Measurements of pO_2 in Zebrafish Embryos

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Abstract

Zebrafish embryos (*Danio rerio*) are common model organisms for *in vivo* studies and are used more and more in the radiobiology community. The *in vivo* and *in vitro* measurement of pO_2 is an important method to characterize the role of hypoxia during irradiation. Previous research showed that a low pO_2 level in the medium around zebrafish embryo during irradiation resulted in a more pronounced radioprotective FLASH effect than at higher pO_2. To link the measurements in the medium with biological response, we present here a possible way to measure the pO_2 inside and outside of the chorion of zebrafish embryos as well as data and practical insides on the upsides and limitations of our measurement system.

Intrachorional pO_2 measurements in zebrafish embryos had to be established. Zebrafish embryos (wildtype AB) in the 1-cell stadium were injected with the phosphorescent probe Oxyphor PtG4 directly into the yolk sack. Different concentrations and injection volumes of the Oxyphor probe were tested out to see if the probe had an influence on the development of the embryos. The embryos were observed over a time period of 5 days and the survival as well as their bodylength was compared to non-injected control zebrafish embryos to evaluate the toxicity of the Oxyphor. After the suitable concentrations and volume for low Oxyphor toxicity were found, the intrachorional pO_2 of the zebrafish embryos was measured using the Oxyled system.

Our typical sample setup consisted of 30 zebrafish embryos (age 24 hours post-fertilization) enclosed in a 500 µl Eppendorf tube filled with 200 µl agarose, and ~300 µl of E3 medium. For this setting, we tested different compositions of injected and non-injected zebrafish embryos to identify the necessary number of embryos for stable measurements. Moreover, an optimized setup was built according to the requirements for irradiation experiments at the ELBE electron accelerator. The extrachorional pO_2 was measured by the Oxylite system.

The optimal concentrations and injection volumes for Oxyphor to get enough healthy zebrafish embryos as well as a good signal-to-noise ratio were 20 µM and 1 nl. Compared to other compositions, the usage of 30 injected zebrafish embryos with 20 µM oxyphor and 1 nl injection volume showed the least amount of variance and will be used in future. Both systems (Oxylite and Oxyled) showed similar extrachorional pO_2 values, whereas the intrachorional pO_2 measurements were noisier than the extrachorional measurements.

It is possible to measure pO_2 inside and outside of the zebrafish embryo chorion. This allows to further correlate radiation induced hypoxia with other biological endpoints during and after irradiation. The possibility to use this system during irradiation at the ELBE accelerator will be evaluated soon. The results of this study will be presented.