Video Article

Retro-orbital Injection in Adult Zebrafish

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Abstract

Drug treatment of whole animals is an essential tool in any model system for pharmacological and chemical genetic studies. Intravenous (IV) injection is often the most effective and noninvasive form of delivery of an agent of interest. In the zebrafish (*Danio rerio*), IV injection of drugs has long been a challenge because of the small vessel diameter. This has also proved a significant hurdle for the injection of cells during hematopoeitic stem cell transplantation. Historically, injections into the bloodstream were done directly through the heart. However, this intra-cardiac procedure has a very high mortality rate as the heart is often punctured during injection leaving the fish prone to infection, massive blood loss or fatal organ damage. Drawing on our experience with the mouse, we have developed a new injection procedure in the zebrafish in which the injection site is behind the eye and into the retro-orbital venous sinus. This retro-orbital (RO) injection technique has been successfully employed in both the injection of drugs in the adult fish as well as transplantation of whole kidney marrow cells. RO injection has a much lower mortality rate than traditional intra-cardiac injection. Fish that are injected retro-orbitally tend to bleed less following injection and are at a much lower risk of injury to a major organ like the heart. Further, when performed properly, injected cells and/or drugs quickly enter the bloodstream allowing compounds to exert their effect on the whole fish and kidney cells to easily home to their niche. Thus, this new injection technique minimizes mortality while allowing efficient delivery of material into the bloodstream of adult fish. Here we exemplify this technique by retro-orbital injection of Tg(*globin*:GFP) cells into adult *casper* fish as well as injection of a red fluorescent dye (dextran, Texas Red) into adult *casper* fish. We then visualize successful injections by whole animal fluorescence microscopy.

Protocol

Part 1. Preparation of injection material

1. Tg(*globin*:GFP) cells:
2. Bleed adult donor fish using 10ul pipette tip coated in heparin (1unit/ul) to puncture fish behind gill.
3. Aspirate red blood cells and dispense in cell buffer (0.9X PBS + 5% FBS + 1% Pen/Strep).
4. Filter cell suspension over 40uM mesh and determine concentration of cells using hemocytometer as previously described by LeBlanc et al.
5. Spin down and resuspend in cell buffer at desired concentration such that final injection volume is not more than 5ul. Here we inject 1.5-2 million cells/recipient.
6. For the dextran, Texas Red®, dissolve the dye in DPBS for a final injection concentration of 10-12 mg/mL. Plan to inject 4 uL per fish.

Part 2. Injection

1. Anesthetize fish in tricaine (4.2ml (4mg/ml) tricaine/100ml fish water).
2. Wash Hamilton syringe 3-4 times prior to injection using 70% ethanol. Rinse 3-4 times with 0.9X DPBS.
3. Place fish dorsal side up and facing right on damp sponge.
4. Hold the Hamilton syringe with your right hand and with your index finger on the plunger. Gently stabilize the body of the fish with your left hand.
5. Position the needle with the bevel facing up such that if the fish s eye were a clock, the needle would be pointing at the 7:00 position and at a 45 degree angle to the fish (figure 1).
6. Gently insert needle 1-2 mm into 7:00 position and slowly depress the plunger.
7. Allow the fish to recover in fresh E3 water.
8. Wash needle, as described above, between injections of different reagents.
9. Flick cell solution to resuspend cells every few minutes to prevent cell clumping.
10. Keep fish off flow for 1 week with daily water changes to avoid infection. We keep fish in ICU water for this period (10mL Stress coat + 5mL pemafix + 5mL melafix per 38 liters E3 water).

Part 3. Representative Results

When performed correctly, it is possible to visualize injected material if it has been labeled in some way. For example, Tg(*globin*:GFP) red blood cells should be seen under a fluorescent dissection microscope circulating in the vasculature of recipient *casper* fish soon after injection as shown in figure 2. Likewise, injection of a 70kDa dextran, conjugated to Texas Red® can be visualized in the vasculature of transparent fish immediately following retro-orbital injection (figure 3). Fluorescent kidney cells from Tg(*β-actin*:GFP) donor fish can also be injected retro-orbitally into irradiated *casper* recipient fish. These cells will eventually home to the recipient marrow (figure 4a) and may then go on to repopulate the kidney after several weeks (figure 4b).

If the injection is too shallow or the angle of the needle bypasses the retro-orbital venous sinus cavity, injected material may be visualized pooling around the eye or flowing out of it. Alternatively, if the injection is too deep, fish may experience tissue damage or excessive bleeding, though they may still recover. When performed correctly, mortality after retro-orbital injection should be less than 5% of total injected fish and success of delivery to the bloodstream should be greater than 90% of total injected fish.

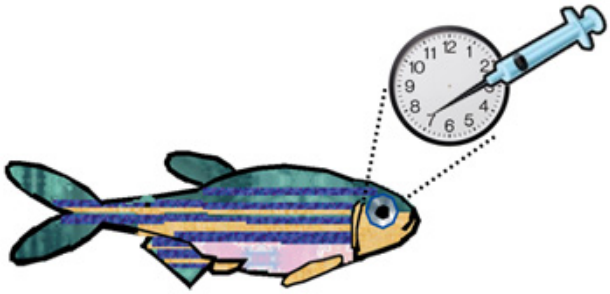

Figure 1: Illustration of retro-orbital injection technique. The right eye of the fish is represented as an analogue clock in which the seven o clock position corresponds to the correct injection spot.

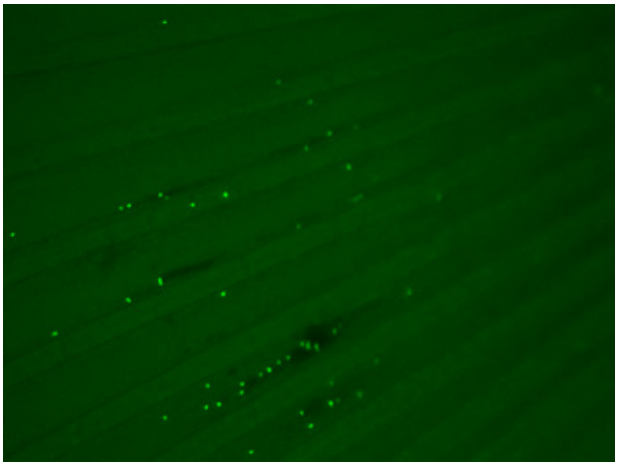

Figure 2: Tg(*globin:*GFP) red blood cells circulating in the vasculature of the tail of an adult *casper* fish three days after retro-orbital injection.

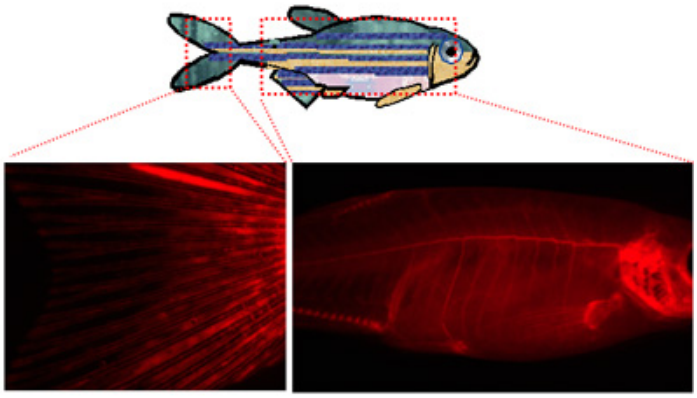

Figure 3: Successful injection of a 70kDa dextran conjugated to Texas Red® used as dye produces fluorescent vasculature in the transparent adult fish, *casper* and is readily viewed using standard whole animal fluorescence microscopy.

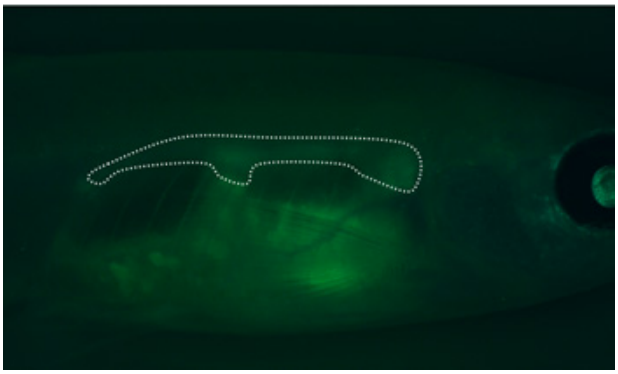

Figure 4a: Three days following retro-orbital injection of whole kidney marrow cells from Tg(*β-actin*:GFP) donor fish, the cells can be visualized homing to the kidney marrow of irradiated adult *casper* recipient fish.

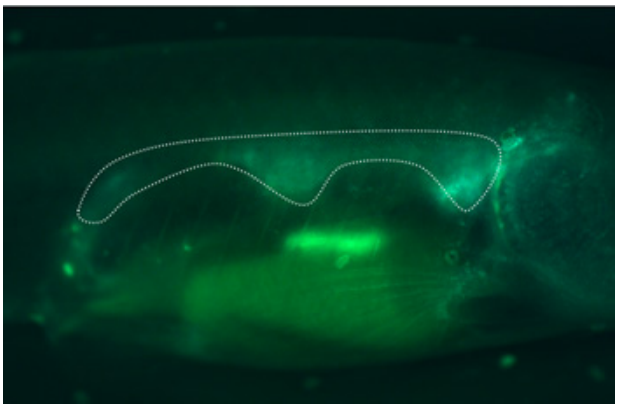

Figure 4b: Four weeks following retro-orbital injection of whole kidney marrow cells from Tg(*β-actin*:GFP) donor fish, the repopulation of the recipient kidney with green donor whole kidney marrow cells can be visualized in irradiated adult *casper* recipient fish.

Discussion

Retro-orbital injection in adult zebrafish gives the highest efficiency of delivery of injected material to the bloodstream with the lowest incidence of mortality. Because of the nature of the injection spot, the puncture appears to heal quickly, decreasing the occurrence of infection and amount of blood loss. The site may even be repeatedly injected during a short time for daily injections of drugs, for example.

Large quantities of drugs must be purchased for traditional treatment methods in adult fish in which fish are simply immersed and soaked in water containing the drug of interest. However, if the drug is injected directly into the bloodstream of the animal, a much smaller quantity is required for treatment, thereby decreasing experimental costs. For certain compounds that are too large to enter the fish circulation through soaking, in the case of certain peptides, retro-orbital injection may also be advantageous.

Further, since RO injection is faster and easier than intracardiac injection techniques in the adult fish, another application of RO injection may include adult chemical genetic screening. Thus, retro-orbital injection may greatly facilitate zebrafish research requiring chemical or chemical delivery.

Acknowledgements

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References

1. Steel, C.D., Stephens, A.L., Hahto, S.M., Singletary, S.J., Ciavarra, R.P. Comparison of the lateral tail vein and the retro-orbital venous sinus as route of intravenous drug delivery in a transgenic mouse model. *Lab Animal*. 37, 26-32 (2008).
2. Pinkerton, W., Webber, M. A method of injecting small laboratory animals by the ophthalmic plexus route. *Proc. Soc. Exp. Bio. Med.* 116, 959-961, (1964).
3. White, R.M., Sessa, A.S., Burke, C., Bowman, T., LeBlanc, J., Ceol, C., Bourque, C., Dovey, M., Goessling, W., Burns, C.E., Zon, L.I. Transparent adult zebrafish as a tool for in vivo transplantation analysis. *Cell Stem Cell.* 2, 183-189, (2008).
4. LeBlanc, J., Bowman, T.V., Zon, L.I. Transplantation of whole kidney marrow in adult zebrafish. *J. Vis. Exp.* 2, 159, (2007).

