Repair Kinetics of DSB-foci induced by proton and helium ion microbeams of diferent energies

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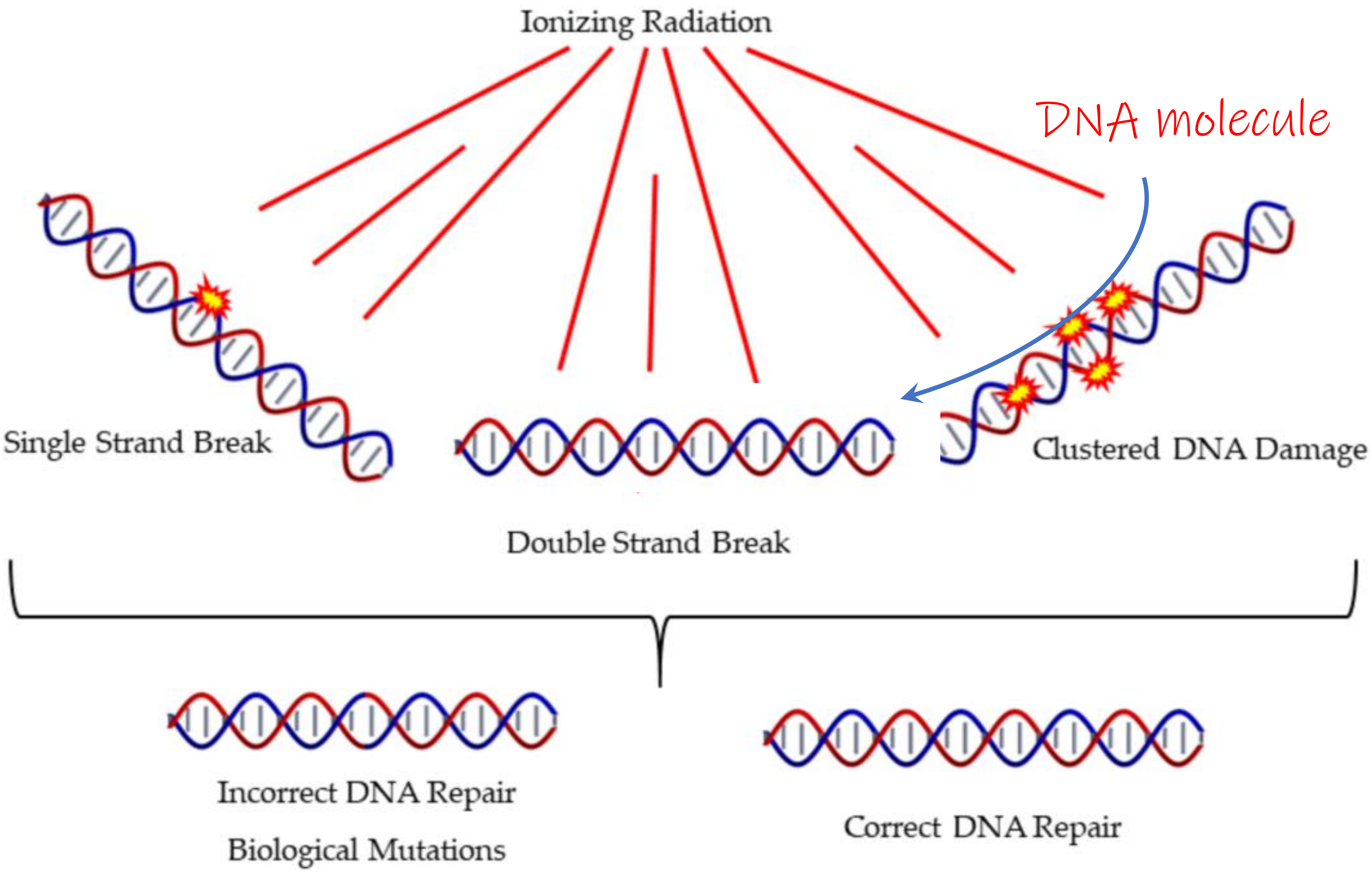
BioQuaRT SIB06-REG3

Introduction



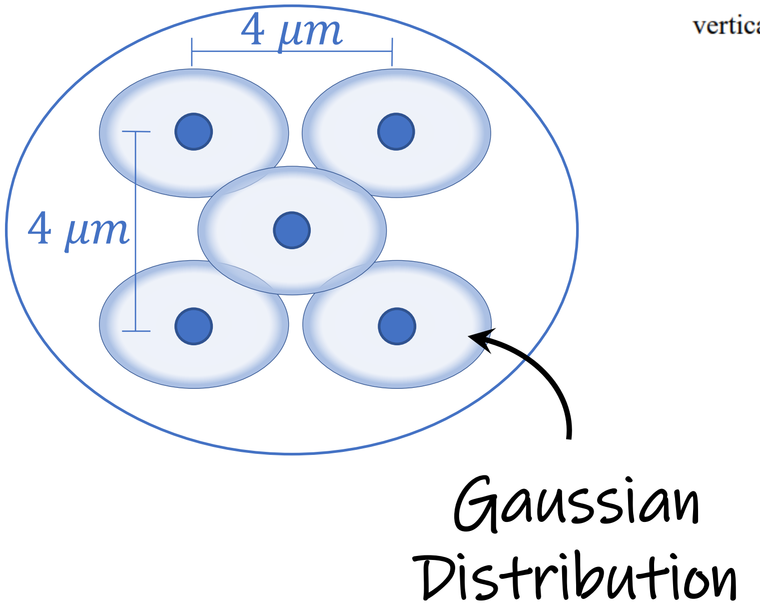
Chatzipapas, K.P., Papadimitroulas, P., Emfietzoglou, D., Kalospyros, S.A., Hada, M., Georgakilas, A.G., & Kagadis, G.C. (2020). Ionizing Radiation and Complex DNA Damage: Quantifying the Radiobiological Damage Using Monte Carlo Simulations. Cancers, 12.

Motivation

- Study the induction and repair of radiation-induced 53BP1 foci in cells exposed to low- and high-LET particle radiation

Materials and Methods (1)

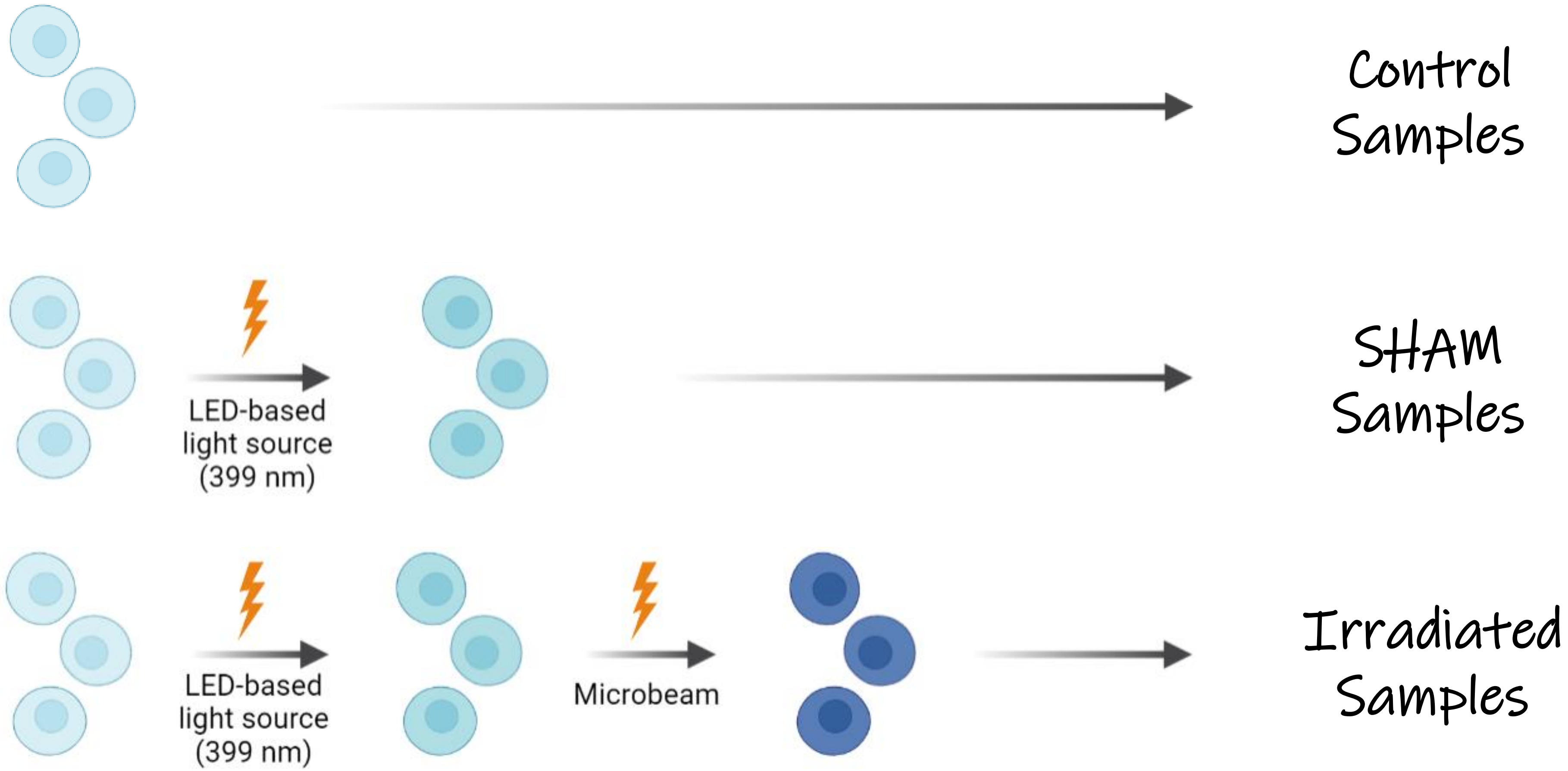
Radiation quality	α-particles 20 MeV	α-particles 10 MeV	α-particles 8 MeV
beam			
horizontal FWHM / µm	4.2	4.5	4.5
vertical FWHM / µm	3.9	3.5	3.5



$$\begin{cases} \sigma_x = FWHM_h / 2\sqrt{2ln2} \\ \sigma_y = FWHM_v / 2\sqrt{2ln2} \end{cases}$$

Gonon et al. (2019) From Energy Deposition of Ionizing Radiation to Cell Damage Signaling: Benchmarking Simulations by Measured Yields of Initial DNA Damage after Ion Microbeam Irradiation Radiat. Res. 191 566–84

Materials and Methods (2)



Materials and Methods (3)

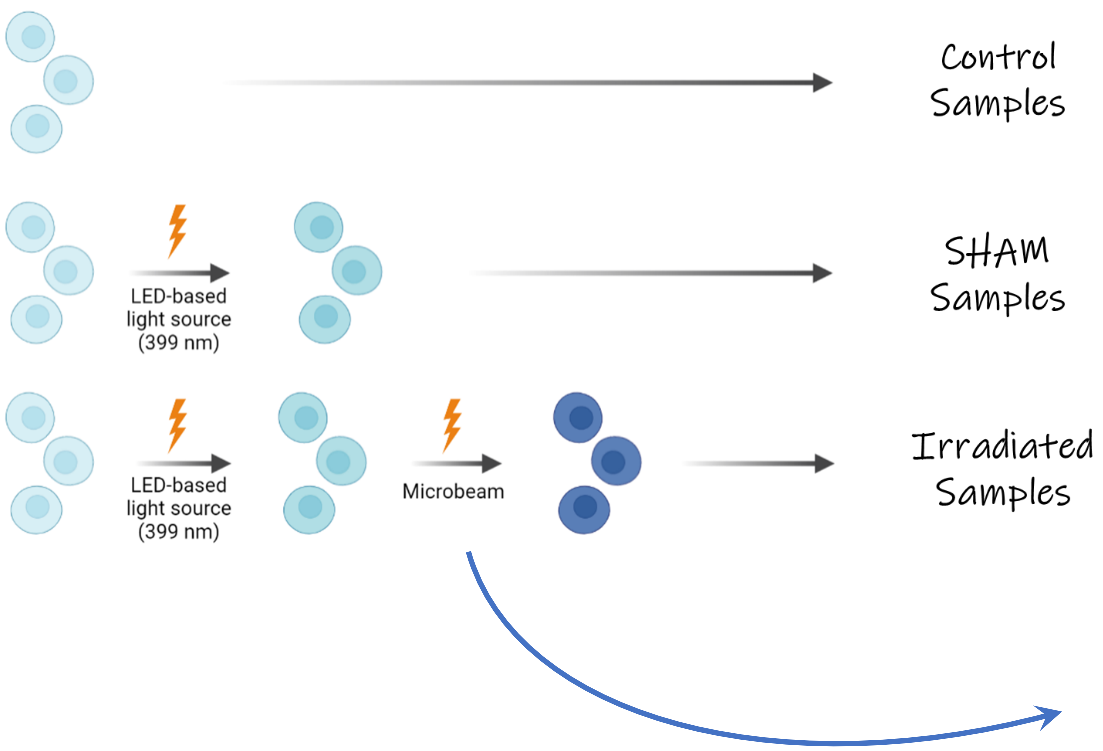


Table 1. Energy and LET values of the different types of radiation used for cellular irradiation.

Particle type and beam energy	Estimated energy at cell nucleus center (MeV)	Estimated LET at cell nucleus center (keV/µm)
α-particles		
20 MeV	17.8 ± 0.2	36 ± 1
10 MeV	5.5 ± 0.4	85 ± 4
8 MeV	1.9 ± 0.6	170 ± 40
Protons		
3 MeV	1.6 ± 0.2	19 ± 2

Materials and Methods (4)

- Foci assay protocol was followed for the following time points after irradiation:
  - 0.5 h
  - 2 h
  - 4 h
  - 8 h
  - 24 h

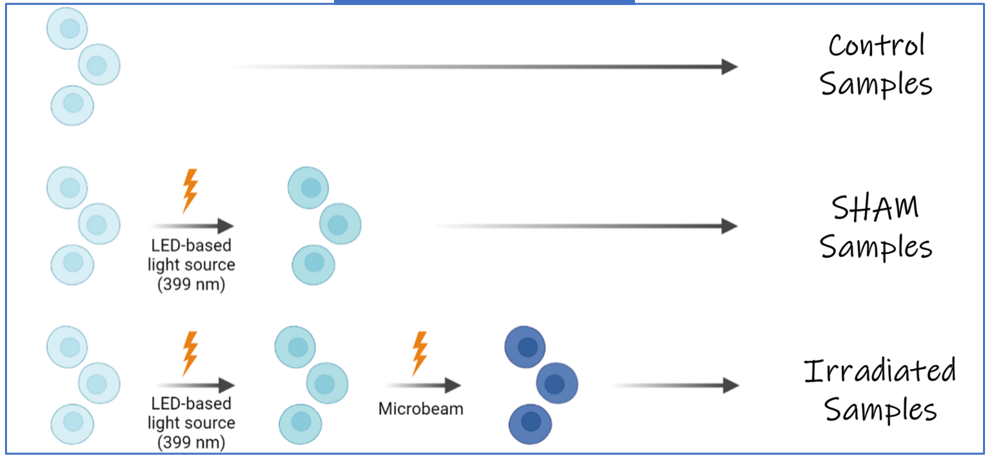
- 53BP1 foci.

- Images were acquired with fluorescence microscopy and analysed with CellProfiler software.

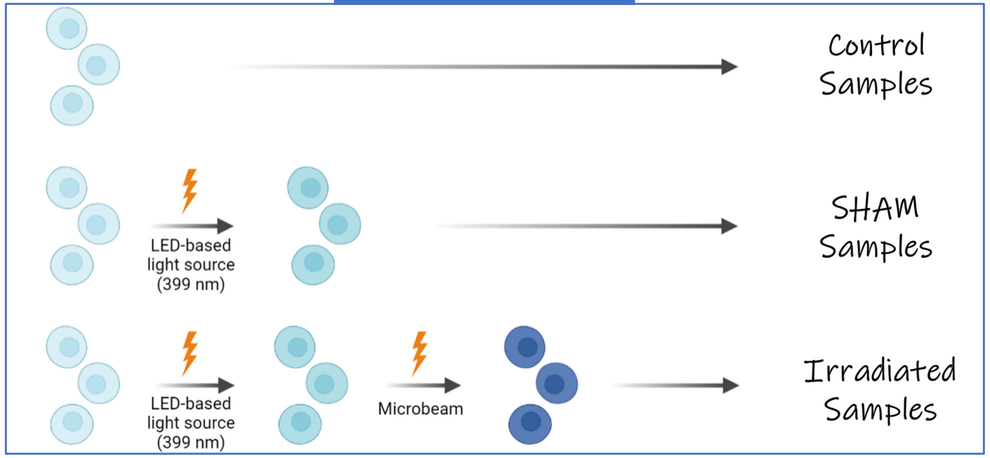
Materials and Methods (5)

- For each time point and radiation quality

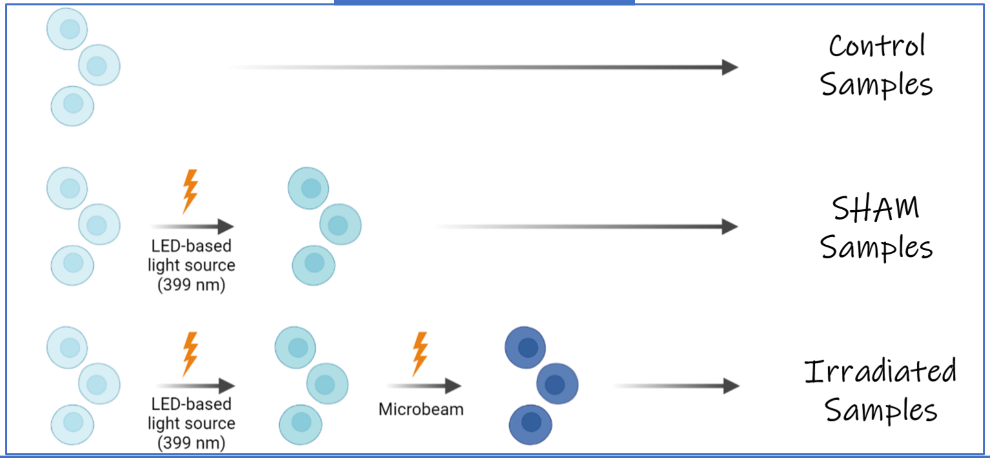
Experiment 1



Experiment 2



Experiment 3



Data Analysis (1)

- Assumptions:

1. The expected number of foci per nucleus and the respective uncertainty for a radiation condition were estimated, respectively, as the mean and the sample standard deviation of the mean values found in the three replicate experiments.

2. The possibility that several foci are formed within an ion track and are indistinguishable is considered. The number of foci formed in an ion track is assumed to be Poisson distributed.

3. The mean number of tracks in proximity (leading to indistinguishable foci) were determined by a simulation of the irradiation, separately for each possible number of ions in such a track “cluster”.

Data Analysis (2)

- Assumptions:

4. It is assumed that radiation-induced foci and foci induced by non-radiation causes occur statistically independently.

5. SHAM irradiated foci are assumed to be always repairable whereas for radiation-induced foci it is possible that foci are persistent.

6. Repair of foci is assumed to follow first order kinetics with a repair rate independent of radiation quality.

Data Analysis (3)

$$m_s\,(t) = \bar{n}_b + \bar{n}_s e^{-\beta_0 (t - t_M)}$$

Mean number of foci observed at time t after irradiation in sham-irradiated cells

Time after irradiation

Time after irradiation when the maximal number of foci is observed

Mean number of background foci

Mean number of foci at $t = t_M$ due to sham irradiation

Repair rate of sham irradiation-induced foci

* foci = foci per nucleus

Data Analysis (4)

Mean number of foci observed at time t after irradiation in irradiated cells

$$m_Q(t) = m_s\,(t) + m_{r,Q}(t)$$

Mean number of foci observed at time t after irradiation in sham-irradiated cells

Mean number of observed foci produced by ions of radiation quality Q

* foci = foci per nucleus

Data Analysis (5)

Mean number of clusters of n_i ions

$$m_{r,Q}(t) = \sum_{n_i} P_Q(t|n_i)\bar{k}_Q(n_i)$$

Mean number of observed foci produced by ions of radiation quality Q

Probability of observing a focus at time t at the location of a cluster of n_i ions, of radiation quality Q, traversing the nucleus in proximity

* foci = foci per nucleus

Data Analysis (6)

Number of ions in the cluster

Two classes of foci: normal and persistent

$$P_Q(t|n_i) = 1 - e^{-n_i \bar{n}_Q \left[(1-p_Q)e^{-\beta_1(t-t_M)} + p_Q e^{-\beta_2(t-t_M)}\right]}$$

Mean number of radiation-induced foci formed along an ion track inside the cell nucleus at time t_M

Data Analysis (7)

Repair rate of normal RIF

Repair rate of persistent RIF

$$P_Q(t|n_i) = 1 - e^{-n_i \bar{n}_Q[(1-p_Q)e^{-\beta_1(t-t_M)}+p_Q e^{-\beta_2(t-t_M)}]}$$

Fraction of persistent foci

*RIF = Radiation-Induced Foci

Data Analysis (8)

$$P_Q(t|n_i) = 1 - e^{-n_i\left[(\bar{n}_Q - \bar{p}_Q)e^{-\beta_1(t-t_M)} + \bar{p}_Q e^{-\beta_2(t-t_M)}\right]}$$

Mean number of persistent foci

Data Analysis (9)

- In all equations, t_M was set equal to 0.5 h (Rogakou *et al* 1998, Anderson *et al* 2001, Mosconi *et al* 2011)

Data Analysis (10)

$$m_{r,Q}(t) = \sum_{n_i} P_Q(t|n_i)\bar{k}_Q(n_i)$$

Mean number of clusters of n_i ions

- The values of $\bar{k}_Q(n_i)$ were determined by simulation assuming two foci were indistinguishable when their distance was less than 2 µm (Gonon *et al* 2019).

Data Analysis (11)

1. Separate non-linear regression of each data set (SHAM, Proton, $\alpha' s$)

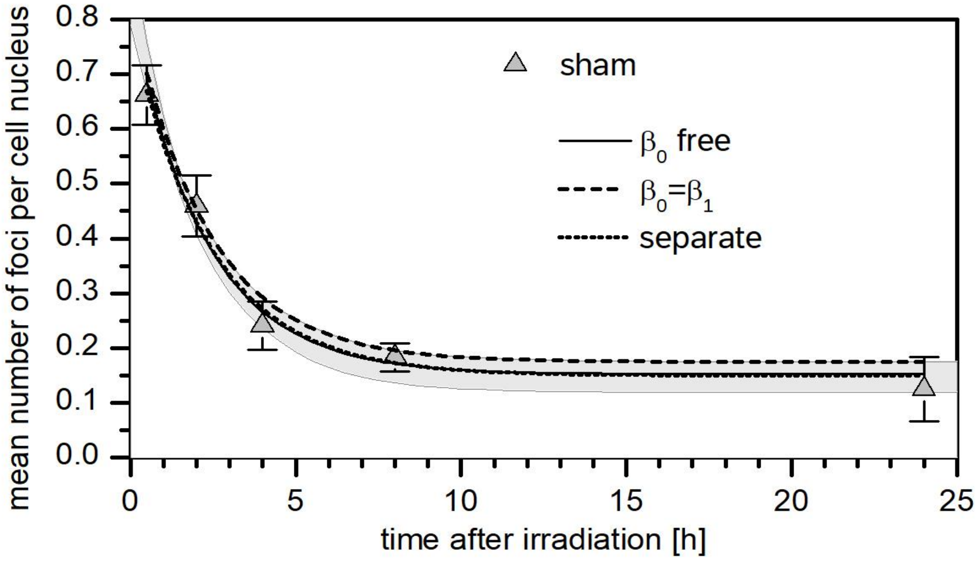
Find $\boldsymbol{\beta_0, \beta_1, \beta_2}$, $\{\boldsymbol{\bar{n}_Q, \bar{p}_Q, p_Q}\}$

2. Simultaneous non-linear regression:
   1. β_0 as free parameter
   2. $\beta_0 = \beta_1$

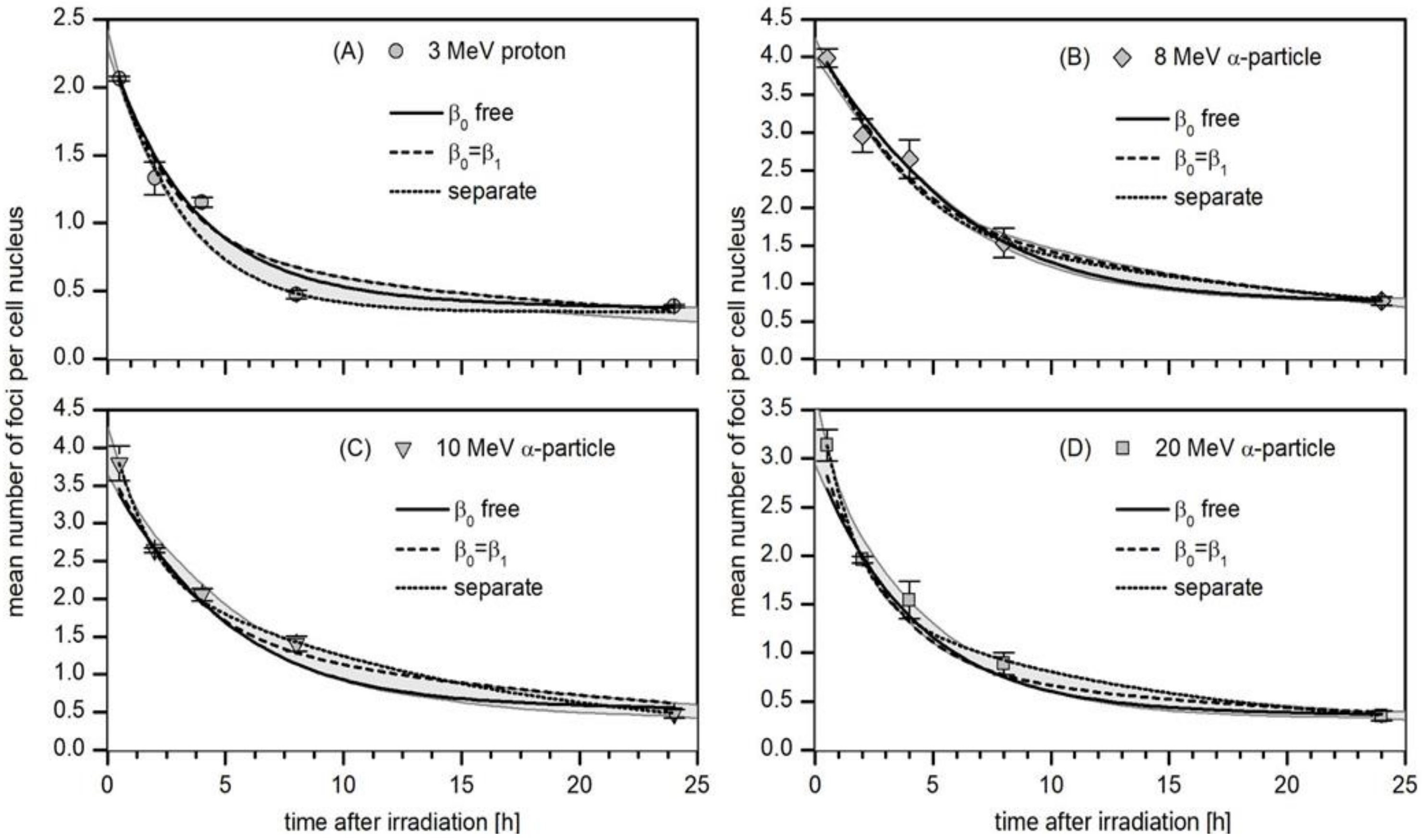
$$P_Q(t|n_i) = 1 - e^{-n_i \bar{n}_Q[(1-p_Q)e^{-\beta_1(t-t_M)}+p_Q e^{-\beta_2(t-t_M)}]} \quad \text{(v1)}$$

$$P_Q(t|n_i) = 1 - e^{-n_i [(\bar{n}_Q-\bar{p}_Q)e^{-\beta_1(t-t_M)}+\bar{p}_Q e^{-\beta_2(t-t_M)}]} \quad \text{(v2)}$$

Results (1)

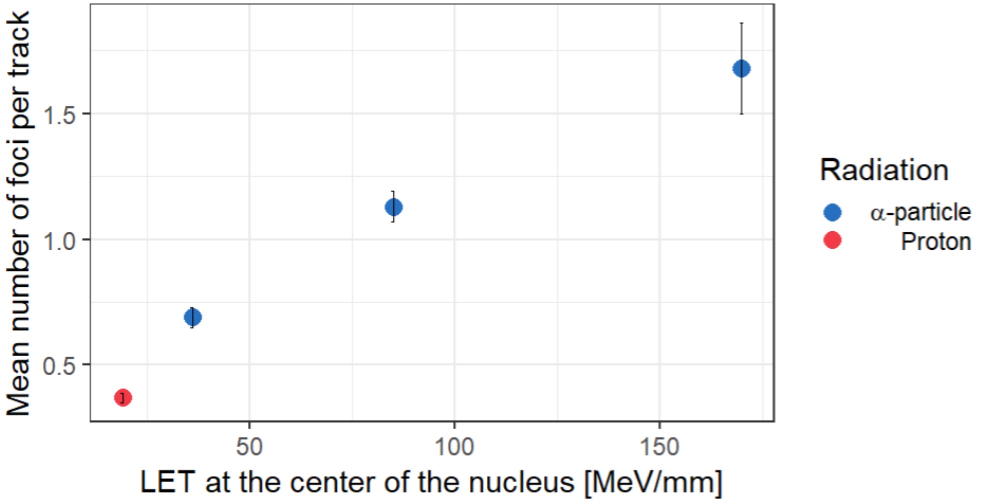


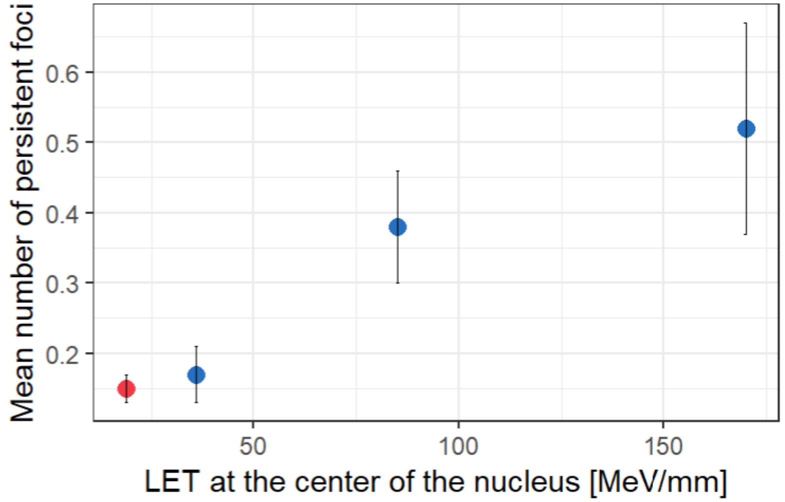
sham
β_0 free
$\beta_0=\beta_1$
separate
mean number of foci per cell nucleus
time after irradiation [h]

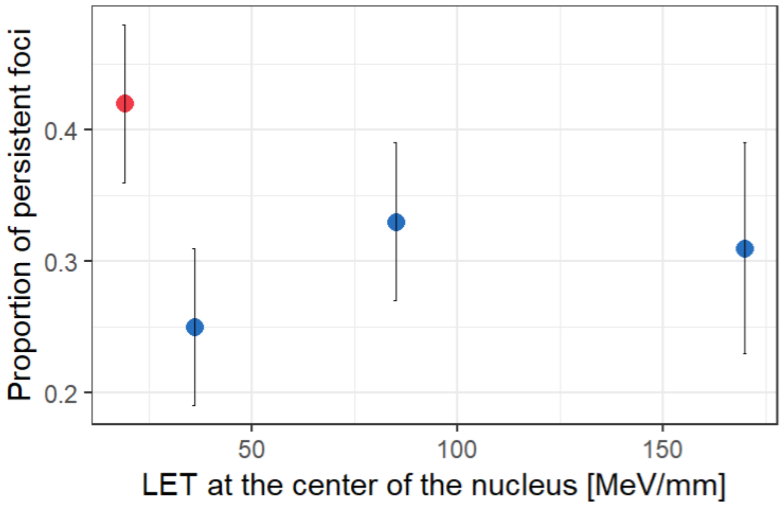


(A) 3 MeV proton
(B) 8 MeV α-particle
(C) 10 MeV α-particle
(D) 20 MeV α-particle
β_0 free
$\beta_0=\beta_1$
separate
mean number of foci per cell nucleus
time after irradiation [h]

Results (2)







From the simulataneous non-linear regression using (v1)

Discussion

- A reliable determination of the repair kinetics is only possible when performing a simultaneous regression of all datasets.
  - Variation of repair rates and of the fractions of foci following different repair kinetics is giving the model too much freedom and less specificity.

- It is not the fraction of persistent foci that increases with LET but their absolute number.

- Protons seem to produce more persistent foci as compared to helium ions of even higher LET
  - LET may not be the best suited parameter to characterize radiation quality (Rucinski et al 2021)

Future Work

- A number of differences related to cells and foci characteristics were found between this work and Gonon *et al* (2019) which motivates comprehensive investigation with a more sophisticated data science approach that will be elaborated in a following paper.
  - foci counts but also other parameters such as the geometry of foci and cell nuclei, variation of track length, density of foci per track length.

- What does an exponential time dependence of the number of radiation-induced foci means?
  - To which factors is the repair time related? Foci size? Others?

- Nanodosimetry correlation/approach (João F. Canhoto PhD thesis)

Acknowledgments

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