



Exposure of *Deinococcus radiodurans* to both static magnetic fields and gamma radiation: observation of cell recuperation effects

Henriette Righi^{1,2} · João D. T. Arruda-Neto¹ · José G. C. Gomez² · Luiziana F. da Silva² · Elizabeth S. R. Somessari³ · Aline C. C. Lemos²

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Abstract

The extremophilic bacterium *Deinococcus radiodurans* displays an extraordinary ability to withstand lethal radiation effects, due to its complex mechanisms for both proteome radiation protection and DNA repair. Published results obtained recently at this laboratory show that *D. radiodurans* submitted to ionizing radiation results in its DNA being shattered into small fragments which, when exposed to a “static electric field” (SEF), greatly decreases cell viability. These findings motivated the performing of *D. radiodurans* exposed to gamma radiation, yet exposed to a different exogenous physical agent, “static magnetic fields” (SMF). Cells of *D. radiodurans* [strain *D.r.* GY 9613 (R1)] in the exponential phase were submitted to ⁶⁰Co gamma radiation from a gamma cell. Samples were exposed to doses in the interval 0.5–12.5 kGy, while the control samples were kept next to the irradiation setup. Exposures to SMF were carried out with intensities of 0.08 T and 0.8 T delivered by two settings: (a) a device built up at this laboratory with niobium magnets, delivering 0.08 T, and (b) an electromagnet (Walker Scientific) generating static magnetic fields with intensities from 0.1 to 0.8 T. All samples were placed in a bacteriological incubator at 30 °C for 48 h, and after incubation, a counting of colony forming units was performed. Two sets of cell surviving data were measured, each in triplicate, obtained in independent experiments. A remarkable similarity between the two data sets is revealed, underscoring *reproducibility* within the 5% range. Appraisal of raw data shows that exposure of irradiated cells to SMF substantially increases their viability. Data interpretation strongly suggests that the increase of *D. radiodurans* cell viability is a sole *magnetic physical effect*, driven by a *stochastic process*, improving the efficiency of the rejoining of DNA fragments, thus increasing cell viability. A type of *cut-off dose* is identified at 10 kGy, above which the irradiated cellular system loses recovery and the cell survival mechanism collapses.

Keywords *Deinococcus radiodurans* · Gamma radiation · Surviving curves · Static magnetic fields · DNA shattered fragments · Cell recuperation effects

✉ João D. T. Arruda-Neto
arruda@if.usp.br

1 Introduction

The extremophilic bacterium *Deinococcus radiodurans* has been a biomodel high on the agenda of cellular and molecular biologists, mostly due to its intriguing and complex mechanisms for both proteome radiation protection and DNA repair, following exposure to high doses of ionizing radiations [1–3].

It is a well-known fact that the main target of high-dose ionizing radiation hitting a cell is its genome. At doses of gamma radiation such as those dealt with in this study, cell DNA is shattered into small fragments, as shown by results obtained elsewhere for *D. radiodurans* [4] and at this laboratory with DNA plasmids using atomic force microscopy [5].

Exposure of *D. radiodurans* to both ionizing radiation and an exogenous physical agent such as SEF was, for the first time, studied at this laboratory [6]. SEF was found to be a high-performance radio sensitizer, when acting on *D. radiodurans*, subsequent to irradiation, a significant depletion of the repairing shoulder was observed for doses from 4 to 8 kGy. On the basis of a biophysical approach, it was concluded that the SEF scrambled the reassembling of small DNA fragments, thus difficulting repair processes [6]. Similar results of radio sensitization by SEF in radiation exposure of more sensitive eukaryotes and prokaryotes cells were also obtained at this laboratory [7, 8].

These findings obtained with SEF motivated the present study, in the sense of carrying out another detailed study of *D. radiodurans* exposed to ionizing radiation followed by exposure to a *diverse* exogenous physical agent: *static magnetic field*. This choice was governed by the plethora of bioeffects induced by magnetic fields [9, 10].

In this work use was made of SMF with intensities 0.08 T and 0.8 T or 80 mT and 0.8 T (moderate intensity magnetic fields). The classification for magnetic field intensities, as proposed by Dini and Abbro in their review article [9], is (a) weak intensity, > 1 m T; (b) moderate intensity interval, 1 mT–1 T; (c) strong intensity interval, 1–5 T; and (d) ultrastrong intensity, > 5 T.

The “targets” inside the cell available for the action of a (or exposure to) SMF, after irradiation with high-dose ionizing radiation, are cell “debris” [56]. These are mostly DNA fragments, since the *D. radiodurans* proteome is relatively more protected than its genome which, to a great extent, explains its extremophilic character [1–3].

Due to the transparency of matter to gamma radiation, the cell membrane should be physically preserved throughout the irradiation process. Furthermore, effects induced by a SMF in an *ex post facto* scenario (post-high-dose irradiation) should, in essence, be physical in nature. In this case, the interpretation of experimental results obtained from exposure of high-dose irradiated cells to a SMF should be supported by aspects of interaction of a SMF with moving charges (DNA fragments, in the present study).

In this case, the role played by SMF is at variance with experiments carried out exposing *whole and healthy cells* to this field. In short, it would be expected that SMFs act physically in cells in a post-irradiation scenario, *with minor or no induced bioeffects*, except for those associated with cell viability.

The demonstration of such a hypothesis is one of the goals of this study, in addition to the search for the kinds of new physical effects possibly induced.

2 Materials and methods

2.1 Bacterial culture

The strain of the bacterium *D. radiodurans* 9613 (R1) was provided by Dr. Carlos E. B. Almeida, from the Institute for Radioprotection and Dosimetry/IRD in Rio de Janeiro. Cells were cryopreserved in glycerol (50%–80 °C). One milliliter of inocula was grown in 50 mL of TGY (1% tryptone, 0.2% glucose, 0.6% yeast extract) and aired with 200 rpm shaker at 30 °C for 24 h. Next, 5 mL of the starting medium was added to 50 mL of a new TGY medium, up to the exponential phase (2 to 8 h). One milliliter of medium in the exponential phase was saved in 1.5-mL plastic tubes for each sample. For each irradiation dose, four samples were prepared for the following procedures: control, only irradiation, and irradiations plus exposure to 0.8 T and 0.08 T magnetic fields.

2.2 Gamma irradiation

Cells in the exponential phase were submitted to ^{60}Co gamma radiation by a gamma cell (0.69 kGy/h) at the Center of Radiation Technologies, Energetic and Nuclear Research Institute (CTR-IPEN), under the Nuclear Energy National Commission (CNEN). Samples were exposed to doses in the interval 0.5–12.5 kGy, while the control samples were housed next to the irradiation setup.

2.3 Exposure to static magnetic fields

Exposures to SMF were carried out with the following settings: (1) by means of a device projected and assembled by our group with niobium magnets, delivering SMF with mean intensity of 0.08 T. Such intensity corresponds to the lower limit of the conventional moderate intensity SMF interval and (2) with an electromagnet (Walker Scientific) from the Magnet-Optics and Non-Linear Spectroscopy Laboratory/Physics Institute-USP. This equipment generates static or variable magnetic fields, with intensities ranging from 0.1 to 0.8 T. In the present study, use was made of 0.8 T, thus reaching the upper limit of moderate intensity SMF interval.

2.4 Viability curves

Total samples submitted to a variety of exposures, as well as the control, were diluted through decimal dilutions in saline solution (NaCl 0.85%, pH 6.8), immediately plated in solid medium (TGY) and placed in a bacteriological incubator at 30 °C for 48 h. After incubation, the counting of colony-forming units (CFU/mL) was performed. All measurements were performed in triplicate for each irradiation dose in the interval 0–12.5 kGy. Samples were collected from a single culture, and three independent irradiations were performed for each dose.

2.5 Survival functions

The determined number of viable cells as function of the radiation dose D , $N_V(D)$, is usually expressed as a *fraction* of viable cells; that is,

$$S(D) = N_V(D)/N_V(0) \quad (1)$$

called the *survival function*, where $N_V(0)$ is the initial number of cells (before exposure to radiation).

Error bars showing up in figures represent external standard deviations. Data handling procedure consisted solely of data averaging; thus, only the external standard deviation of the averaged values was calculated, a simple and conventional parametric statistic in the normal model [11].

3 Results

A mere visual inspection of the *D. radiodurans* survival functions (Figs. 1 and 2) reveals, principally, a remarkable similarity between the two data sets in terms of both shape and magnitude (within experimental uncertainties). Importantly too, these two data sets were taken in two independent experimental runs and both in triplicate.

3.1 Irradiated cells exposed to SMF—surviving functions

The results obtained from irradiations *without* exposure to SMF, with gamma radiation doses from 0 to 12.5 kGy, exhibit the well-known characteristics of *D. radiodurans* radiological survival functions; that is, a slowly decreasing behavior up to 10 kGy, approximately, is identified as the dose interval where cell repairing mechanisms are still effective, followed by an abrupt decrease interpreted as a consequence of the large amount of DNA damage combined with the depletion of the repairing protein pool [1–3].

Exposure to SMF with intensities of 0.08 T and 0.8 T did not alter the shape of the survival curves but increased the magnitude of all data points (Figs. 1 and 2). Such a highly unexpected

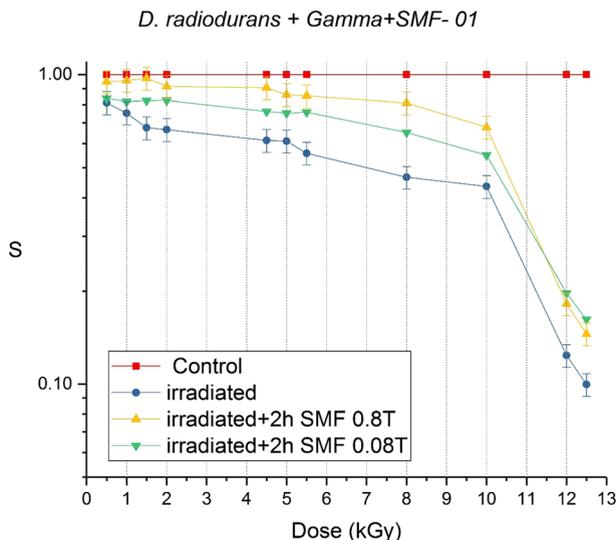


Fig. 1 Survival functions log plots (data points) of *D. radiodurans* following both exposure to gamma radiation, in the dose range from 0.5 to 12.5 kGy, and to static magnetic fields (0.08 T and 0.8 T). The lines in this and in the other figures serves only as eye guidelines

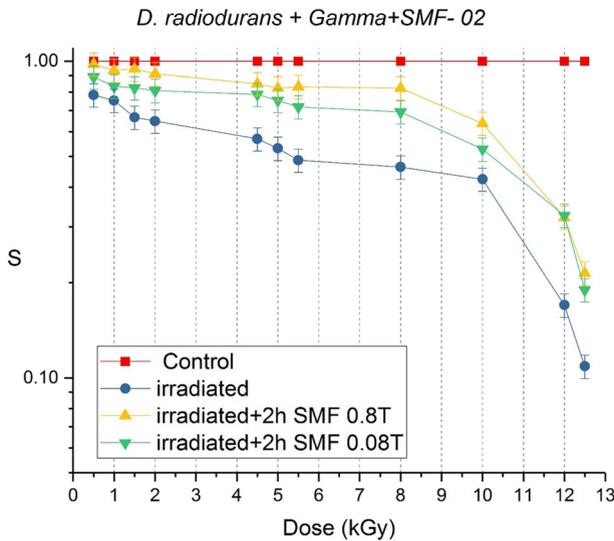


Fig. 2 The same as in Fig. 1 but for a different experimental run (details in the text)

result strongly suggests that SMF, an exogenous physical agent, interferes positively, in some sense, with the cell recuperation process. In order to both quantify and interpret this first time observed biophysical effect we consider, initially, the ratio

$$r = S(\gamma + B)/S(\gamma), B = 0.08 \text{ T or } 0.8 \text{ T} \tag{2}$$

where $S(\gamma)$ and $S(\gamma + B)$ are survival functions for exposure to gamma radiation and for exposure to gamma radiation following exposure to SMF, respectively.

Since $S(\gamma + B) > S(\gamma)$ we have that

$$S(\gamma + B) = S(\gamma) + \Delta S(B) \tag{3}$$

where $\Delta S(B)$ is the proliferation recovery and the relative proliferation recovery is here introduced as

$$R(B) = \Delta S(B)/S(\gamma) \tag{4}$$

which from now on is referred to as the cell recuperation coefficient.

3.2 Cell recuperation coefficient

Results for $R(B)$ and $S(\gamma)$, both averaged over the two primary data sets (Figs. 1 and 2), $\langle R(B) \rangle$ and $\langle S(\gamma) \rangle$ respectively, are shown in Fig. 3. This figure, together with Table 1, is intended to provide an overview of the main trend of the results, *vis-à-vis* the two SMF intensities, by means of four representative doses: 2, 5, 8, and 10 kGy, since the dose intervals 2–5 kGy and 8–10 kGy are here identified as the lowest and the highest dose regimes, respectively. However, full results for $\langle R(B) \rangle$ and $\langle S(\gamma) \rangle$ are shown in Figs. 4 and 5.

An evaluation of the statistical significance of $[S(\gamma + B) - S(\gamma)] = \Delta S(B)$, Eq. (3), the main ingredient of $R(B)$, Eq. (4), is provided by the ratio R/σ_{sd} , where σ_{sd} is the corresponding standard deviation of $R(B)$. Results for R/σ_{sd} are shown in Table 2. Except for the lowest dose

(2 kGy), such a ratio is $\geq 2x\sigma_{sd}$ up to a maximum $5x\sigma_{sd}$, indicating, thus, a confidence level $\geq 95\%$ [11].

The following features associated with $\langle R(B) \rangle$ *vis-à-vis* $\langle S(\gamma) \rangle$, and as function of doses, are rather salient:

- Except for the magnitude, the behavior of $\langle R(B) \rangle$ for the two SMF intensities is similar.
- $\langle R(B) \rangle$ increases monotonically up to 5 kGy, followed by an abrupt increase from 5 to 8 kGy.
- A maximum is reached at 8 kGy, and then for $D > 8$ kGy, an accentuated decrease of $\langle R(B) \rangle$ was observed up to a total fading way for $D > 12$ kGy.
- The cell recuperation coefficient $R(B)$, Eq. (4), does not respond linearly as a function of the magnetic field intensity B . When B is increased by one order of magnitude, from 0.08 to 0.8 T, the corresponding recuperation improves by an average of only 20%.

These *cell recovery aspects*, associated with exposure of damaged cells to SMF, are addressed below (paragraph 4.2).

4 Discussion

The material presented in this section is organized as (1) discussion of the **Results** from *this study* and from ad-hoc data of the literature; (2) **Theoretical background**, based on well-known interaction properties of macromolecules exposed to SMF; and (3) data interpretation, by intertwining **Results**, **Theoretical background**, and conjectures supported by biological concepts for *D. radiodurans* reassembly of shattered chromosomes.

As discussed by Zahradka and collaborators [4], the recuperation of *D. radiodurans* is accomplished by *homology-based reassembly* of shattered chromosomes (see also Blasius et al. [12]). In fact, *D. radiodurans* cells are grouped in pairs or in tetrads—their DNA is not

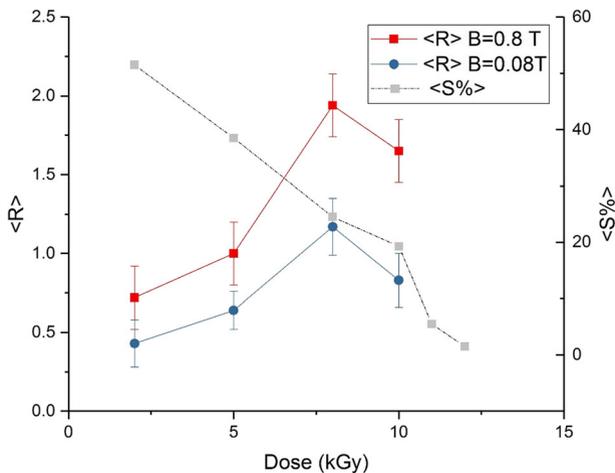


Fig. 3 The averaged cell recuperation coefficient (left-handed scale), $\langle R \rangle$, and the averaged survival function (right-handed scale), $\langle S \rangle$, of *D. radiodurans* as function of the doses and exposed to SMF (0.08 T and 0.8 T)

Table 1 $\langle S \rangle$ is the averaged survival function, and $\langle R \rangle$ is the averaged cell recuperation coefficient corresponding to SMF equal to 0.08 T and 0.8 T

B = 0.08 T		B = 0.8 T	
2–5 kGy	8–10 kGy	2–5 kGy	8–10 kGy
$\langle S \rangle 0.61 \pm 0.05$	0.45 ± 0.03	0.61 ± 0.05	0.45 ± 0.03
$\langle R \rangle 0.28 \pm 0.03$	0.35 ± 0.04	0.44 ± 0.05	0.65 ± 0.07

dispersed within the cytoplasm but, instead, keeps its toroidal shape, which facilitates its homolog recombination following, e.g., radiation damages.

The results here obtained, as expressed by the *cell recuperation coefficient*, raise the question: *how a static magnetic field would interfere in a recuperation mechanism such as the homology-based reassembly in order to render it more efficient?*

4.1 Theoretical background

4.1.1 Interactions of magnetic fields with macromolecules

Many and diverse non-specific biological responses have been observed by exposure to very weak magnetic fields (intensities up to 0.1 mT). The majority of the findings occurs through interactions of magnetic fields with the magnetic moments in rotating macromolecules. A recent scientific report, by Binhi and Prato [10], extensively and intensively addresses and discusses this issue.

The interaction energies (U) of very weak magnetic fields (\vec{B}) with molecular magnetic moments ($\vec{\mu}$), and given by $U = \vec{\mu} \cdot \vec{B}$ are, therefore, small down to the quantum level,

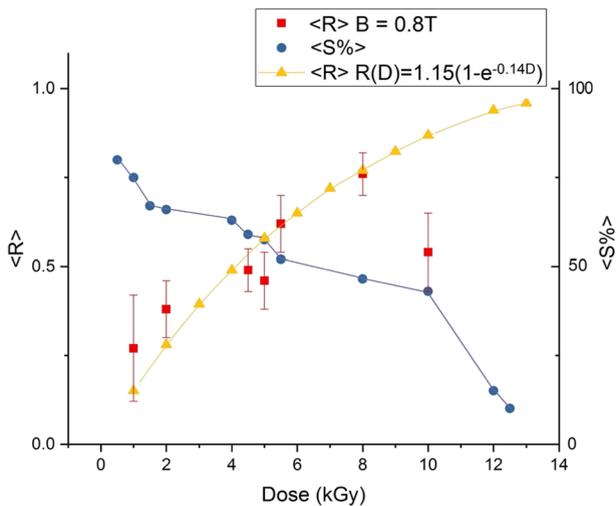


Fig. 4 *Data points:* the averaged cell recuperation coefficient (left-handed scale), $\langle R \rangle$, corresponding to a SMF intensity equal to 0.8 T. *Curves:* (a) *decreasing curve*, the averaged survival function (right-handed scale), $\langle S \% \rangle$; (b) *increasing curve*, obtained by the fitting of the statistical expression for the cell recuperation coefficient, Eq. (9), to the data points corresponding to B = 0.8 T (details in the text)

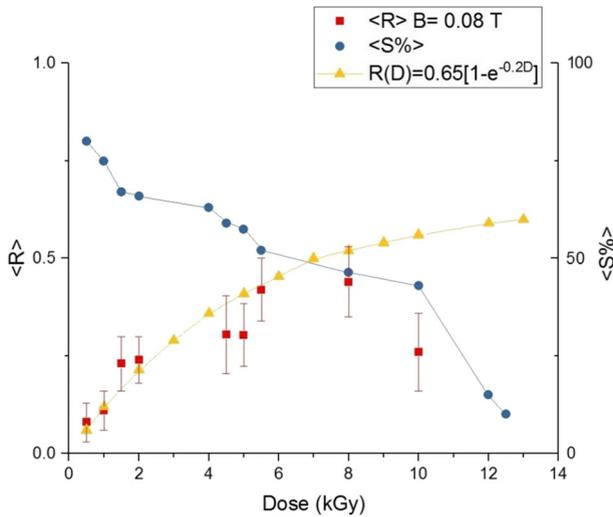


Fig. 5 The same as in Fig. 4 but for the SMF with B = 0.08 T

leading to the degeneracy of magnetic moment levels. This circumstance is at the heart of biophysics processes driven by magnetic moments (see Binhi and Prato [10] and references therein).

Magnetic field intensities in the present study, 0.08 T and 0.8 T, are up to eight orders of magnitude higher than those reported elsewhere as associated with non-specific biological responses [10]. In this case, the magnitude of the corresponding interaction energy is also orders of magnitude higher than the magnetic moment levels spacing, thus hampering any influence arising from the quantum level. On the other hand, such high-intensity magnetic fields sense bulk physical properties of macromolecules such as moving and *unbalanced charge distributions*, mostly, and electric dipole moments (in polar molecules).

4.1.2 Exposure of DNA fragments to SMF

As mentioned above, the DNA of *D. radiodurans* exposed to high doses of gamma radiation is severely shattered [4], a circumstance corroborated by experiments with DNA plasmids carried out in this laboratory [5]. The fragmentation profiles from plasmids DNA exposed to gamma radiation show that shattering starts more severely from 3 kGy on, where the most abundant sizes correspond to lengths of 50 to 100 nm (see Appendix, Fig. A, in González et al. [5]).

These small DNA fragments were produced by double-strand breaks, a process leaving the fragments with unbalanced electric charges. A SMF interacts only with moving charges, which could be manifesting themselves as (a) translational displacements or as (b) rotation loops.

For the [a]-possibility, each charge (q) experiences a magnetic force given by

$$\vec{F}_m = q \vec{v} \wedge \vec{B} \tag{5}$$

where \vec{v} is the charge velocity. As \vec{F}_m is perpendicular to \vec{v} , the resulting trajectory is a circular path, where its radius is inversely proportional to the magnitude of \vec{B} . Actually, the action of \vec{F}_m on q could be better described as taking place by a torque given by

Table 2 The ratio R/σ_{sd} as function of the dose (D), where R is the *cell recuperation coefficient* and σ_{sd} is its *standard deviation*, for the two SMF setting (see text for details)

D(kGy)	–	0.08 T	–	0.8 T
2	–	1.5	–	1.9
5	–	2.5	–	2.7
8	–	3.3	–	5.0
10	–	2.5	–	4.3

It is important to stress that R was calculated from the averaging of $\sigma(\gamma + B)$ and $\sigma(\gamma)$ corresponding to the two independent runs shown in Figs. 1 and 2

$$\vec{\tau}_a = \vec{r} \wedge \vec{F}_m \tag{6}$$

where \vec{r} is the position vector of q relatively to the center of the trajectory.

Thus, if I_{in} is the DNA fragment moment of inertia, under the action of $\vec{\tau}_a$, the fragment acquires rotational motion with angular acceleration α given by

$$\alpha = \frac{\tau_a}{I_{in}} \tag{7}$$

Also, for the (b) possibility of a rotational path results, since the magnetic field \vec{B} interacts with the magnetic moments ($\vec{\mu}$) of the charge rotation loops by means of a torque

$$\vec{\tau}_b = \vec{\mu} \wedge \vec{B} \tag{8}$$

Likewise, angular acceleration also is

$$\alpha = \frac{\tau_b}{I_{in}} \tag{9}$$

4.2 The rationale for the findings (see Section 3.2)

4.2.1 Supporting conceptual background

Considering the physical reasonings presented in paragraphs 3.1 and 3.2, exposure of *D. radiodurans* cells almost simultaneously to ionizing radiation and SMF puts into evidence the following:

- (i) Production, at the genomic level, of small and charged DNA fragments.
- (ii) Circular trajectories, as the only kind of movements induced by SMF on these DNA fragments.
- (iii) DNA fragments have relatively small moments of inertia. Their exposure to intense magnetic fields leads them to perform *small radius trajectories*.
- (iv) A jamming of many shattered DNA fragments, confined to move in *small radius trajectories*, takes place. In this case, the proximity among these fragments is considerably increased. Such a circumstance would facilitate fragments toward homology matching, which is at the heart of the cell recovery process.
- (v) Since a SMF equally illuminates all the shattered DNA fragments, the *cell recuperation coefficient* (R) should obviously be proportional to the number of shattered DNA

fragments (N_f), that is, $R \sim N_f$. However, $S \sim \frac{1}{N_f}$, also an obvious radiobiological concept, leading to $R \sim \frac{1}{S}$.

4.2.2 Cell rehabilitation and physical reasonings

Also listed in Section 3 are the most relevant features associated with $\langle R(B) \rangle$, the averaged *cell recuperation coefficient*. Below outlined is the rationale associated with all of these features in terms of physical reasonings presented in paragraphs 3.1, 3.2, and 4.2.1.

Feature (a). *Except for the magnitude, the behavior of $\langle R(B) \rangle$ for the two SMF intensities is remarkably similar.*

- (1) As discussed in 4.2.1–[v], $R \sim \frac{1}{S}$ where S , in principle, drives the shape of the curve $R(B) \times D$ (see Fig. 3).
- (2) The action of two SMF with intensities B_1 and B_2 on the DNA fragments, e.g., 0.08 T and 0.8 T, is the induction of circular movements in these fragments with radii $r(B_1)$ and $r(B_2)$, respectively.
- (3) The sole action of SMF does not change the number of fragments (these are produced in the irradiation process).
- (4) The fact that $B_1 < B_2$ implies $r(B_1) > r(B_2)$ which, according to 4.2.1–[iv], would facilitate fragments toward homology matching leading, thus, to $R(B_2) > R(B_1)$, as dramatically demonstrated by the experimental results (Figs. 1, 2, and 3).

Feature (b). *$\langle R(B) \rangle$ increases monotonically up to 5 kGy, followed by an abrupt increase from 5 to 8 kGy.*

It was pointed out in 4.2.1–[v] that $R \sim \frac{1}{S}$. Therefore, the abrupt increase of $\langle R(B) \rangle$, from 5 to 8 kGy is a consequence of an equally abrupt decrease of S in this dose range (Fig. 3).

Feature (c). *A maximum is reached at 8 kGy, and then, for $D > 8$ kGy one observed $\langle R(B) \rangle$ decreasing in accentuated fashion up to a total fading way for $D > 12$ kGy.*

- (1) Figure 3 shows that the *D. radiodurans* surviving function, averaged over the two data sets (Figs. 1 and 2), $\langle S \rangle$, shows a huge decrease in the 5–8 kGy dose interval.
- (2) For $D > 12$ kGy N_f , the number of DNA fragments produced immediately after irradiation at 12 kGy, is *residual*. Therefore, there is a rather small number of cells eligible for recovery by the SMF.

Feature (d). *The cell recuperation coefficient does not respond linearly as a function of the magnetic field intensity B . When B is increased by one order of magnitude, from 0.08 to 0.8 T, the corresponding rehabilitation improves by an average of only 20%.*

- (1) The role played by the SMF, as described and discussed above (Section 4.2.1–[iv]), is the squeezing up of the shattered DNA fragments into small and limited-size regions in the cell interior (which works as a facilitator toward homology matching).
- (2) These sizes are of the order of $r(B)$, the radius of circular movements induced by the SMF in the DNA fragments.

- (3) The higher the intensity of the SMF is, the smaller $r(B)$ is and, consequently, the higher $R(B)$ would be.
- (4) However, this is a kind of dynamics taking place in a medium with viscosity, making linear responses uncertain at short periods of time (see Appendix A).

4.3 Unraveling the *stochastic* nature of results for $R(D)$

On the basis of the random character of DNA breakage, a stochastic evaluation of the reassembly stage is proposed. The only assumption in this approach is that all fragments have approximately the same reassembly probability.

According to the setup of the present experiment, as discussed above (Materials and Methods), a SMF equally “illuminates” all the DNA fragments produced in the irradiation process. In this sense, the cell recuperation coefficient $R(D)$ (Sections 3.1 and 3.2) should be proportional to the number of shattered DNA fragments or, equivalently, to the number of radiation damaged cells, N_d . That is,

$$R(D) \sim N_d(D) \text{ or } R(D) = K \cdot N_d(D) \quad (10)$$

where K is the proportionality constant.

More specifically, K is a fractionary magnitude, between 0 and 1, expressing the *recuperation efficiency*; that is, $K = 0$ implies zero recuperation ($R = 0$), while if $K = 1$ means that all the damaged cells, N_d , were recovered ($R = N_d$), a 100% *recuperation efficiency*. K is a function, of course, of the interaction peculiarities between the *exogenous physical agent* (SMF in this case) and the *population of damaged cells*, N_d .

If N_0 is the number of cells at the beginning of the experiment (when $D = 0$), the number of surviving cells is given by the following *surviving function* (details in the Appendix B):

$$N_S(D) = N_0 \cdot e^{[-\lambda \cdot D]} \quad (11)$$

Therefore, $N_d(D) = N_0 - N_S(D)$, and substituting in Eq. (10),

$$R(D) = R_0 \cdot \left[1 - e^{(-\lambda \cdot D)} \right] \text{ with } R_0 = K \cdot N_0 \quad (12)$$

Shown in Fig. 4 is the experimental data for $R(D)$, corresponding to $B = 0.8$ T, and the phenomenological function for $R(D)$, Eq. (12), fitted to the data by means of a bi-parametric (parameters R_0 and λ) least square fitting, providing

$$R_0 = 1.15 \pm 0.12 \text{ and } \lambda = 0.14 \pm 0.02 \text{ (kGy)}^{-1} \quad (13)$$

Taking into account the uncertainties associated with the experimental data, $R(D)$, plus the number of data points (five, origin inclusively), it is reasonable concluding *cum grano salis* that the fitted function (Eq. (12)) describes the *statistical nature* of the *cell recuperation coefficient* for $B = 0.8$ T (Eq. (1)).

Likewise, with the data set measured for $B = 0.08$ T, the $R(D)$ fitted function provided (Fig. 5)

$$R_0 = 0.65 \pm 0.08 \text{ and } \lambda = 0.20 \pm 0.03 \text{ (kGy)}^{-1} \quad (14)$$

The meaning of the *constant relative probability* in the D-phase-space, as implied by Eq. (11), that is,

$$\left(\frac{1}{N_S}\right) \cdot \left(\frac{dN_S}{dD}\right) = -\lambda, \quad (15)$$

is straightforward (see Eq. (21) in Appendix B). In fact, the *probability* per dose interval unity and per unity of surviving cells *is the same at all doses*. According to the *theoretical approach* here proposed and developed, the viability increase of irradiated *D. radiodurans*, induced by exposure to SMF, is a consequence of an improvement of the DNA fragment rejoining, a process endowed with a predominant *stochastic component*.

The constant λ is a sole property of the system (samples of *D. radiodurans* cells, in this case) and of the exogenous physical agent (SMF). Also, $D_0 = \lambda^{-1}$ is the dose necessary to damage 63% of the initial cell population N_0 ; that is, $N_S(D_0) = 0.37 \cdot N_0$, while the *cell recuperation efficiency* is equal to 0.63 K. N_0 (the conceptual meaning of K was discussed above).

From the fitting results reported above, the following is obtained for D_0 :

- (a) If $B = 0.8$ T, $D_0 = \lambda^{-1} \approx 7$ kGy
- (b) If $B = 0.08$ T, $D_0 = \lambda^{-1} \approx 5$ kGy

4.3.1 Random variable and the phase space concept

The statistical nature of $R(D)$ was demonstrated in Section 4.3, but *statistical characterization* of data also requires identifying the right underlying *stochastic process*. The definition here used for the concept of a *stochastic process* is “any process in which there is a *random variable*”. Thus, one is led to the search for the random variable.

The cell recuperation process of *D. radiodurans*, here investigated, was induced by exposure to SMF which is responsible for the DNA fragments jamming in a small radius region in the cell interior. Such fragment jamming is described by the well-known *random walk* process, as a Brownian motion [13].

It is worth pointing out that in a system constituted of elements interacting by means of short-range forces, as in a random walk process, each of these elements interacts only with the elements of the system in close proximity to it. Due to the short-range character of the interaction, each element (DNA fragments in this approach) undergoes random jumps to one of its nearest-neighbor sites, where the jump lengths (ℓ) are small. The rate of interactions (collisions), ω (s^{-1}), is inversely proportional to ℓ , or $\omega \sim \ell^{-1}$. Therefore, the jump length ℓ is the *random variable*.

In terms of a more formal physical *parlance*, the *phase space* for DNA fragments is a plane containing points defined by coordinate pairs (ℓ, p) , where ℓ is the relative distance between two fragments undergoing *successful rejoining* and p represents the fragments linear momenta.

In this sense, since exposure to SMF increases cell rehabilitation (cell viability), it is said that this exogenous magnetic field is responsible for a *phase space widening*.

All this strongly indicate that the *phenomenon* here reported (damaged cells viability increasing induced by SMF) is basically of *non-deterministic physical nature*.

4.4 Comparing the role of two exogenous physical agents, SEF, and SMF, in the viability of *D. radiodurans*

As commented in the [Introduction](#), a recent publication from this laboratory showed that plasmid DNA irradiated with gammas has its DNA shattered into small fragments [5]. When *D. radiodurans* is exposed, immediately after irradiation, to a static electric field, its cell viability greatly decreased, mostly at the “repairing shoulder” [6].

In fact, diversely from a SMF, a SEF accelerates charged DNA fragments through straight line paths all along the direction of the electric field. In this case, the chances for homology matching of DNA fragments are very small, since all fragments are constrained to move along the same direction. This circumstance, of course, scrambles reassembling of small DNA fragments, leading to a less efficient repair process and, consequently, to a decrease in viability [6].

Static magnetic fields, as discussed and demonstrated in this study, induce fragments to move in circular paths, which confine movements to take place in regions delimited by the circular path radii. Such a dynamic condition increases the *phase space* for the reassembling of DNA fragments, thus contributing to an increase in viability.

5 Conclusions

1. Exposure of *D. radiodurans* cells to static magnetic fields, following high doses of irradiation, increases their viability.
2. The irradiated cellular system loses its physical and biological characteristics for doses higher than 10 kGy, where recovery by the SMF is damped and cell survival mechanisms collapse.
3. The role played by the SMF is to increase the *phase space* for reassembling of DNA shattered fragments, thus favoring an increase in viability.
4. The increase in viability of the irradiated *D. radiodurans* cells induced by SMF is a stochastic process, whose random variable is the relative separation lengths of DNA fragments.
5. The phenomenon of damaged cells viability increasing induced by SMF is of *non-deterministic physical nature*.

6 Final remarks

6.1 The role played by endogenous magnetic fields

Although this work deals with exogenous magnetic fields, it would be interesting to point out that endogenous magnetic fields have the same physical interaction characteristics verified for their exogenous counterparts. As commented elsewhere [14], one of the functions endogenous magnetic fields may have in biology involves their action in imposing radial trajectories and accelerations on charges traversing DNA and/or protein helices. Also, these endogenous fields may exert influences holistically, where torque actions may be functional in motility.

6.2 The role played by nucleoid compactness

It is worth pointing out that compact nucleoids would be fragmented by the ionizing radiation in *larger pieces*, and their *higher inertia* certainly constrains *random walk diffusion*. In fact, larger DNA pieces would undergo random jumps with lengths much smaller (see 4.3.1). Such a proximity among DNA fragments would facilitate their homolog recombination, increasing therefore the efficiency of the repair process. This may explain why many radiation-resistant bacteria possess a particularly compact nucleoid.

In the case of *D. radiodurans*, the compactness of its DNA, associated with its peculiar and complex mechanisms for both proteome radiation protection and DNA repair [1–3], is responsible for this bacterium being recognized as one of the most resistant existing organism.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Appendix A

Assuming a constant Magnetic Force $F_m(B)$ and a viscous force inside the cell proportional to the velocity, $f = \gamma v$ (γ is the viscosity coefficient), both acting on a DNA fragment with mass m , from Dynamics it gives

$$F_m - f = m \cdot a \rightarrow F_m - \gamma v = m \frac{dv}{dt}, \quad (16)$$

whose solution is

$$v(t) = \frac{F_m}{\gamma} \cdot \left\{ 1 - e^{-\frac{\gamma}{m}t} \right\}, \quad (17)$$

Therefore, the velocity of each DNA fragment would increase asymptotically up to $\frac{F_m}{\gamma}$, after exposure to SMF over a *long period of time*.

Appendix B

Be $N(D)$ the number of surviving cells after irradiation with a dose D . If the dose is changed by a differential increment dD , the number of surviving cells decreases by dN_s , that is,

$$dN_s = N_s(D + dD) - N_s(D) < 0. \quad (18)$$

Also, dN_s is directly proportional to both $N_s(D)$ and dD ; thus,

$$dN_s \propto N_s(D)dD \text{ or } dN_s = -\lambda N_s(D)dD \rightarrow \frac{dN_s}{N_s} = -\lambda dD, \quad (19)$$

and integrating Eq. (19) from $(0; N_0)$ to $(D; N_s)$ renders

$$N_s(D) = N_0 \cdot e^{-\lambda \cdot D}. \quad (20)$$

Interestingly, Eq. (20) contains an important *statistical concept* as evidenced by rewriting Eq. 19 as

$$\frac{1}{N_s} \cdot \left(\frac{dN_s}{dD} \right) = -\lambda. \quad (21)$$

We note that $\frac{dN_s}{dD}$ is the variation of N_s per unit dose interval, which is a *probability* in the phase-space. Dividing this probability by N_s gives a *relative probability*, or, a *normalized probability*, $\frac{1}{N_s} \cdot \left(\frac{dN_s}{dD} \right)$, where its absolute value is a *constant* (λ).

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Affiliations

Henriette Righi^{1,2} · João D. T. Arruda-Neto¹ · José G. C. Gomez² · Luiziana F. da Silva² · Elizabeth S. R. Somessari³ · Aline C. C. Lemos²

Henriette Righi
henrighi@gmail.com

José G. C. Gomez
jcgomez@usp.br

Luiziana F. da Silva
lukneif@usp.br

Elizabeth S. R. Somessari
esomessa@ipen.br

Aline C. C. Lemos
aline.lemos@usp.bra

¹ Physics Institute, University of Sao Paulo, Sao Paulo, Brazil

² Institute for Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil

³ Nuclear and Energy Research Institute, IPEN- University of Sao Paulo, Sao Paulo, Brazil