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A Fast, Simple, and Affordable Technique to Measure Oxygen Consumption in Living Zebrafish Embryos

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Abstract

In all animal species, oxygen consumption is a key process that is partially impaired in a large number of pathological situations and thus provides informative details on the physiopathology of the disease. In this study, we describe a simple and affordable method to precisely measure oxygen consumption in living zebrafish larvae using a spectrofluorometer and the MitoXpress Xtra Oxygen Consumption Assay. In addition, we used zebrafish larvae treated with mitochondrial respiratory chain inhibitors, antimycin A or rotenone, to verify that our method enables precise and reliable measurements of oxygen consumption.

Keywords: respiration, zebrafish larvae, oxygen consumption

Introduction

Respiration is a key cellular function, which is altered in a great number of disorders and animal disease models, thus providing a meaningful window to interrogate the physiology of living cells and organisms.[1] Automated devices allowing precise measurement of both mitochondrial respiration (oxidative phosphorylation) and glycolysis have been developed in recent years, but their price and cost of usage restrain their use on a daily basis. To overcome this limitation, we describe here a simple and affordable method that allows precise measurement of oxygen consumption in living zebrafish larvae, using a simple spectrofluorometer and the MitoXpress Xtra Oxygen Consumption Assay (Agilent), a quantitative test relying on a fluorescent probe, which is quenched in a dose-dependent manner by O_2 molecules (Fig. 1A).

Results

To assess the ability of this method to detect fine changes in O_2 consumption in living zebrafish larvae, we measured O_2 consumption of pools of seven 5 days post-fertilization wild-type individuals treated with either vehicle (1% dimethyl

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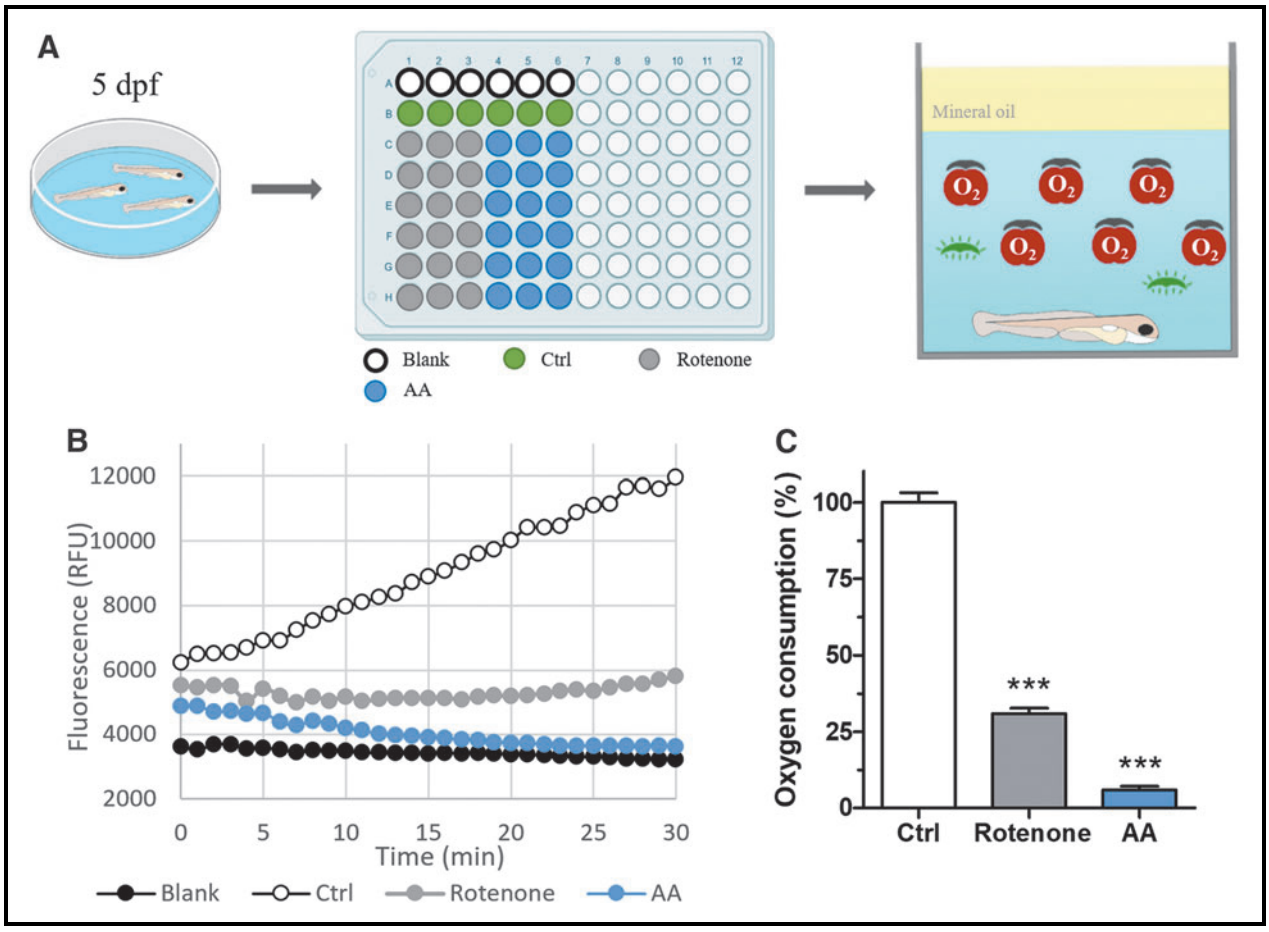

FIG. 1. Real-time measurement of oxygen consumption in 5 dpf zebrafish larvae. **(A)** Scheme of the experimental setup used to measure oxygen consumption *in vivo* in zebrafish larvae, treated or not with MRC inhibitors, with an example of a filled 96-well microplate. Pools of seven 5 dpf larvae treated with MRC inhibitors or untreated (control) were transferred to a 96-well microplate (Greiner Bio-One International) and then incubated in the presence of the MitoXpress Xtra probe, (Supplementary Data) a fluorescent molecule whose fluorescence is quenched in a dose-dependent manner by O_2 through direct molecular interactions, thus making fluorescence intensity inversely proportional to the amount of free O_2 dissolved in the medium. **(B)** Real-time measurement of the oxygen consumption in larvae exposed to MRC inhibitors or untreated, and blank (E3 medium without larva). **(C)** Quantification of the oxygen consumption of larvae exposed to either 2 μM rotenone or 10 μM AA. Whiskers represent the standard error of the mean. Rotenone ($n=36$), AA ($n=35$), control ($n=18$), and blank ($n=24$), one-way ANOVA with Tukey's Multiple Comparison Test: ***$p<0.0001$. AA, antimycin A; ANOVA, analysis of variance; dpf, days post-fertilization; MRC, mitochondrial respiratory chain.

sulfoxide [DMSO]), 2 μM rotenone, a specific inhibitor of the mitochondrial respiratory chain (MRC) complex I,[2] or 10 μM antimycin A (AA), a specific inhibitor of the MRC complex III (Fig. 1A).[3,4] As previously reported, we found that 1% DMSO concentration had no effect on larval development.[5]

Results showed that oxygen consumption of larvae exposed to 2 μM rotenone and 10 μM AA was 31% and 6%, respectively, of that observed in non-treated controls (Fig. 1B, C, Supplementary Data), indicating that the method described allows reliable measurements of oxygen consumption. Thus, this simple and direct method can be used to measure respiration *in vivo* in zebrafish intoxication and disease models.

Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Data

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