Observation of Hypersensitivity Phenomena in E-Coli Cells after Irradiation by Alpha Particles

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ABSTRACT

In order to study the hypersensitivity phenomena in single strand cells, E-Coli bacteria cultures were irradiated by alpha particles emitted from Am^{241} point source. The cells showed hypersensitivity at 0.05 Gy. Survival curves models were fitted to the data. The recent hypersensitivity models fitted the data much better than the old conventional models.

Keywords: E-coli, α-particles, hypersensitivity, survival models

ظهور ظاهرة فرط الحساسية في خلايا **E-coli** بعد تشعيعها بجسيمات ألفا

E-coli

. 0.05 Gy   . Am^{241}

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                                                                    – :

INTRODUCTION

Low-dose irradiation is taking a particular importance nowadays, especially because of the observation of the hypersensitivity phenomena in which the cells gains some resistance after irradiation to about 0.5 Gy, where the usual nearly exponential shape of the survival curve, represented by the survival versus dose in Gy, starts to increase at about 0.5 Gy to a higher survival as the dose increases before falling down in a usual manner. This phenomena has been observed in conventional irradiation experiments and in micro beam experiments (Marples *et al*., 1994, Schettino *et al*., 2001, Bohrnsen *et.al* 2002, Linda. 2002b, , Tsoulou *et al*., 2002, Xiao *et al*., 2006). Hence, the traditional survival curves models such as the Single Hit Single Target (SHST), and the Molecular model represented by the linear quadratic equation (LQ), both are described by equations below, are no more useful to describe the low dose data:

The SHST model is a simple exponential equation (Alpen, 1990) :

$$S = \exp(-\alpha D)$$ .... (1)

Where S is the survival, D is the dose, which has the same definition in all models and α is the damage factor.
And a quadratic term is added in the LQ equation as (Alpen, 1990):

$$S = \exp(-\alpha D - \beta D^2)$$ .... (2)

Where α and β are the fitting parameters

The LQ model has been modified by (Joiner and Johns, 1988) to be known as the Inducible Repair model (IR) which is assumed to be applicable to survival curves which show hypersensitivity phenomena as well as those which do not show it, The equation that describe the IR model is given by (Dasu and Denekamp, 2000):

$$S = \exp\{-\alpha_R[1 + (IRR - 1)e^{-D/D_c}]D - \beta D^2\}$$ .... (3)

Where IRR = α_S / α_R
α_S is the steep initial slope at very low doses to describe the hypersensitivity region. It changes exponentially as the damage inflicted leads to the induction of more efficient repair, and transformed to a more resistant, shallower slope of the shoulder region after repair has fully induced (characterized by α in the LQ classical model, and now called α_R) . The quadratic β term takes over at progressively higher doses.
D_C is the dose at which 63 %(i.e. 1-1/e) of the transition has occurred.
The IR model is so successful in describing survival curves in the low-dose region, however, it describes the high dose region poorly, and a new statistical model, the Repairable Conditionally Repairable model (RCR) was predicted by Linda Persson, in which the survival is given by(Linda, 2002a):

$$S = e^{-aD} + bDe^{-cD}$$ .... (4)

a, b and, c are fitting parameters
This model is able to describe survival curves at low and high dose reasonably well.
In this study, single strand E-Coli bacteria were irradiated by alpha particles emitted from a point source in order to see if they show a hypersensitivity to radiation. Very low doses were used for irradiation (<0.1 Gy), the survival curve obtained has a shape similar to those which show hypersensitivity in mammalian cells but the inflection point occurs at 0.05 Gy instead of 0.5 Gy as in mammalian cells.

Method

E-coli bacteria were isolated from human colon and cultured for 24 hours at 37 ^{o}C in Agar-Agar medium. Diluted suspensions were prepared using Nutren-Broth, and a point Am^{241} source which emits alpha particles of energy 5.485 MeV was used for irradiation (Al-Taii , 2006)The source was placed at 2 cm distance from the Petri dish. The dose rate at the dish surface was calculated according to the relation (Tait, 1980):

$$D(Gy/sec) = \Phi_o \times 1.602 \times 10^{-10}(dT/\rho dX) \quad \dots\dots\dots\dots \quad (5)$$

Where Φ_o is the flux in units of particles/cm^2.sec
ρ is the air density and equals to 0.00129 g/ cm^3
dT/dX is the linear energy transfer in KeV/μm units
The energy E_α at 2-cm distance from the source was calculated using the energy range relationship(Mahesh and Mustafa, 1976):

$$E_\alpha^{(x)} = E_\alpha^{(0)}\left(1 - \frac{x}{R}\right)^{\frac{2}{3}} \quad \dots\dots\dots\dots\dots\dots\dots \quad (6)$$

$E_\alpha^{(x)}$ is the initial alpha particle energy
$E_\alpha^{(0)}$ is the energy after traveling a distance x
R is the range which is given by

$$R = (0.005 + 0.285)E^{3/2} \quad \dots\dots\dots\dots\dots \quad (7)$$

The range was found to be 4.007 cm at which $E_\alpha^{(x)} = 3.45 MeV$

This value of $E_\alpha^{(x)}$ was used in SRIM2003 program (Ziegler, 2003) to calculate dT/dX which is found to be 97.7 KeV/μm
To calculate Φ_o, assume that N is the number of particles emitted over a 4Π solid angle per second. This simply represents the activity of the source. Hence, the number of particles at 2 cm distance from the source in all directions will be

$$\Phi_o = N/4\pi r^2 \quad \dots\dots\dots\dots\dots\dots\dots\dots.. \quad (8)$$

An ^{241}Am source of activity 1 μCi was used in this experiment, This gives $\Phi_O = 763.5$ Particles/cm^2.sec
Substituting the values of Φ_O and dT/dX in equation (5), we can calculate the dose rate at 2-cm distance.
Assuming 10% loses due to backscatter , geometrical arrangements and efficiency of the measurements. The dose rate was found to be 1*10^{-4} Gy/sec.
After irradiation, carried out in medium at room temperature, the samples were washed, diluted with fresh medium and visible colonies were scored for survival evaluation using the method of point counting where each dark point represents a colony (see figure 1) . In every experiment two independent samples were irradiated for a given dose with a control Petri dish for each.

Control dish (unirradiated)
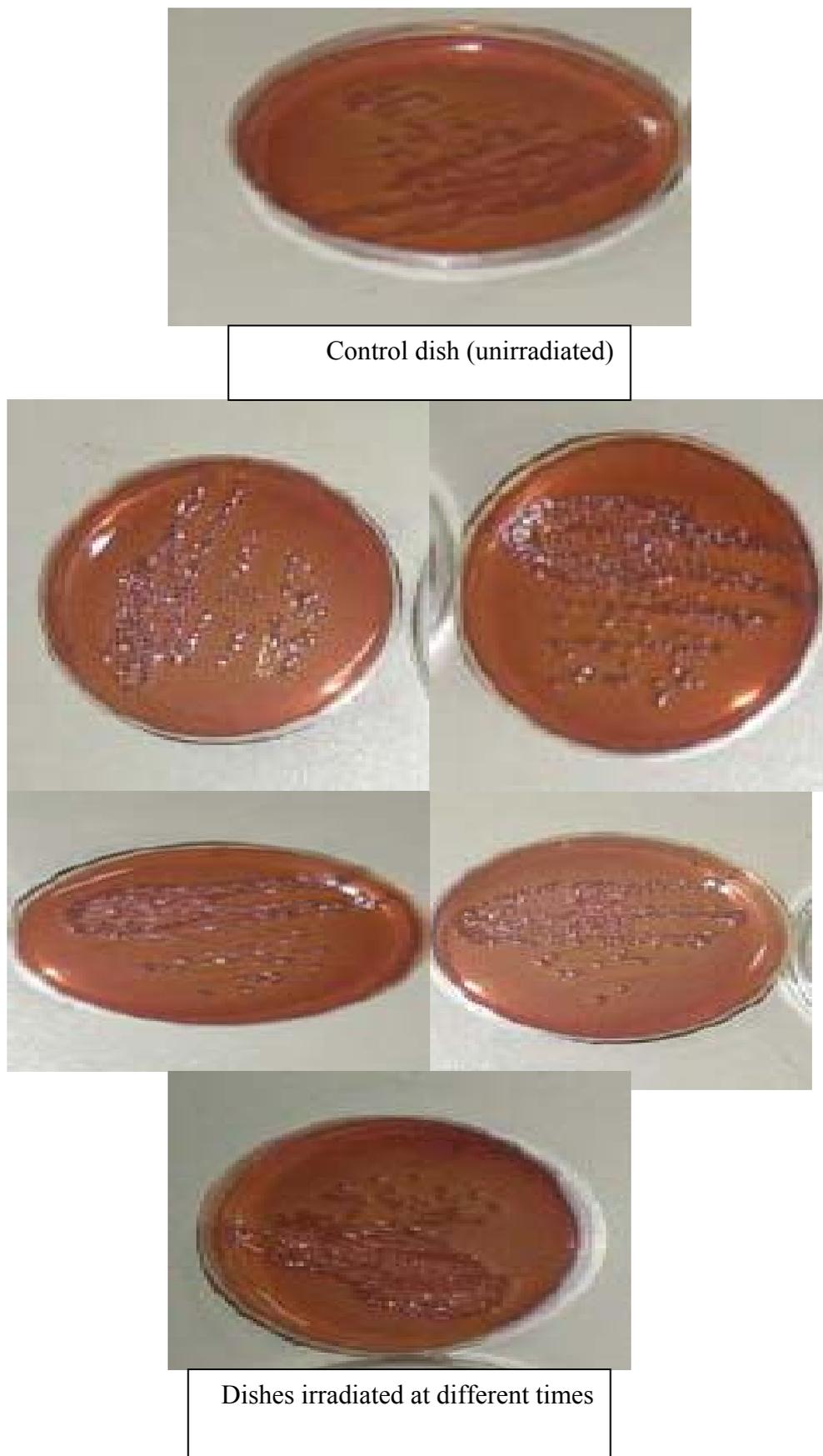
Dishes irradiated at different times

Fig. 1: Control and irradiated Petri dishes. The dark points indicate colony formation

RESULTS AND DISCUSSION

The survival versus dose for irradiation of E-Coli cells by 3.45 MeV alpha particles is shown in figure (2). It is clear that, the curve does not take the usual semi exponential shape of the survival curve. The survival falls down to 0.15 at 0.05 Gy and starts to increase to about 0.25 before it starts to decrease again slowly. This implies that the cells have gained some resistance against radiation similar to what have been seen in mammalian cells hypersensitivity which occurs at about 0.5 Gy.

To test for hypersensitivity phenomena we used four survival curves models; These are: the SHST model and the LQ model, which both are used to describe data of usual survival curves which show no peculiar structure, represented by equation 1 and 2 respectively, and the IR model and the RCR model which were predicted to describe the hypersensitivity phenomena and represented by equations 3 and 4 respectively,

The fit of these models to the data is shown in figure (3). To test the goodness of fit, four statistical quantities have been examined; These are ; the Sum of Squares due to Error (SSE) which should be close to zero, the R-square (RSQ) value which should be close to 1 or it is better to use the Adjusted R-square (ADJ) value which takes into account the number of degrees of freedom (ν) where $\nu = n - m$, n is the number of fitted points and m is the number of fitted parameters, also the Root Mean square Error (RMSE) should have a value close to zero (see Mat-Lab help for details). As clearly seen from figure 3, the SHST model and the LQ model do not fit the data in the 0.05 Gy region,. and as seen from table 1, the statistical quantities that describe the goodness of fit for these models are far from acceptable, although we have tried our best to improve them. We have learned from our experience with Matlab fitting programs that when SSE is greater than 0.1 or ADJ is less than 0.9 the fit is bad. However, the IR model and the RCR model describe the shape of the curve much better , where SSE for these two models is less than 0.1 and the ADJ, although it is not very close to 1, but a value of about 0.9 is acceptable. This means a hypersensitivity phenomena is present in single strand cells as E-Coli bacteria and not restricted to double stranded cells as it was thought before.

It is difficult to compare our results with previous data since almost all the hypersensitivity measurements have been carried out on mammalian cells (i.e. double stranded) while E-coli bacteria cells are simple single stranded cells. But, in general, the damage factor which describe the cell kill at the initial slope of the survival curve is about the same for a given type of radiation at a given energy. This factor is represented by α in the SHST model and the LQ model and by α_R in the IR model and as seen from table 1 it has similar values in these models.
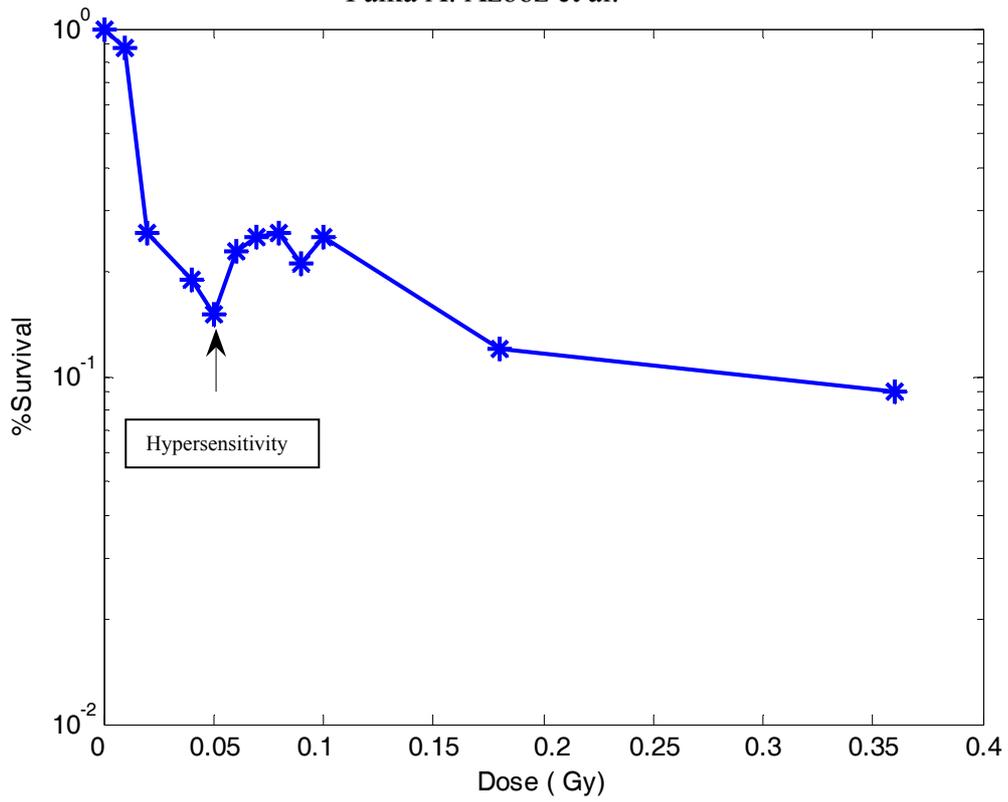
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Fig. 2: Experimental data points for the survival fraction of E-coli cells as a
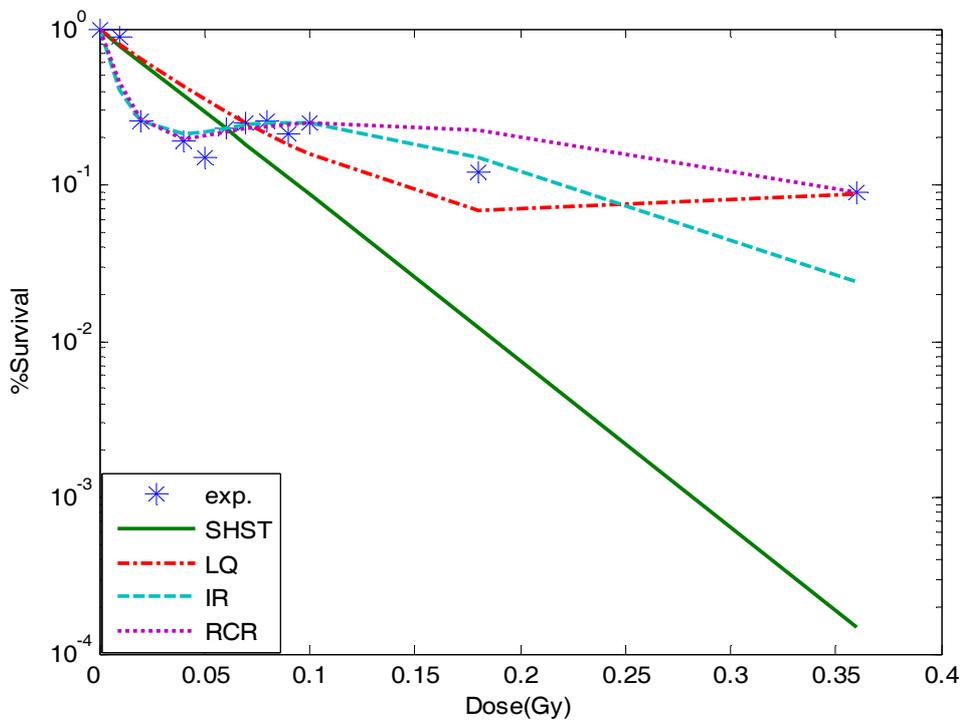function of Dose

Fig.3: The survival fraction of E-coli cells as a function of
dose Fitted to SHST, LQ, IR, and RCR equations.

Table 1: Biophysical and Statistical parameters obtained from the fit.

			E =3.45 MeV		LET=97.7 (KeV/μm)	
			SSE	RSQ	ADJ	RMSE
SHST	α	2.45 (+0.92, -0.92)	0.124	0.765	0.733	0.0538
LQ	α	2.24 (+0.87, -0.87)	0.077	0.797	0.782	0.0752
	β	-0.4523 (+0.098, -0.098)				
RCR	a	9.205 (+2.87,-3.2)	0.039	0.9502	0.9392	0.0725
	b	0.58 (+0.115,- 0.21)				
	c	0.8855 (+0.258,- 0.198)				
IR	$α_R$	2.39 (+0.78,-0.69)	0.076	0.91	0.905	0.082
	$α_S$	3.79 (fixed at bound)				
	β	0.0 (fixed at bound)				
	D_c	0.033 (fixed at bound)				

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