REGENERATION ASSAY

INTRODUCTION

Biobide is a biotechnology company offering drug discovery services to Pharma, Biotech, Chemical and Cosmetic companies. Our services are based on the zebrafish model and the capacity to offer highly efficient tailor made assays.

The zebrafish model is gaining relevance in pre-clinical trials due to its small size, transparency, ease to manipulate and rapid development. This model has a high genetic homology with humans (over 85%) as well as important parallels in organogenesis and functional mechanisms.

Due the high regenerative capacity of the model, the zebrafish is selected to evaluate whether its regenerative potential upon new specific active ingredient or mixes could reach an increase in its regenerative capacity. Zebrafish can regenerate several organs such as the tail fin, heart, central nervous system, and photoreceptors. This property makes it an interesting tool to evaluate the effect of test items, after injury.
In fact, it has been tested the regenerative potential of nut oil using zebrafish caudal fin injury (1).

In our assay, zebrafish is used to evaluate whether the regenerative capacity of zebrafish until 5dpf is increased in presence of test compounds.

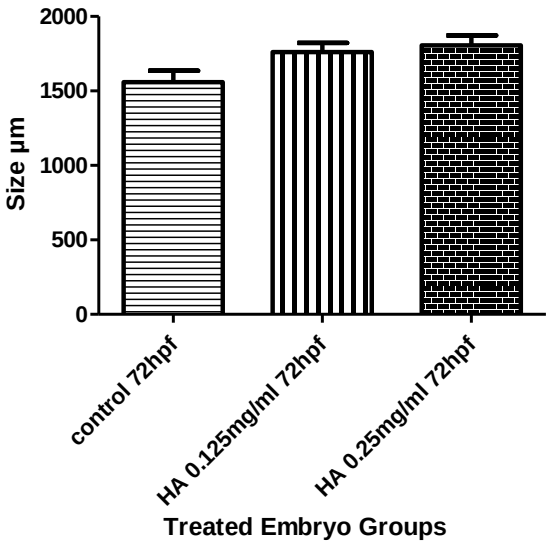

Figure 1. tail regeneration from 10 independent embryos from 3 groups (control treated with vehicle, and Hyaluronic acid 0,125 and 0,250 mg/ml) after 24 hours post injury.

METHOD DESCRIPTION

The assay consists on the following steps:

Treatment: 24 hpf embryos are dechorionated and treated with the compound object of study, at the desired concentration. Depending on the previous knowledge and the number of products to test, different assays are proposed:

- Concentration-response curves: an initial curve of six concentrations is proposed: 0.01, 0.1, 1, 10, 30 and 100 µM. For IC50 calculation, a second appropriate curve will be done if necessary.
- Screening: only one concentration per compound will be tested.
- Assays opened to client interest.

Incubation time: The item to be tested is incubated for a total period of 96 hours.

Data collection: After 24 hours of treatment, 48hpf embryos are placed in agarose plates, individual embryos are visualized under the microscope and pictures are taken.
Tail surgery is performed upon 48hpf embryos sedated, and pictures are taken after that, to register the injury.

Pictures time points: 48hpf before surgery (BS), 48hpf after surgery (AS), 72hpf, 96 hpf, 120 hpf.

Analysis: Image analysis is done individually:

Images are analyzed using Axiovision 4.8 software
tail measurement is manually perform in each time point and the measurement is compared with its control time point.

[1] Rojo, 2010. J. Cosmet Dermatol. 9(3), 185-195