

Custom-built prototype experimental set-up to investigate light-induced damage in retina of zebrafish animal models

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Abstract

Custom-built experimental set-ups have served the purpose before advanced machines were put into use in almost all laboratories across the globe. Prototype designs of machines in every walk of science have a chance to evolve and become the best of their kind in design and function. Zebrafish have nearly 70% of their genes coding for human protein, including disease-causing genes, and this has made them an important cost-effective animal model. Research involving exposure to varied intensities and spectral frequencies of light utilizes zebrafish animal models and these studies need an experimental set-up that could help researchers study retinal damage in these animals. Thus the idea of devising an experimental set-up that could help to explore light-exposure-induced retinal damage in zebrafish was born. Commonly available low-cost materials like polyvinyl chloride (PVC) pipes, light-emitting diode (LED) panels from bulbs, glass aquarium tanks and insulated wires were used to build the experimental set-up. Lux meters and UV meters were used to determine the spectral frequency or intensity of choice for the experiment intended. Our custom-built device will suit any researcher interested in studying the effect of high-intensity visible light or UV rays on zebrafish. The set-up is easy to build and consumes very little electricity. Replicability of the model is high and requires less space, time and money. Our custom-built experimental set-up is being reported at this time of increasing research with zebrafish animal models in India which will be very useful for aspiring young researchers.

Keywords: experimental tool, retinal pigment epithelium, zebrafish

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Introduction

Custom-built experimental set-ups have served the purpose before advanced machines were developed and came into use in almost all laboratories across the globe. Prototype designs of machines in every walk of science have a chance to evolve and become the best of their kind in design and function. Zebrafish have nearly 70% of their genes coding for human protein, including disease-causing genes, and

this has made them an important cost-effective animal model in scientific research.

Our knowledge on the role of genetics in human disease has led to the development of various promising zebrafish animal models.¹ Apart from the genetic disease models, it is possible to develop and standardize research methods using zebrafish as an animal model for many other diseases that humans develop. Introduction of custom-built research tools for the scientific community will produce an impact

on the research output from developing and poor countries.

Due to the non-availability of a suitable experimental set-up to study the effect of intensity and frequency of light on the retina of zebrafish animal models, we devised and developed one for our use. The model that we developed could also be used to study the effect of high intensity light-induced retinal damage or the effect of UV light exposure on the retina of zebrafish or even behavioral studies on zebrafish models upon exposure to anxiety, stress etc. Keeping in mind the extent of research opportunities that could be taken up and the easy replicability of our experimental prototype, we decided to publish our prototype model. Our objective was to design a custom-built experimental set-up that could help in studying high intensity light-exposure-induced retinal damage in zebrafish.

Materials and Methods

This study was done with the permission of the Head of the Department. The following low cost materials sourced from the local aquarium and hardware shops were used: (i) One glass tank of size 12"X 6" X 6" (ii) PVC pipes - 2m length having 1" diameter (iii) LED bulbs - four (7watts/20K lux) (iv) Lux meter (v) Aerator (vi) Insulated wires (vii) Lab thermometer (viii) pH meter (ix) Insulation tape.

Procedure

1. Measure and cut the PVC pipes to form a framework that rests over the glass tank as show in Figure 1.
2. Make circuits for the LED bulbs in a parallel connection from a single AC power source.
3. Use flexible plastic material that eases the bending of the LED panel to increase or decrease the distance between it and the water tank to get the desired lux inside the glass tank (Figure 2).
4. Use lux meter to ensure uniform intensity of light in all areas of the glass tank (water filled, with the luxmeter covered in polythene bag).
5. Zebrafish can be grouped and exposed to the desired intensity of light for a fixed period of study (15 days to a month).

6. The effect of exposure to selected high intensity light exposure can be explored by studying the histopathological changes in retinal pigment epithelium of the eye or, to be more accurate, using qt PCR exploration on the damaged genes.

Figure 1: The cut PVC pipes forming the framework for the set up

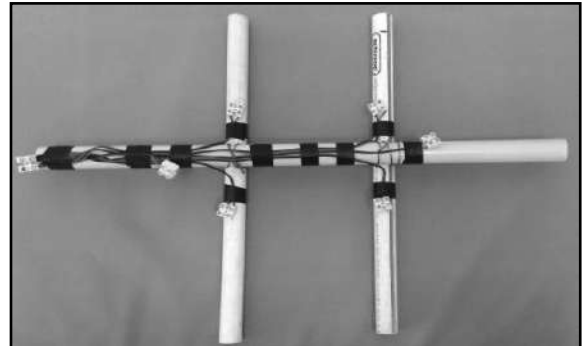
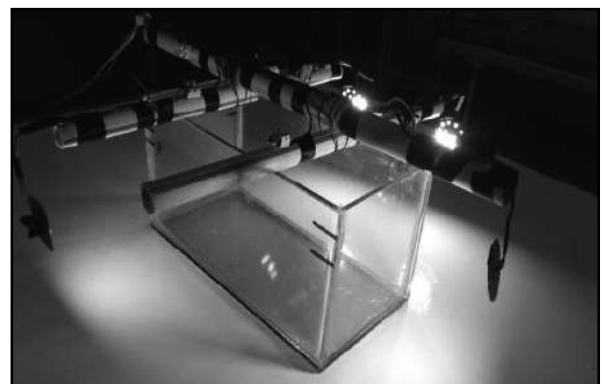


Figure 2: Custom- built experimental set- up to investigate light-induced retinal damage in zebrafish



Guidelines to be followed while using this set-up

1. Proper insulation to be ensured to prevent electric shock. Unauthorized handling of the apparatus to be strictly avoided.
2. Study group of fish to be kept in a dark room and exposed to the artificial light source at particular time intervals taking care not to

alter the normal light/dark cycle of 10 hours light and 14 hours dark.

3. Number of fish in the experimental group must be larger than in the control group as there could be some casualties as happens with any other animal experiments.
4. Since the exposure is manually operated, holidays will be a factor of consideration for continuity of the study period exposure as the zebrafish has an unusual ability of regenerating its damaged retina.
5. Number of fish to be used for the study could be decided based on the quantity of tissue required from the eyes for qt PCR sampling.

Discussion

This experimental set-up utilizes low cost and available materials in the local market and also can be replicated by anyone with a little knowledge of electrical circuits. Our design is sturdy enough to be utilized for research studies with different objectives as well. Researchers conducting studies using this experimental set-up could save time and cost. It can be adopted by individual researchers who could begin their research in zebrafish animal models with a small investment. This set-up can be a tool to explore areas such as controlled light exposure on retina, controlled circadian rhythms against hormones and blood parameters and the effect of increased environmental temperature on behavioural changes in zebrafish.

Light-induced retinal degeneration is a commonly used technique to explore the effect of different intensities and frequencies of light on the retina.² Photochemical damage is the most common form of retinal damage caused by exposure to direct sunlight and several artificial light sources, including ophthalmic instruments.³ Rod photoreceptor loss in zebrafish has been documented in studies done earlier. UV light-induced damage can be studied by assessing the extent of the damage produced using proteomics and real time PCR methods.² Studies using this experimental set-up will save time and cost.

Prototype models in medical science laboratories are not new, however there is a need to have steady progress in improving the features of the prototypes

in terms of affordability, ease of use, ergonomics, safety and effectiveness.⁴

We stress that researchers who intend to conduct research with this experimental set-up at their academic or research setting use it with adequate precautionary measures towards the hazards of electricity as the model is a prototype and it needs to undergo standardization and quality checks.

Limitations: This is a prototype that we have designed. We have plans to revise, validate and standardize the model set-up. This is a pilot test set-up and is being employed by us on another research work studying the effect of controlled high intensity light-induced damage on the retinal pigment epithelium of zebrafish animal models.

Conclusion

Our experimental set-up is easy to make and will be useful for any basic science researcher who wishes to explore the ill effects of high intensity light on retinal pigment epithelium using zebrafish as an animal model.

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Conflicts of Interest: Nil

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