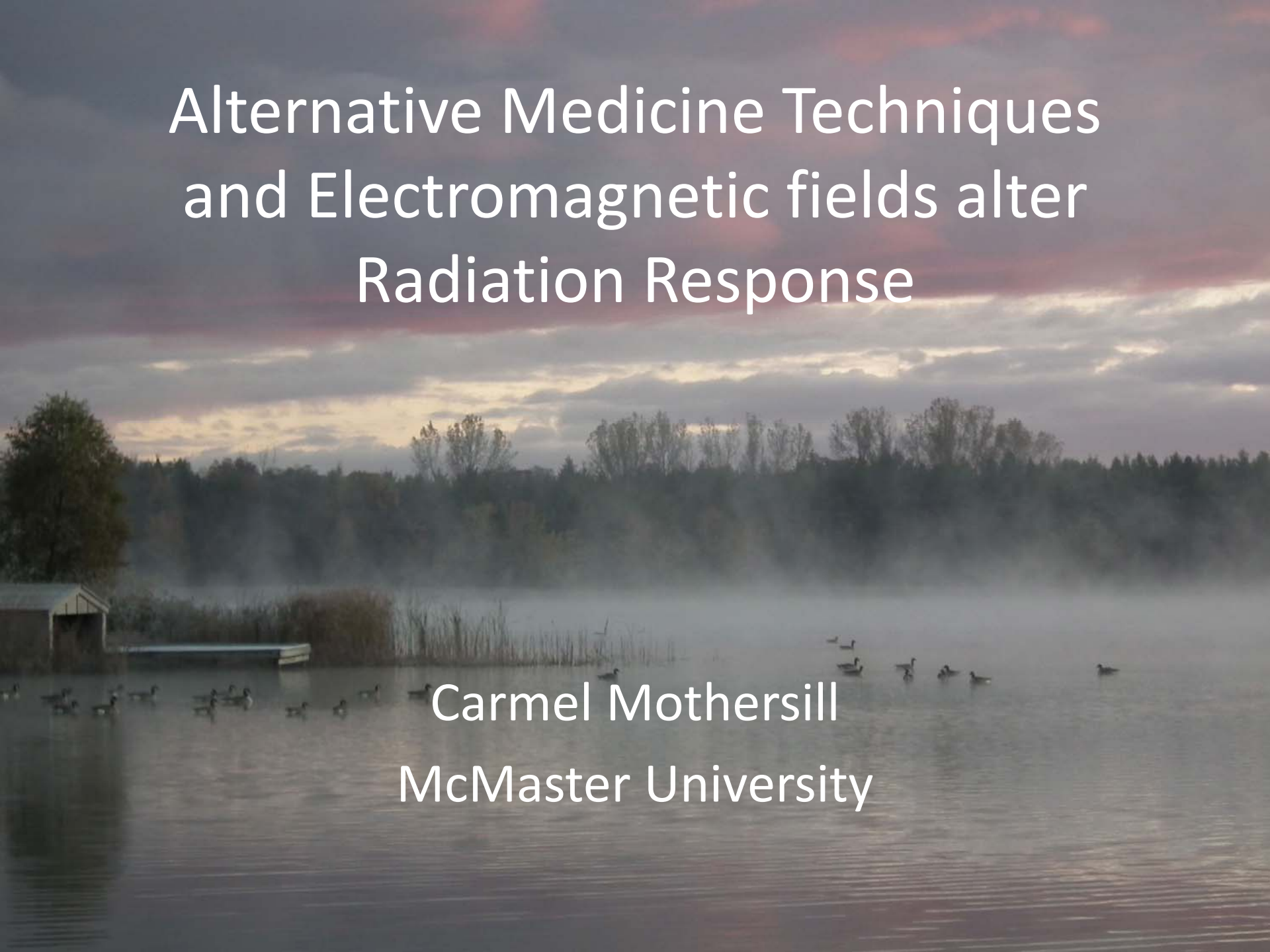


Alternative Medicine Techniques and Electromagnetic fields alter Radiation Response

Carmel Mothersill
McMaster University



Background

- Electromagnetic (EM) fields can perturb biological organisms through effects on neural and immune systems and on cell membranes.
- New non-targeted effects (NTE) radiobiology suggests signaling pathways should be perturbed by EM fields because ion-gated channels and receptors previously thought to be confined to the nervous system are critical.
- Experiments done to examine EM effects and effects of alternative medicine techniques which affect EM fields.

'Non-targeted' radiation effects

Bystander effects

Effects in neighbouring cells



Abscopal effects

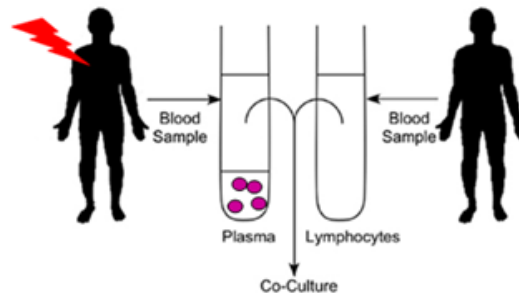
Effects in neighbouring tissues



Inter-animal
signaling

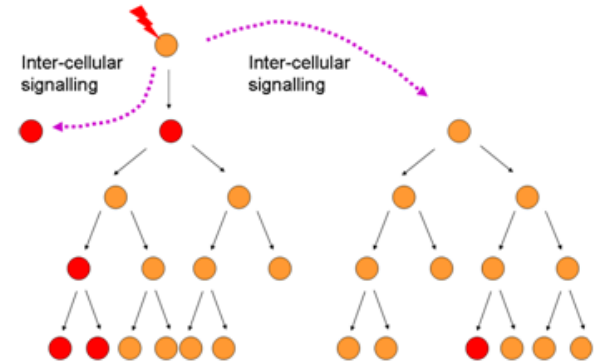
Clastogenic factors

Ex vivo effects in cultured cells



Genomic Instability

Effects in unirradiated descendant cells



Inflammatory Processes
may provide
mechanistic link

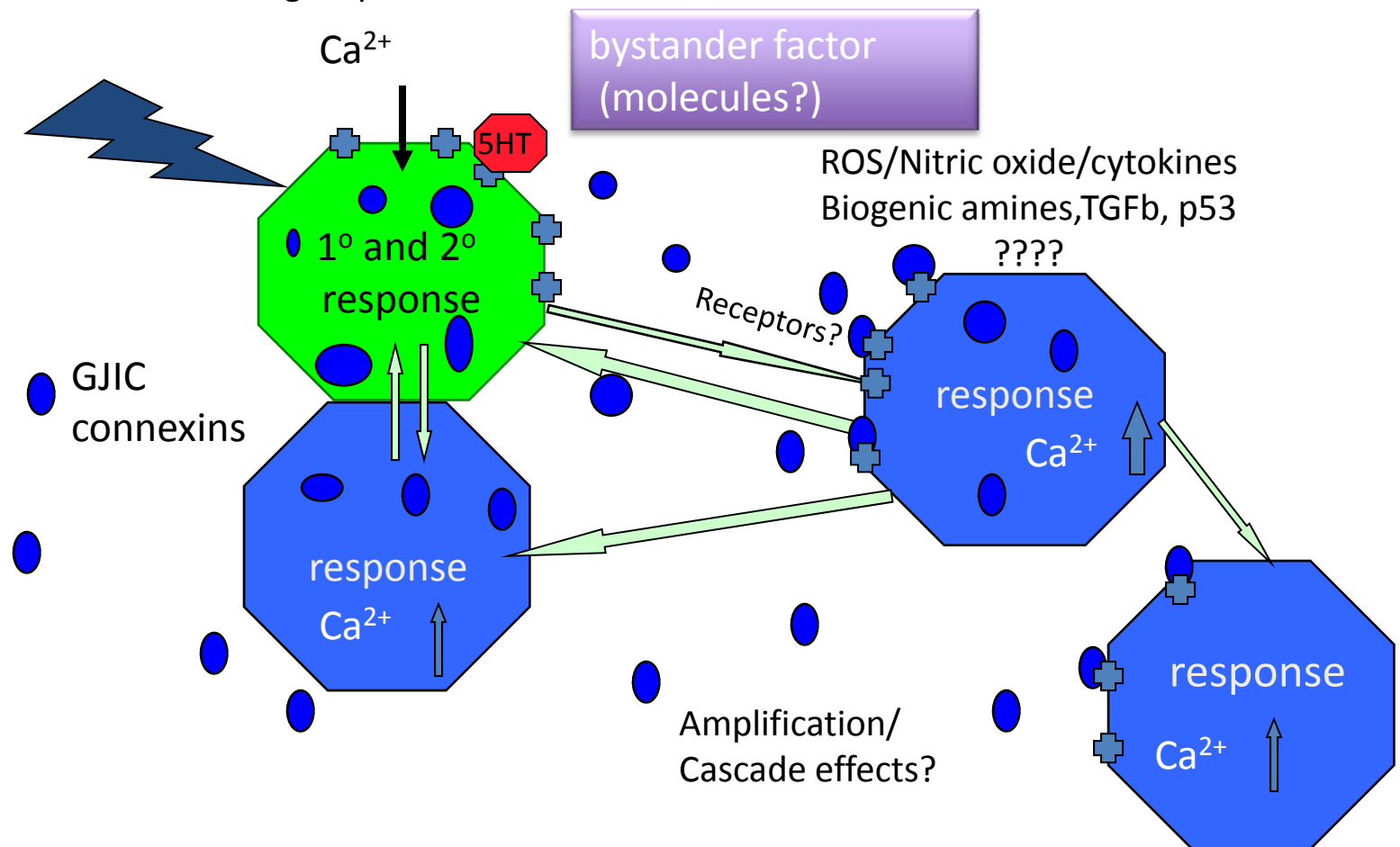
Long-term effects on innate immune
response function may occur

Effects in neighbouring animals



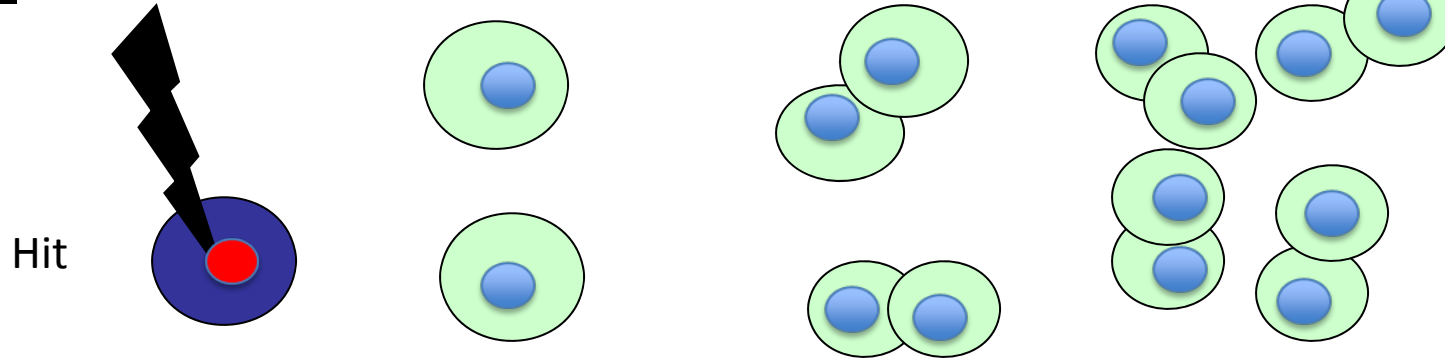
The bystander effect

Ionizing radiation, UVA, UVB, ELF-EMF and heavy metals induce affected cell to signal to others. Responses to the signals include apoptosis, micronucleus formation, transformation, mutation, induction of stress and adaptive pathways. Serotonin (5HT) and Calcium ions known to be involved in signal production.



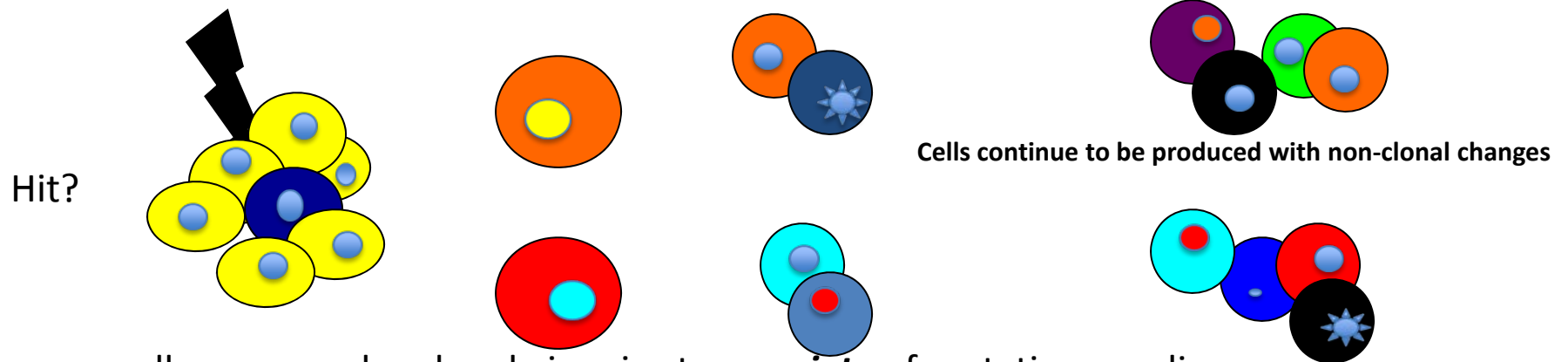
The link between bystander effects and genomic instability – twin pillars of the new paradigm

Old view- clonal outcome



Progeny are all **clonal** i.e. identical and mutation is passed to all progeny

New view-non-clonal, population-determined outcome

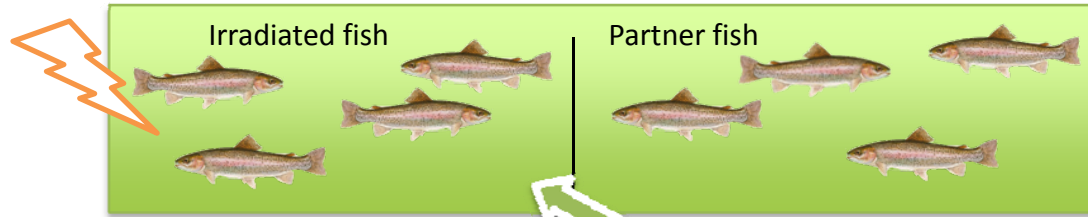


Progeny cells are non-clonal and give rise to a **variety** of mutations or die

Endpoints

- Direct radiation effect (clonogenic assay, calcium flux, proteomics)
- Bystander signaling effect (clonogenic assay, calcium flux, proteomics)
- In vivo fish model for studying bystander effect in a separate organism.

Measuring bystander response to radiation *in vivo* (adapted from Mothersill et al 2006)



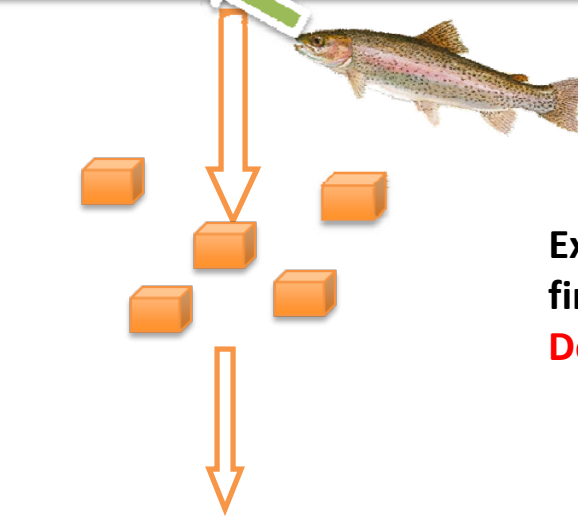
Irradiate or sham irradiate fish, allow to swim with unexposed partner for 2hrs

Unexposed fish introduced into water from irradiated or sham fish
After 2hrs. Dissect tissues

Do proteomics

Explant pieces taken from skin, fin, gill, spleen and kidney

Do tissue culture

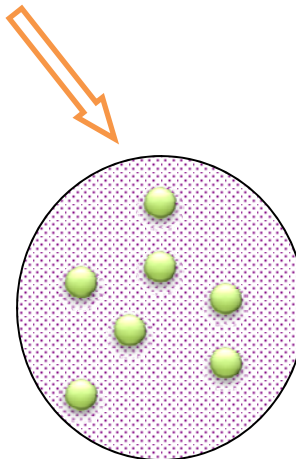
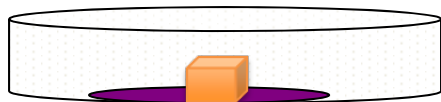


Culture of explants for 2 days

Harvest culture medium for calcium flux, ELISA and clonogenic assays

Grow up culture

examine explant outgrowth do immunocytochemistry



Add medium to unirradiated clonogenic cell line
determine surviving fraction by counting colonies after 10 days

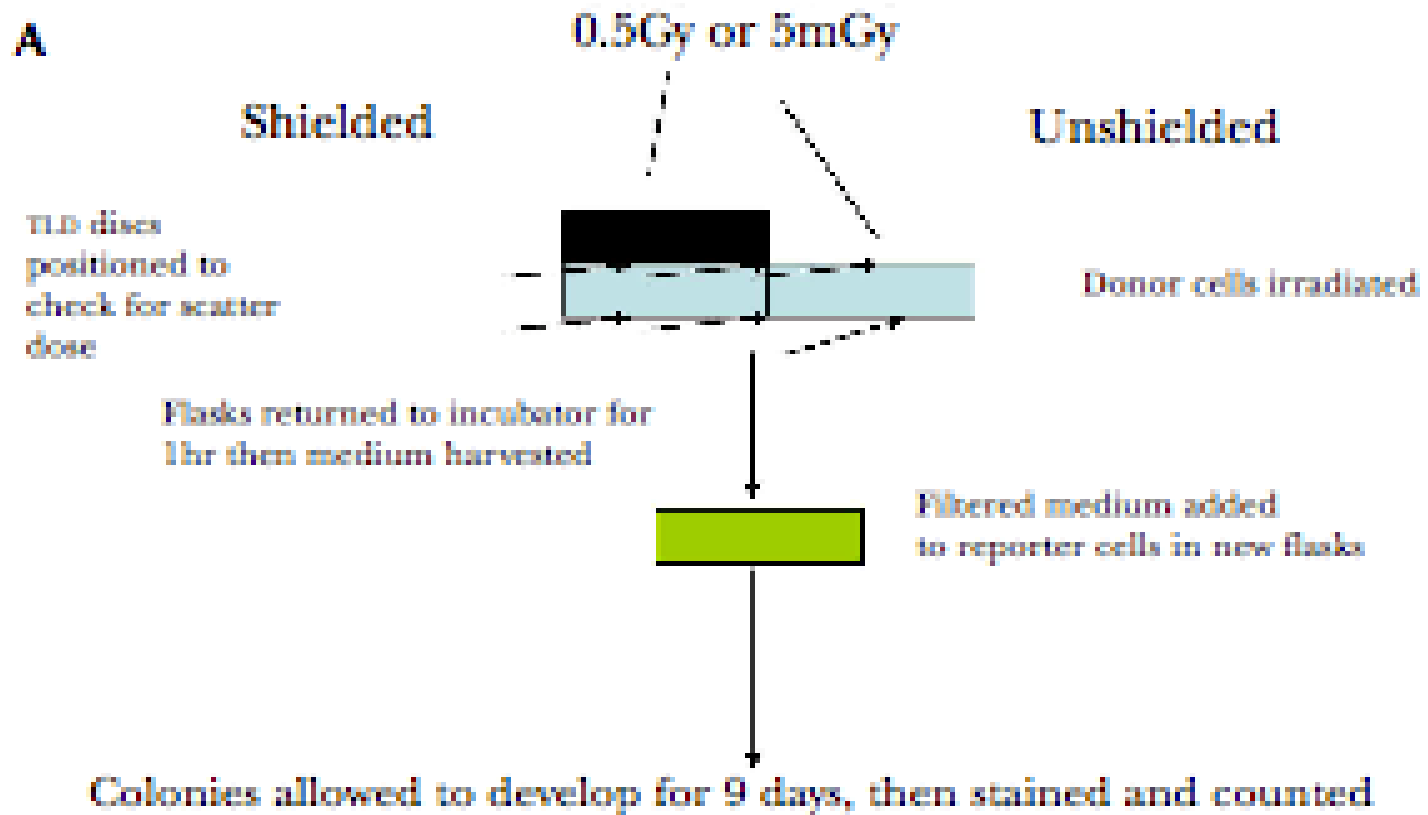
Five different approaches

- Expose cells/organisms to IR without EM field (Faraday cages)
- Expose cells/organisms to MRI
- Expose cells/organisms to IR but prevent chemical transmission of bystander signals
- Expose cells/organisms to IR with inhibitors/stimulators of signaling receptor pathways
- Expose cells/organisms to “alternative” medicine techniques known to involve biofields – acupuncture and reiki

Five different approaches

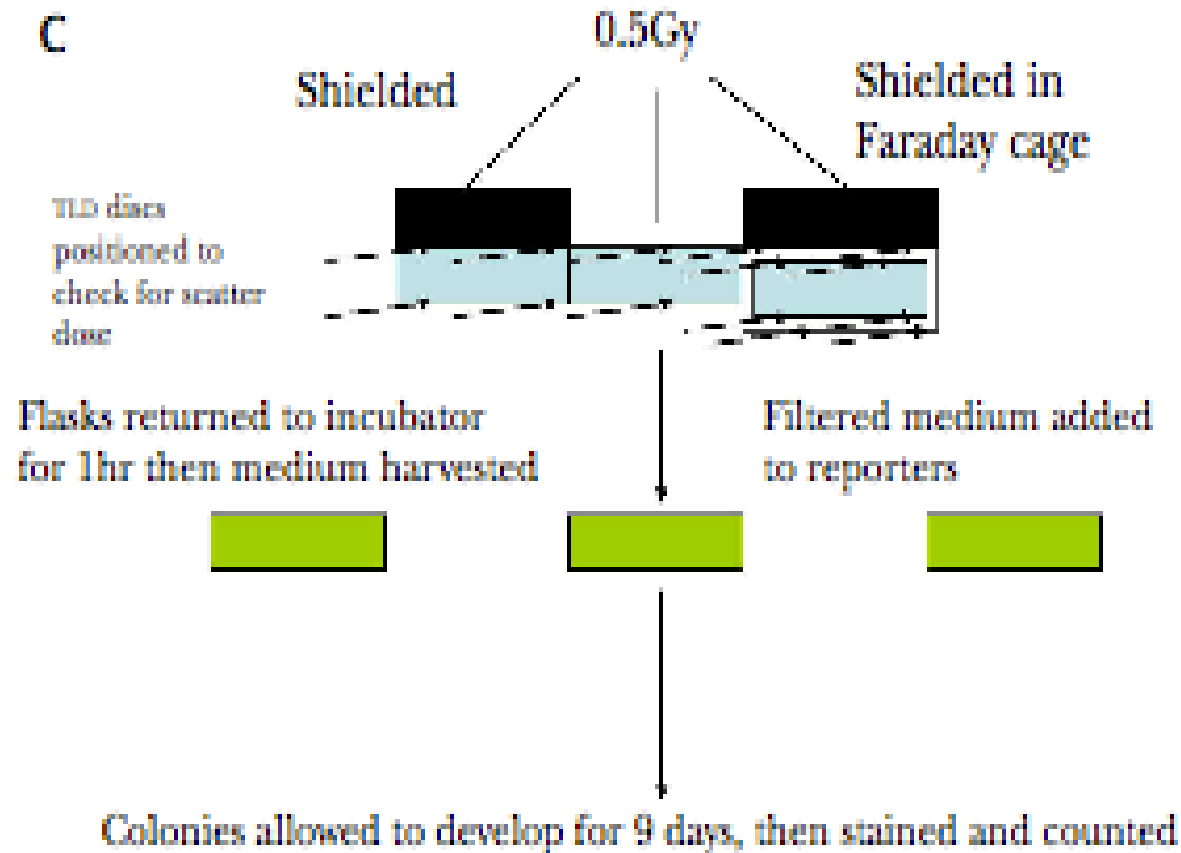
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Protocol for EM experiments 1

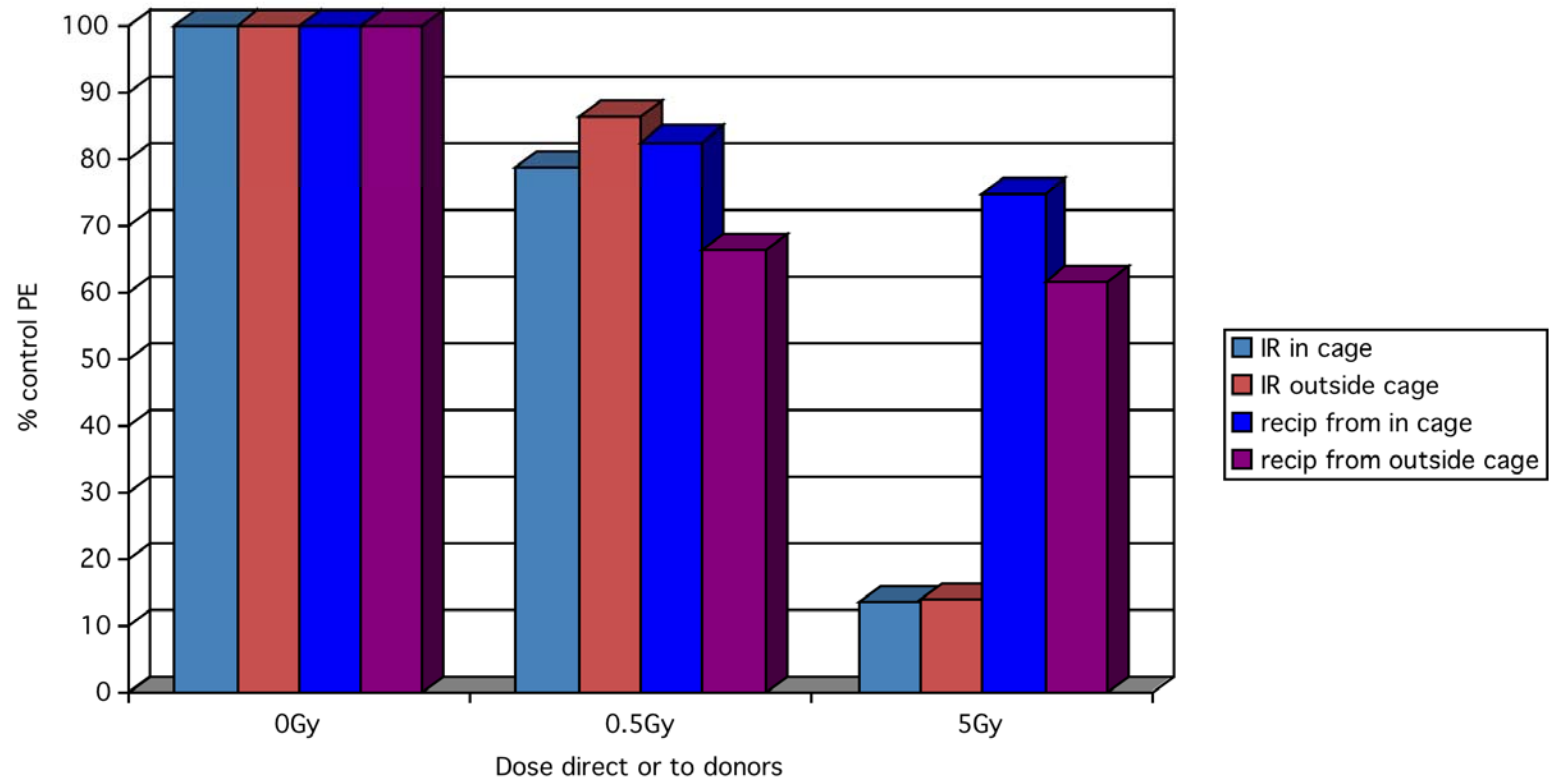


Protocol for EM Experiments 2

C



Effect of using a Faraday cage during irradiation

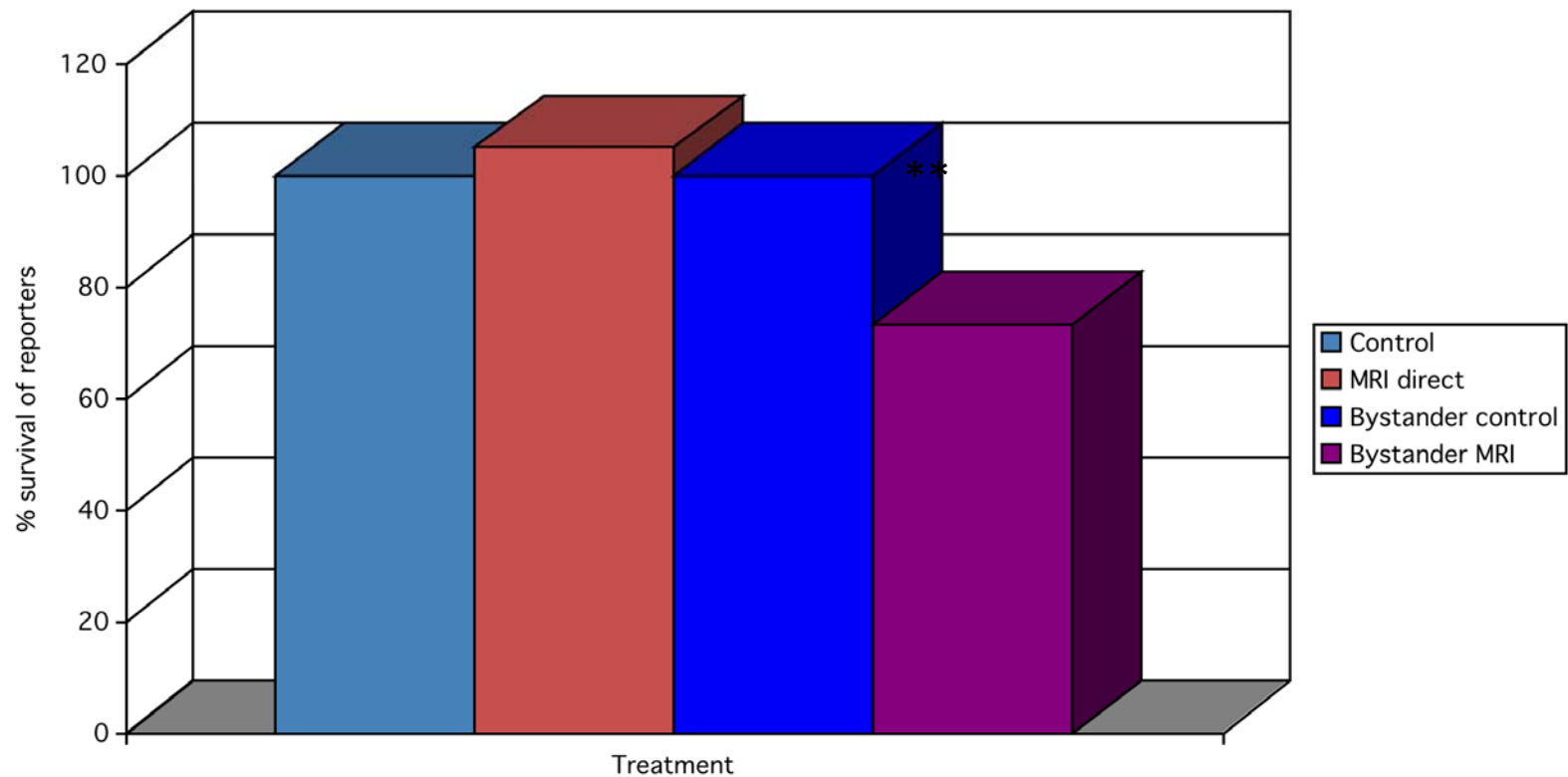




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Figure 3: Effect of medium from MRI exposed cells on survival of reporters



** = $p < 0.01$

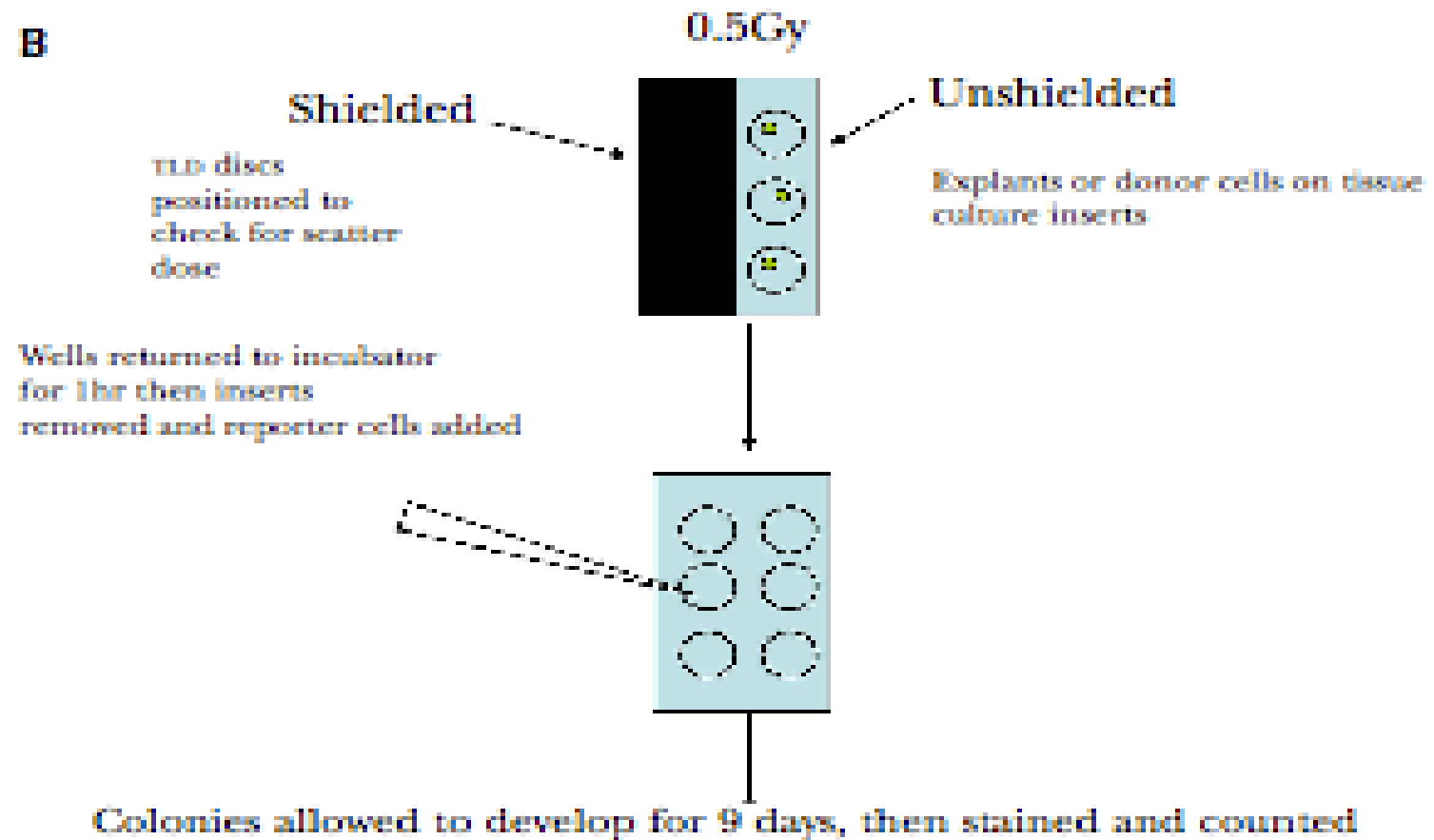


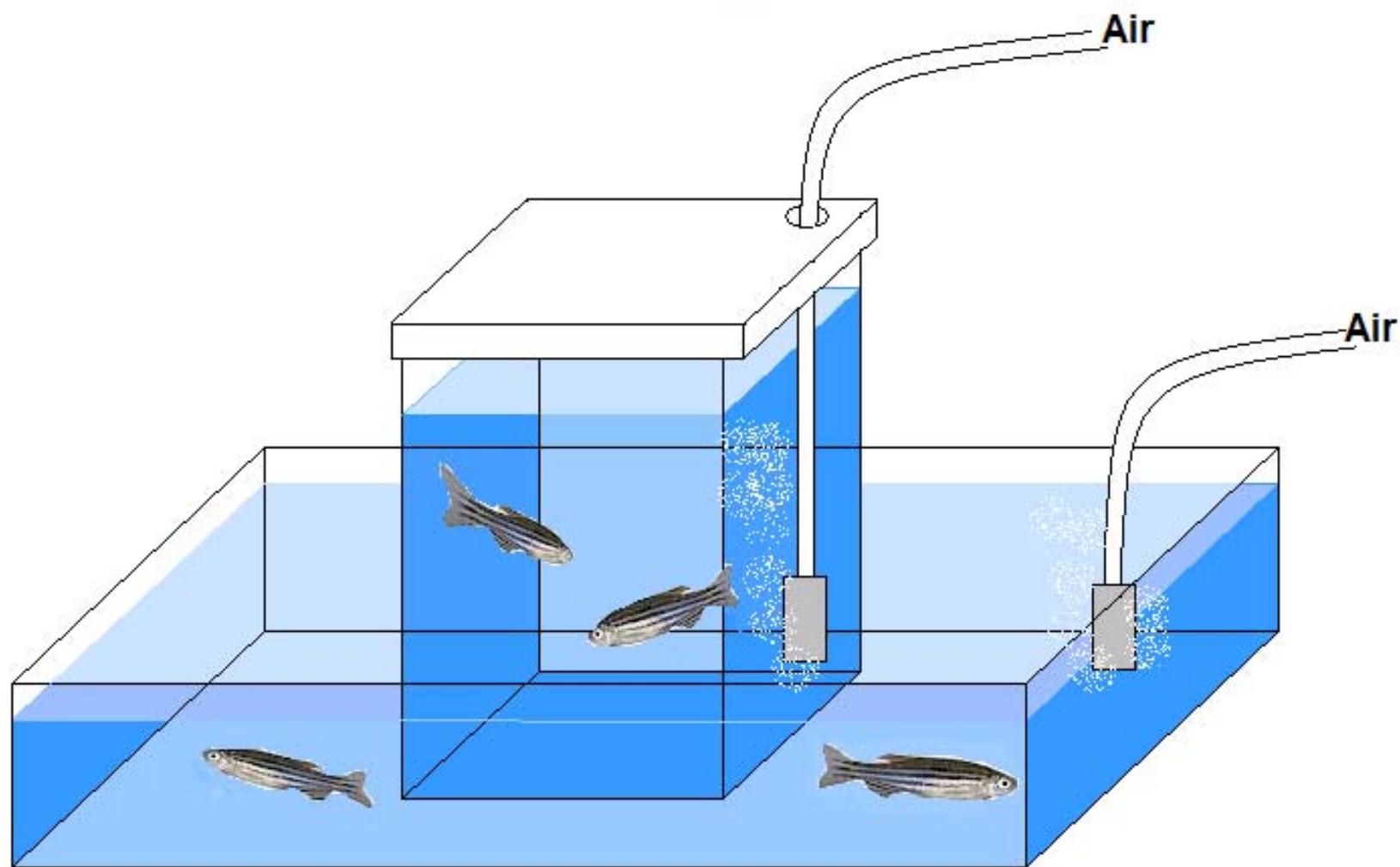
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Protocol for EM experiments

B

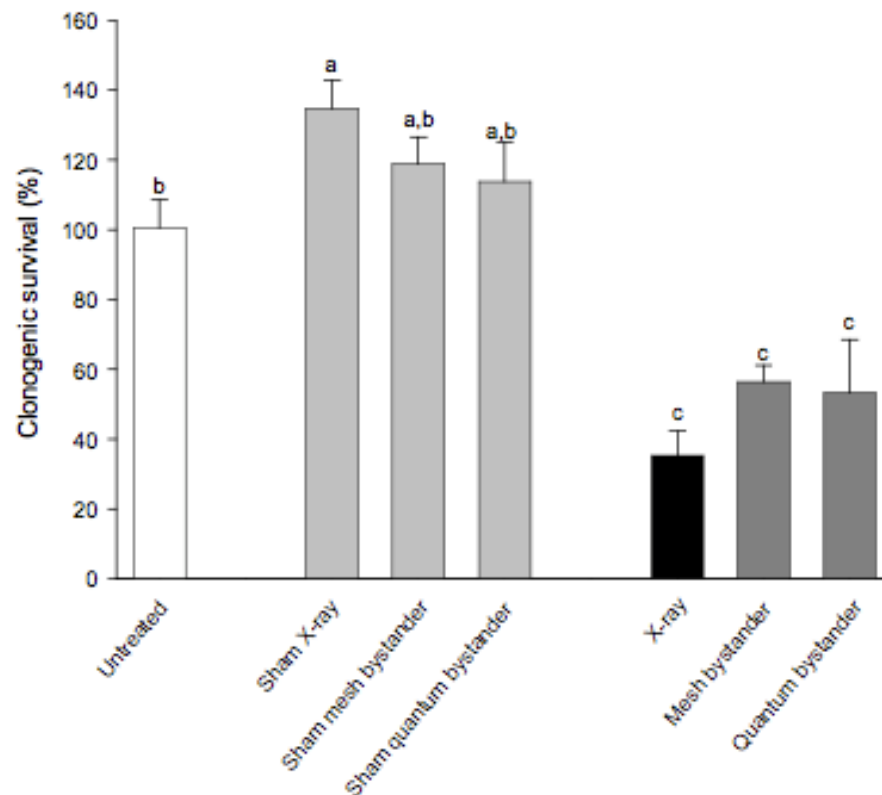




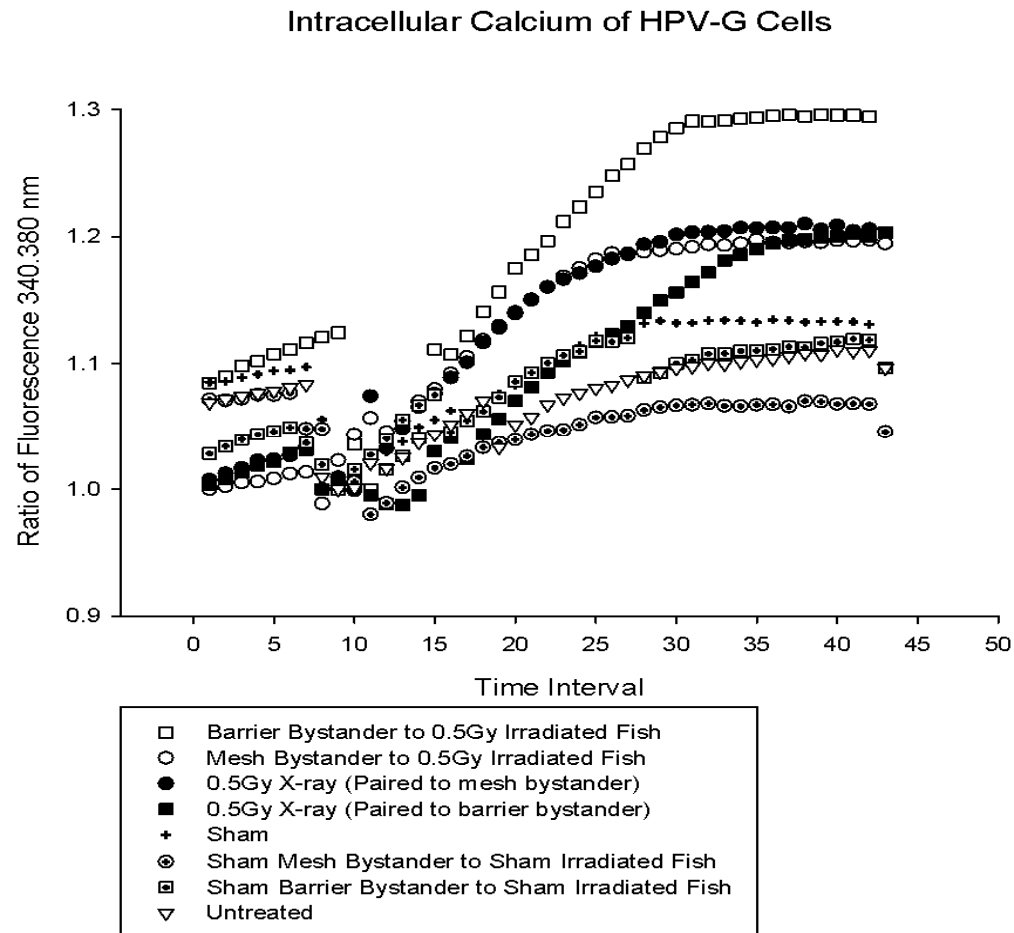


Barrier Bystander Clonogenic Assay

Experiment 1

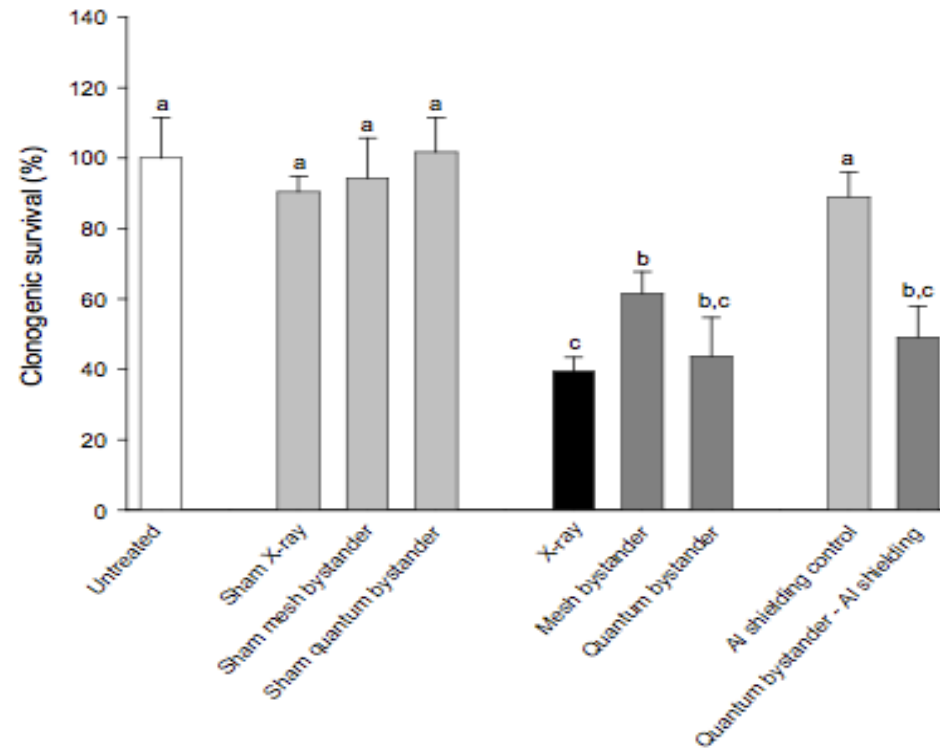


Calcium traces for barrier v mesh experiment



Barrier bystander assay \pm Al foil

Experiment 2



Rat communication of bystander signal to unirradiated cage mates

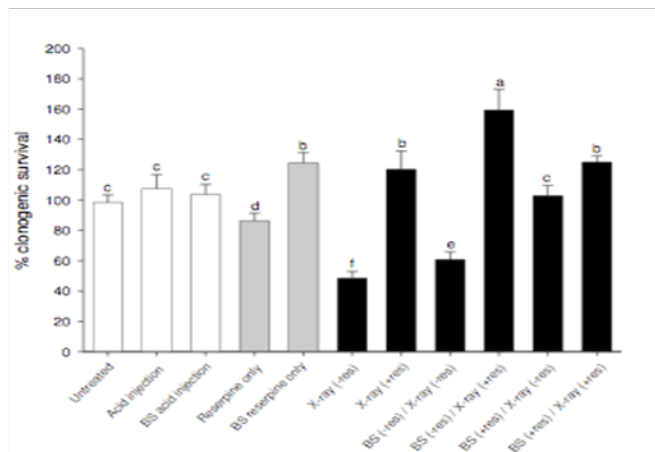




Five different approaches

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Serotonin important in signal generation



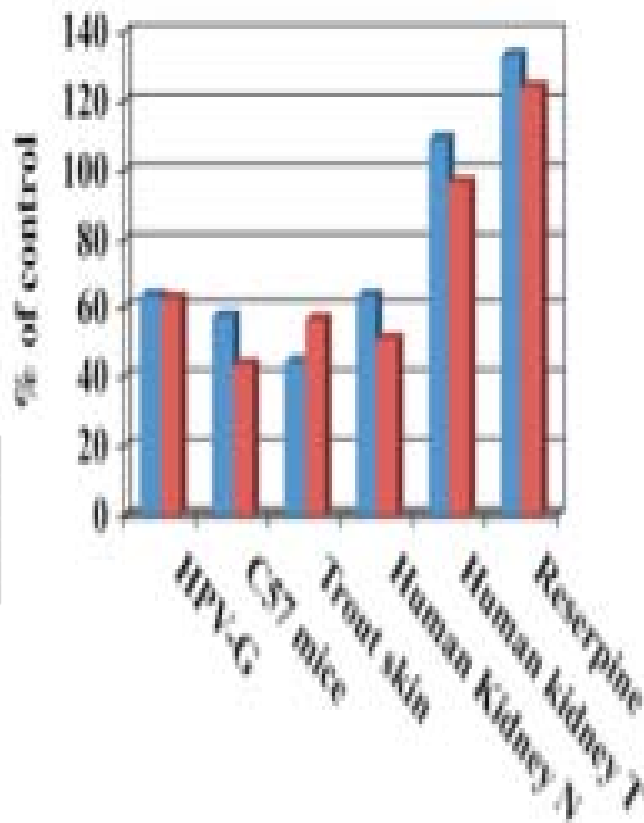
Sham treatment	% clonogenic survival
Sham X-ray (-reserpine injection)	103.8 ± 8.1 ^{*,†,‡}
Sham X-ray (+reserpine injection)	111.7 ± 8.6 [‡]
Bystander (-reserpine) / sham X-ray (-reserpine)	102.0 ± 4.8 ^{*,†}
Bystander (-reserpine) / sham X-ray (+reserpine)	137.0 ± 6.8 ^{*,‡}
Bystander (+reserpine) / sham X-ray (-reserpine)	93.5 ± 3.6 [†]
Bystander (+reserpine) / sham X-ray (+reserpine)	130.6 ± 4.3 [‡]

* significantly different to equivalent X-ray treatment

† significantly different to untreated and reserpine injected fish.

‡ significantly different to reserpine injection only induced bystander effect.

Fish injected with reserpine do not communicate the bystander signal



Reserpine inhibits serotonin binding and prevents the bystander effect in vitro and in vivo

% control 5HT
% bystander

Saroya, R et al, Injection of reserpine into zebrafish prevents fish to fish communication of radiation-induced bystander signals; confirmation in vivo of a role for serotonin in the mechanism, Dose response, in press

Poon RC et al 2007. Bystander effects of ionizing radiation can be modulated by signaling amines. Environ Res. 105(2):200-211.

Importance of serotonin in serum

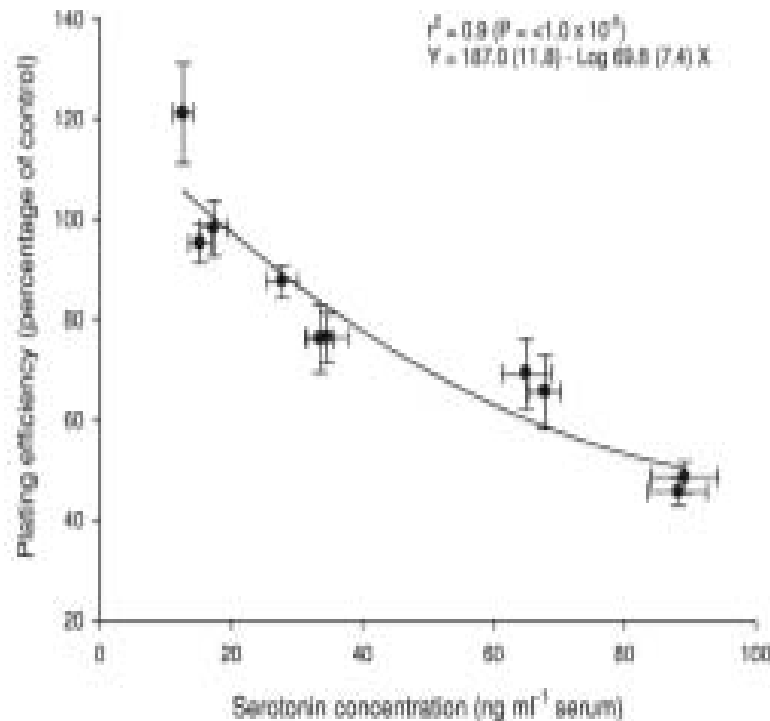


FIG. 1. Serotonin level (ng/ml) in 10 samples of commercially available fetal calf serum as a function of the percentage reduction in plating efficiency of HPV-G-transfected human keratinocytes (HPV-G cells) exposed to bystander signals in medium harvested from 0.5 Gy-irradiated donor cells.

TABLE 1
Serum as the Source of Variability in
Bystander Experiments

Serum origin	Plating efficiency (%)		
	Directly irradiated cells	Reporter cells	Serotonin ng/ml
1. Gibco Canada	93 ± 7	95 ± 4	15 ± 2
2. Gibco Canada	74 ± 6	76 ± 5	35 ± 3
3. Gibco Canada	386 ± 3	46 ± 3	88 ± 4
3. Exposed to sunlight 1 h	94 ± 43	98 ± 7	3 ± 2
3. Exposed to laboratory light 3 h	102 ± 7	100 ± 9	5 ± 1
4. PAA	95 ± 6.0	98 ± 6	17 ± 2
5. PAA	96 ± 8	121 ± 10	13 ± 1
6. PAA	54 ± 4	48 ± 3	89 ± 5
7. Gibco USA	69 ± 6	65 ± 7	68 ± 2
8. Gibco USA	74 ± 5.	69 ± 7	65 ± 4
9. Gibco USA	779 ± 8	88 ± 3	28 ± 2
10. Gibco USA	72 ± 6	76 ± 7	34 ± 2

Notes. Data for concentration of serotonin in 10 batch samples of sera with the corresponding plating efficiency of directly irradiated (0.5 Gy) and ICCM-treated reporter cells. The table includes data showing the effect of light on the level of serotonin in one of the highest samples

Reserpine disrupts calcium pulse and bystander effect in vivo

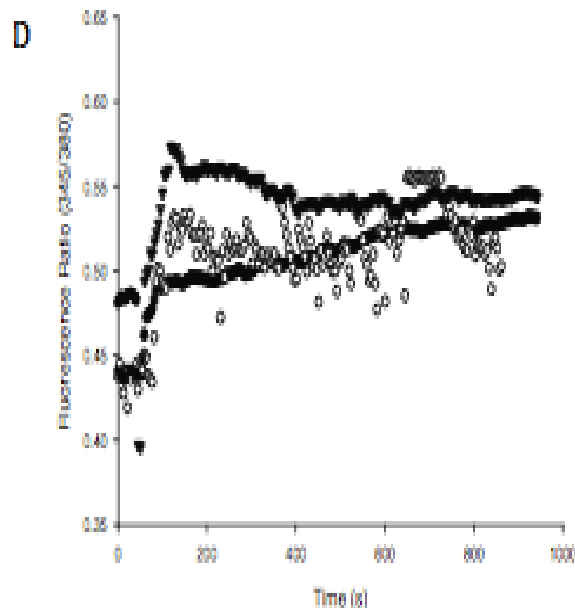


FIGURE 2D: Intracellular calcium trace in HPV-G cells, X-ray + reserpine injected fish. Medium was added from fish that were untreated (●), injected with reserpine and given an X-ray (○), and the bystander fish to the X-ray + reserpine group (▼), 45 seconds after recording. 6 fields were measured per sample. One representative trace is shown.

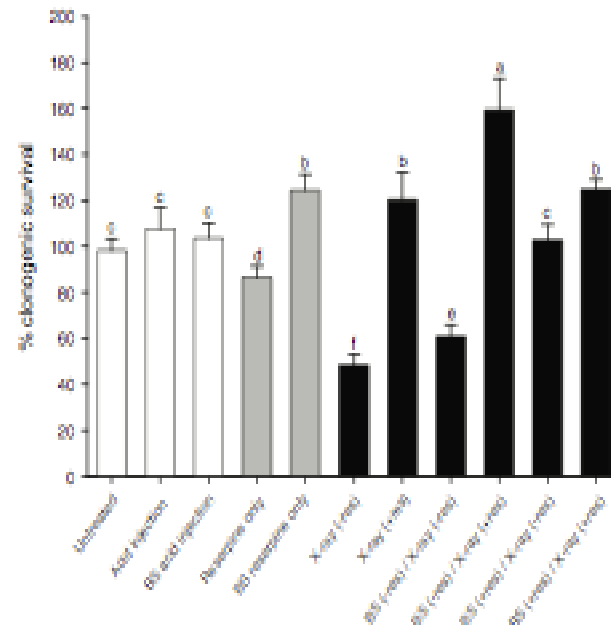
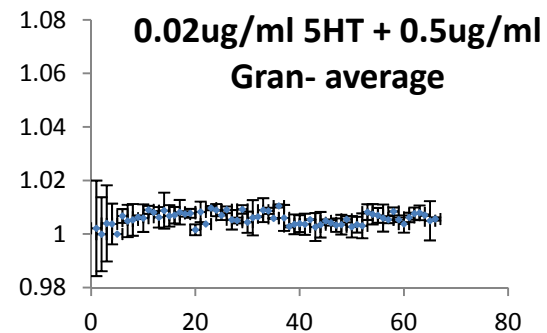
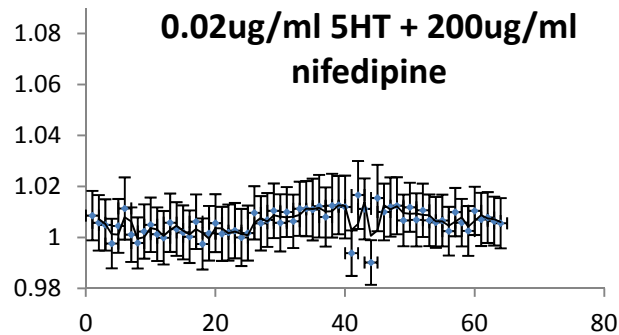
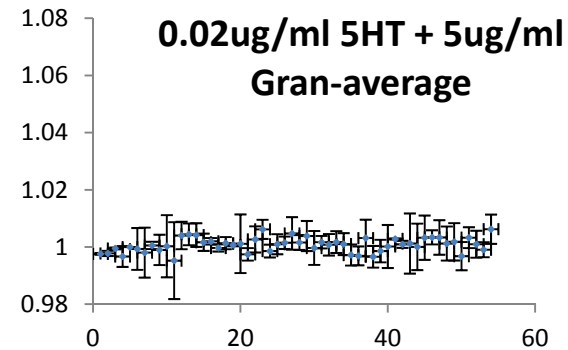
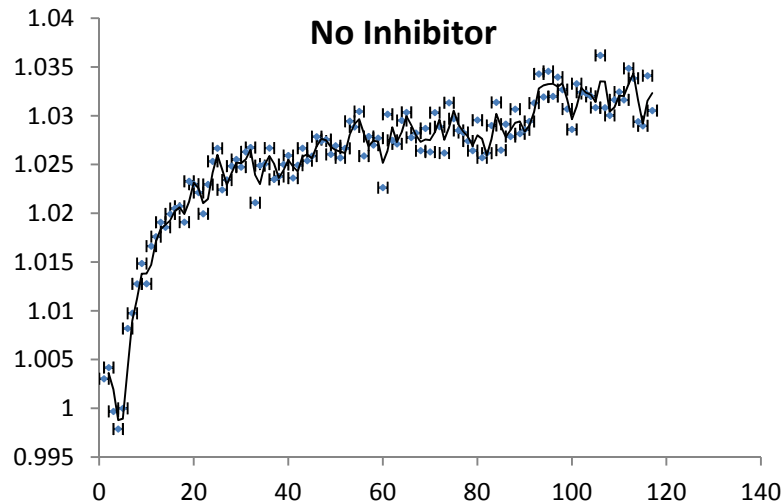


FIGURE 1: Clonogenic survival of HPV-G transfected keratinocyte reporter cells treated with growth medium from zebrafish skin tissue explants. Black bars represent fish that were X-rayed or partnered with X-rayed fish. Grey bars represent treatment or partnership with reserpine only, while the white bars represent untreated and acid injected or partnered control fish. Fish injected with reserpine are indicated by +res and -res indicates those that were not injected. Bars with corresponding letters show statistical similarities. N = 5. BS = bystander fish.

Incubation with Inhibitors Post Radiation- Reporter cell treatment

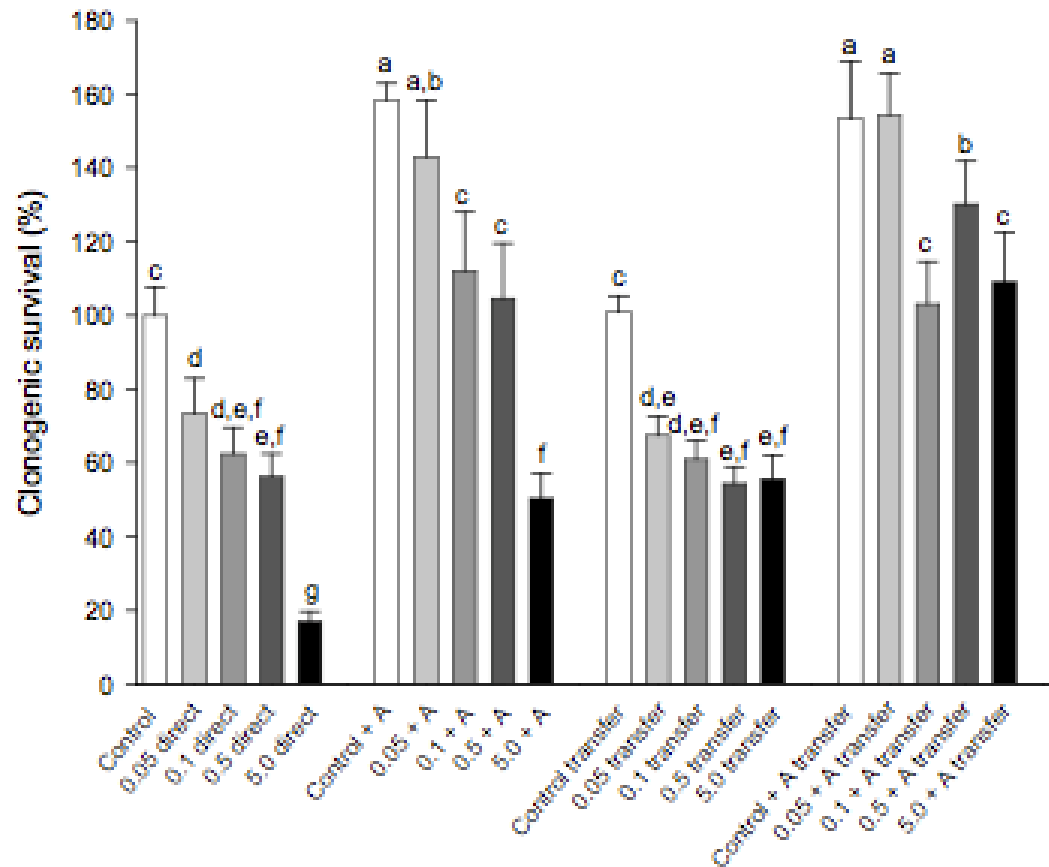




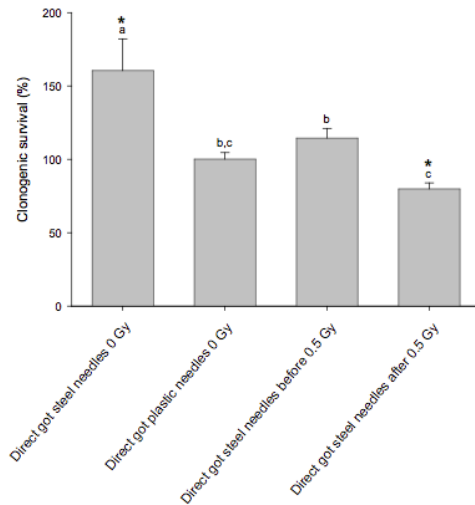
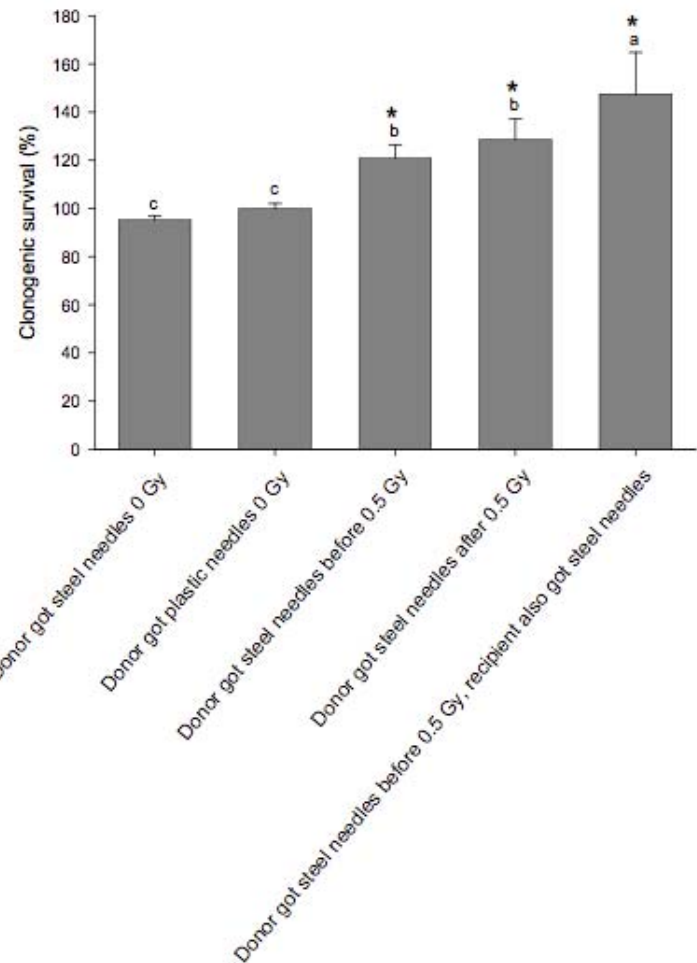
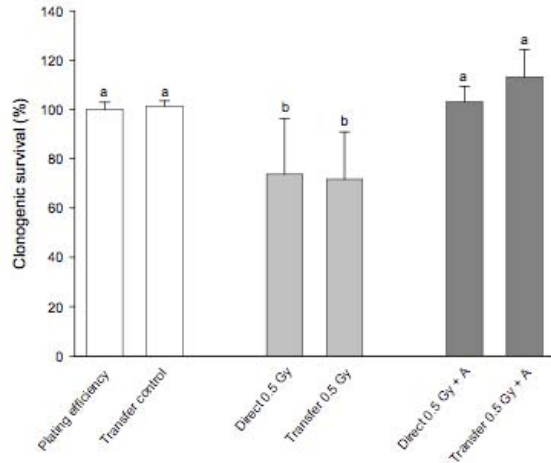
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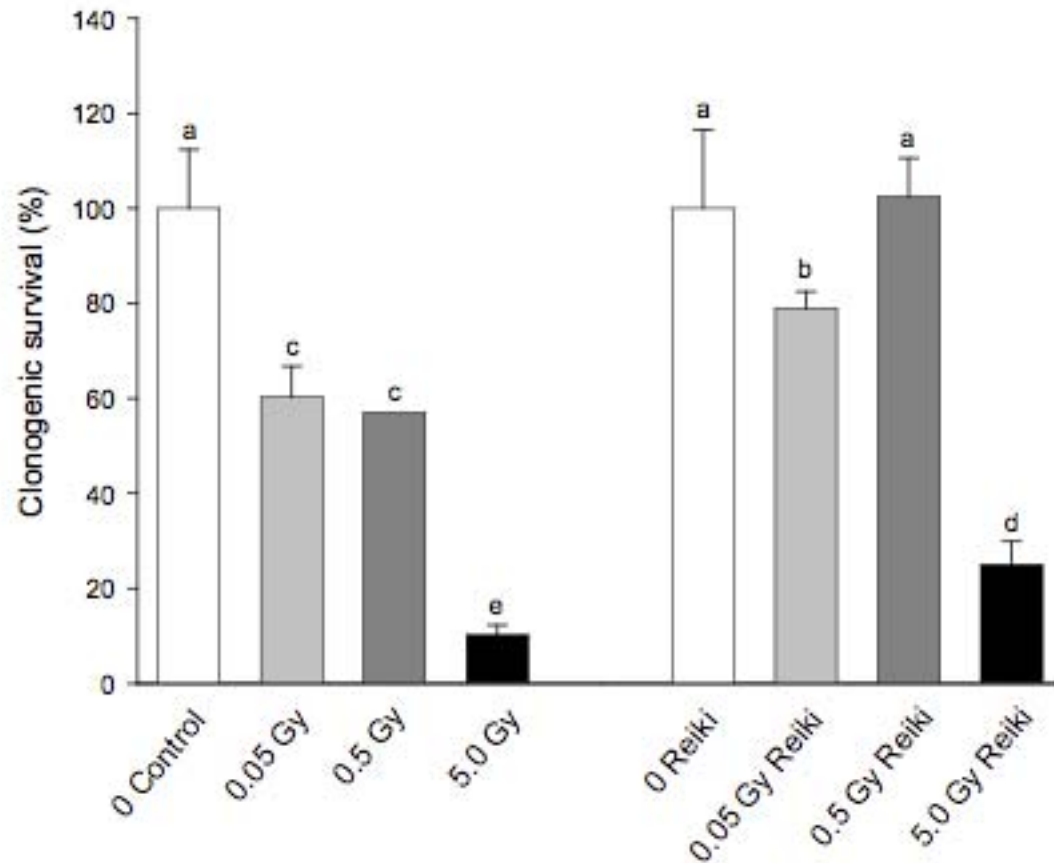
Acupuncture effect dose response



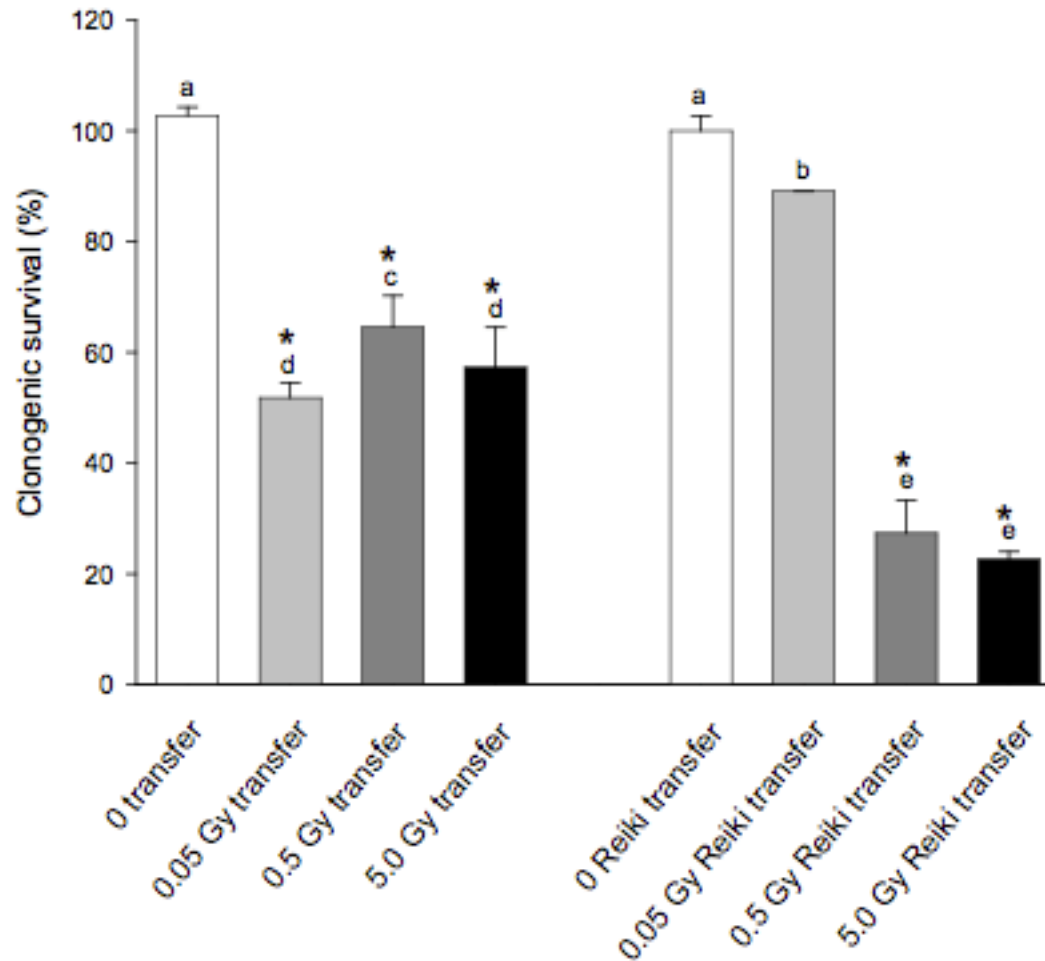
Acupuncture like treatment of irradiated or bystander cells



Effect of Reiki treatment on radiation dose response



Effect of Reiki treatment on bystander dose response





Looking to the future?

Role for alternative techniques in radiation medicine?

New targets for drug development?

Importance of holistic approaches in cancer therapy?

New targets/biomarkers for radiation protection?

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Colin Seymour, Richard Smith, Jenn Fazzari and our McMaster and old DIT Labs. NSERC - IRC, COG, Canada Research Chairs Programme, EU NOTE, ERICA and PROTECT and all the humans, animals, plants and environments that made me think about this!



Thank you!

