Video Article

Transplantation of Whole Kidney Marrow in Adult Zebrafish

Jocelyn LeBlanc[1], Teresa Venezia Bowman[1], Leonard Zon[1]

[1]Children's Hospital, Harvard Stem Cell Institute, Harvard Medical School

Correspondence to: Jocelyn LeBlanc at jleblanc@enders.tch.harvard.edu, Leonard Zon at zon@enders.tch.harvard.edu

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Abstract

Hematopoietic stem cells (HSC) are a rare population of pluripotent cells that maintain all the differentiated blood lineages throughout the life of an organism. The functional definition of a HSC is a transplanted cell that has the ability to reconstitute all the blood lineages of an irradiated recipient long term. This designation was established by decades of seminal work in mammalian systems. Using hematopoietic cell transplantation (HCT) and reverse genetic manipulations in the mouse, the underlying regulatory factors of HSC biology are beginning to be unveiled, but are still largely under-explored. Recently, the zebrafish has emerged as a powerful genetic model to study vertebrate hematopoiesis. Establishing HCT in zebrafish will allow scientists to utilize the large-scale genetic and chemical screening methodologies available in zebrafish to reveal novel mechanisms underlying HSC regulation. In this article, we demonstrate a method to perform HCT in adult zebrafish. We show the dissection and preparation of zebrafish whole kidney marrow, the site of adult hematopoiesis in the zebrafish, and the introduction of these donor cells into the circulation of irradiated recipient fish via intracardiac injection. Additionally, we describe the post-transplant care of fish in an "ICU" to increase their long-term health. In general, gentle care of the fish before, during, and after the transplant is critical to increase the number of fish that will survive more than one month following the procedure, which is essential for assessment of long term (<3 month) engraftment. The experimental data used to establish this protocol will be published elsewhere. The establishment of this protocol will allow for the merger of large-scale zebrafish genetics and transplant biology.

Video Link

The video component of this article can be found at https://www.jove.com/video/159/

Protocol

Irradiation

1. Irradiate WT fish at 25Gy 2 days before transplantation

Kidney dissection

1. Sacrifice appropriate number of fluorescent donors in tricaine (~100ml 4mg/ml Tricaine / liter of fish water) (average of 500,000 cells per donor fish)
2. Isolate kidney into 0.9X PBS + 5%FCS + 1%Penn strep ("PBS+") in tube
3. Break up with 1ml pipet, then with 10ml syringe with 18 1/2 G needle
4. Transfer into 50mL conical and fill up with PBS+
5. Spin down @ 1500rpm 8min
6. Pour out, resuspend pellet in large volume and filter through 40micron filter
7. Spin down @ 1500rpm 8min
8. Pour out, resuspend in 1ml PBS+, transfer to eppendorf tube
9. To count: 10ul cell sol + 90ul trypan blue (now it is diluted 10X)
10. 10ul cell sol + 90ul PBS+ (now it is diluted 10X)
11. Let stand 5 min, add 10ul both sol to either side of hemacytometer
12. Count cells in large box (5x5), account for death percentage if significant
13. Cells/ml = (cell count)(10^4) (x10 dil. factor)
    Example - 60 cells counted on hemacytometer is 6,000,000 cells total
14. Spin down sample @ 1500rpm 8min
15. Calculate volume to resuspend pellet in to get appropriate concentration of cells
16. 5ul total volume to inject - 3ul being WKM and 2ul being peripheral blood
17. Example - have 6,000,000 cells total
    want to transplant 200,000 cells per recipient
    have 20 recipients
    want 66,667 cells/ul (200,000cells / 3ul)

resuspend pellet in 90ul (6,000,000cells / 66,667cells/ul)
only need about 60ul of that (20recip x 3ul)

Peripheral blood extraction

1. Sacrifice appropriate number of WT fish in tricaine (~100ml 4mg/ml Tricaine / liter of fish water) for carrier blood (average of 2,000,000 cells per donor)
2. Anticoagulant: dilute 1ul (3unit/ul) in 1mL PBS+
3. Coat pipet tip with heparin, stab through gill with pipet tip, add blood to PBS+
4. Spin 1500rpm 5min, resuspend in 1.5mL
5. Spin 1500rpm 5min, resuspend in 1mL
6. Filter through 40micron filter
7. Count cells as above
8. Cells/ml = (cell count)(10^4)(x10 dil. factor)
9. Account for death
10. Calculate resuspension volume as above

Injection

1. Inject 5ul into heart of each recipient using a Hamilton syringe
2. Anesthetize fish in tricaine (4.2ml (4mg/ml) Tricaine/ 100ml fish water) and place on damp sponge
3. Insert tip a little behind heart
4. Poke around heart area until you see a small burst of blood (meaning you hit the heart) -> Might not see this but inject anyway

Fish ICU - post-injection

1. Keep fish off flow for 1 week to avoid infection
2. Put 5-10 fish per cage
3. Remove dead and keep record
4. Manually change water each day