

University of Idaho

Animal Care and Use Committee

Standard Operating Procedure (SOP)

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Title Intraocular injection

Species Goldfish

Purpose

The adult goldfish retina contains proliferative cells that are capable of ongoing neurogenesis, and regenerative activities in response to injury. This makes the goldfish eye an ideal location for the study of retinal development and retinal regeneration, in a highly accessible situation. One method for labeling proliferative cell populations is the intraocular injection of the thymidine analog bromodeoxyuridine (BrdU). BrdU is taken up by proliferative cells and incorporated into DNA synthesized during S-phase. It is antigenically distinct and can be visualized later in harvested tissues by a simple immunocytochemical procedure. However, the availability of BrdU to proliferative cells following injection is not known, and recently, alternative methods for BrdU exposure have been established that allow precise knowledge of duration of exposure for the purpose of gaining quantitative data on cell proliferation.

Potential Impact on Animal Subjects

At the concentrations used, there should be no major health risks. However, these experiments will always result in the sacrifice of the animal and collection of tissues for experimental evaluation. Unhealthy animals may be at risk of failing to thrive during the exposure to BrdU.

Description

Goldfish are removed from their home aquaria and transported to the laboratory in shoebox-sized containers with system water. In the laboratory, the water is aerated by portable pumps and aeration stones while the microinjection station is prepared.

All materials that may come into contact with the fish, including the injection needle, forceps, and scalpels, are sterilized with alcohol.

Solutions to be injected are prepared in concentrations that, based on geometry of the eye, a 2-5 ul injection will be calculated to result in the desired intraocular concentration. Solutions for injection are made in sterile 0.9 % saline. These are drawn up into a blunt-end Hamilton syringe, then a small quantity is plunged out to ensure the absence of air bubbles. A blunt-end, flexible needle is used to decrease the likelihood of accidental human injection. Potentially hazardous materials are clearly marked as such, and laboratory personnel are provided with complete information regarding the hazard. Concentrated stock solutions are similarly labeled, and stored in marked containers at appropriate temperatures.

The stage of the stereomicroscope is cleaned with alcohol, then a paper towel, soaked in system water, is placed on the stage. This wet paper towel will prevent loss of the fish protective mucus coat. The fish is submerged in a solution of freshly-made MS-222 anesthetic, usually 0.2% (but less if the fish is smaller than 5 cm from tip to tail). When operculum movement ceases, and when the fish no longer displays a startle response, the fish is placed on the wet paper towel on the stereomicroscope stage.

A small incision is made in the cornea, near the limbus, using a microsurgical scalpel. The injection syringe is then guided by a micromanipulator into the incision, and to the side of the lens. Care must be taken not to strike the retina with the needle as this may cause a proliferative response that will make interpretation of the experiment difficult. The desired volume of solution is slowly injected, then the needle kept in place for a few seconds to prevent backwash. The needle is slowly withdrawn, then the fish is returned to aerated system water for recovery.

If the fish is not breathing and swimming within a few minutes, reviving can be facilitated by washing the mouth and gills with a gentle stream of saline, to remove anaesthetic from the gills and simulate breathing movements. Generally the fish revives within 3-4 minutes, but is kept in the shoebox container for an additional thirty minutes to ensure that it has completely recovered. Then fish are returned to home aquaria, clearly labeled for experimental condition.

References

- Julian D, Ennis K and Korenbrot JI (1998) Birth and fate of proliferative cells in the inner nuclear layer of mature fish retina. *J. Comp. Neurol.* 394:271-282.
- Otteson D and Hitchcock PF. The rod photoreceptor lineage in adult goldfish retina. Submitted to *J. Comp. Neurol.*