Zebrafish embryos for studying radiation response *in vivo*

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INTRODUCTION

Embryos of the zebrafish, *Danio rerio*, are explored as a feasible model for studying radiation response *in vivo*. In recent years, *Danio rerio* has become a preferred model for studying human disease, including carcinogenesis. The greatest advantage is that the human and zebrafish genomes share considerable homology, including conservation of most DNA repair-related genes [1]. Rapid embryonic development is another advantage in that major organ systems become evident within 24 hours post fertilization (hpf). Here, the feasibility is studied through the response of these embryos to low dose of alpha particles.

MATERIALS AND METHODS

The embryos were dechorionated and transferred into a custom-made holder with a 16 μm polyallyldiglycol carbonate (PADC) film as the base. Alpha-particle irradiation was made with a planar ^{241}Am source from the side of the PADC film at 1.25 hpf (see Fig. 1). At this developmental stage, the cells had not assumed differentiated cell fates. The irradiation time was chosen as 30, 60 and 90s, and the average delivered doses to an embryo were calculated as 0.7, 1.4 and 2.1 mGy, respectively.

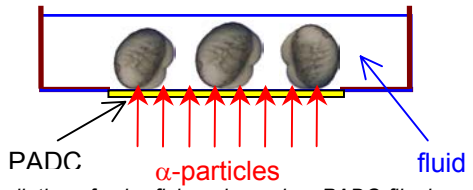

Fig. 1. Irradiation of zebrafish embryos in a PADC-film based holder.

After irradiation, the embryos were returned to the 37°C incubator until they developed into 24 hpf, which was our chosen endpoint for analyses of apoptosis, a highly regulated biological process during embryonic development. At 24 hpf, the embryos were collected and examined by vital dye staining using acridine orange [2]. Images of stained embryos were captured under a florescent microscope, and the number of apoptotic cells for each embryo was counted.

RESULTS AND DISCUSSION

Table 1. The mean difference (± SE) in the number of apoptotic cells obtained in embryos irradiated for different time and in the controls. The p values for comparisons between data for a particular irradiation time with the control are determined using t-test.

	30 s	60 s	90 s
Mean±SE	-28.0±7.8	-26.6±10.6	-2.7±12.1
Sample size	26	24	24
p	0.0197	0.0416	0.435

The difference in the number of apoptotic cells obtained in embryos irradiated for different time and those in the corresponding controls are shown in Table 1 and Fig. 1. The results show a nonlinear dose-risk relationship and a reduction of risk at low doses.

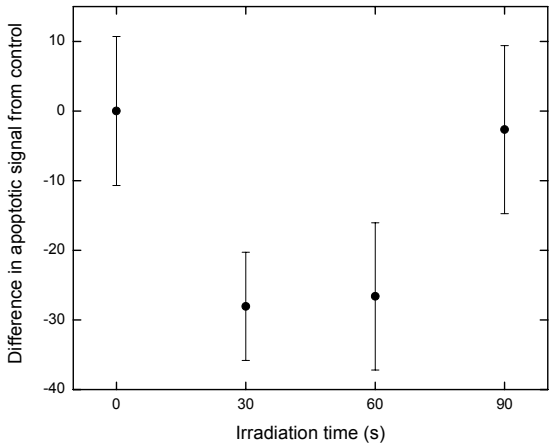

Fig. 2. The mean difference (± SE) in the number of apoptotic cells obtained in embryos irradiated for different time and in the controls.

The results demonstrate that zebrafish embryo is a feasible model for studying radiation response *in vivo*. Nevertheless, due to the random nature of alpha-particle emission, for the low doses used here, the numbers of alpha-particles traversing each cell and thus the resulting doses could vary to a large extent. This will be more serious if we explore the region for even smaller doses. Although it can be argued that bystander effects can distribute the effects so that all cells respond to the same extent, a microbeam facility will help avoid ambiguities caused by non-uniform doses, and help confirm such bystander effects *in vivo*.

CONCLUSION

Our results of a nonlinear dose-risk relationship and a reduction of risk at low doses do not support the "Linear No Threshold" hypothesis. These also demonstrate that the zebrafish embryo is a feasible model for studying radiation response *in vivo*. A microbeam facility will help avoid ambiguities caused by non-uniform doses, and also confirm bystander effects *in vivo*.

REFERENCES

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