Proceedings of ICBioMed 2025 Symposium: Computational Modelling and Simulation for Biology and Medicine
DOI: 10.54254/2753-8818/2025.LD31507

The Influence of High-Pressure Environment on Zebra Fish Heart Regeneration

Xiyao Hu[1], Ziheng Li[2*]

[1]*School of Hefei No.1 Senior High School and Canada British Columbia International School, Hefei, China*
[2]*Nanjing Jinling High School Cambridge A-level Center, Nanjing, China*
**Corresponding Author. Email: zl9814400@gmail.com*

Abstract. This work, compared different oxygen ratios under different water pressures' influence on the heart regeneration of zebrafish. Zebrafish have strong ability of regenerate of the heart. Oxygen plays a really important role in cell repair. And changes in pressure will influence the oxygen content in the water. The solubility of oxygen increases under high pressure, and the solubility of oxygen drops under low pressure. Studying the effect of pressure and oxygen can provide the perspective for understanding how the external factors influence the regeneration. That can inspire the treatment of human diseases and provide strategies for human heart repair. For example, we can control the concentration of oxygen to regulate the regeneration of human's heart. In the experiment, we use the adult zebrafish to establish a hypoxia reoxygenation model to harm the heart of the zebrafish and put them under different pressures and oxygen ratios. We use the dissolved oxygen probe to monitor the oxygen levels and TUNEL assay to check the apoptosis. Then we use pHH3 and PCNA staining to check the proliferation of the heart. The expected results are the high pressure may promote regeneration by activating mechanical sensitive pathway like YAP. Or it may cause cellular apoptosis and delay the regeneration. These can help us optimize the effect of hyperbaric oxygen on wound healing and help divers or people living in high-altitude areas treat heart injuries.

Keywords: Zebrafish, heart regeneration, hydrostatic pressure, TUNNEL assay, hypoxia-reoxygenation (H/R) model

1. Introduction

Subsequent to fibrin clot formation, the zebrafish myocardium avoids the pronounced collagen accumulation and fibrotic scarring characteristic of mammalian cardiac wound healing, thereby maintaining tissue plasticity throughout the regenerative process. Conversely, resident cardiomyocytes undergo undergo active proliferation to regenerate damaged myocardial architecture through compensatory cellular repopulation mechanisms [1]. The proliferation in cardiomyocytes reaches its peak at 14 days post resection. At the 60-day postoperative milestone, near-complete restitution of physiological contractility that approximates baseline hemodynamic performance [2]. Researches in zebrafish have not supported stem cells as the source of regenerating myocardium [3].

DOI: 10.54254/2753-8818/2025.LD31507

Lineage-tracing via Cre-recombinase technology reveals that mature cardiomyocytes disassemble sarcomeric ensembles and undergo phenotypic regression to developmentally primitive states, initiating a ontogenetic recapitulation sequence wherein proliferative reentry precedes structural maturation, followed by cell division and maturation that recapitulates the developmental program [4].

Research about zebra fish under high-pressure water [5] finds that zebrafish died at 12.5 MPa exposure (close to the pressure at 1 250 m underwater), while identifying 18 Mpa (1800-meter depth equivalence) as the maximal survivable threshold under sustained compression. Based on the transcriptome data [6], the activity levels of genes showed major differences in the experimental groups compared to controls. However, these genetic changes were not uniform-each type of tissue displayed its own unique pattern of which genes were affected. Among the four tissues in zebrafish, brain, ovary, eyes and molecules investigated, the ovary demonstrated the greatest resistance to pressure changes, whereas the brain exhibited the highest vulnerability to elevated hydrostatic pressure conditions. These datasets establish critical benchmarks for forecasting the migration of fish to the deep sea and for comprehending the deep-sea fish's adaptive mechanisms.

Water pressure serves as the primary factor controlling how deep marine creatures can live. For every 10 meters deeper in the ocean, the pressure grows by approximately one atmosphere (equal to 0.1 megapascals). Thus, the deep seas pose significantly challenges for both human exploration and marine fish habitation [7]. Microbial studies reveal that high water pressure makes cell membranes less flexible and forces cells to produce more protein-repair molecules, ultimately impairing normal cell functions [8]. Furthermore, high water pressure makes hydrogen bonds stronger, disrupting key genetic processes like DNA copying and protein production [9]. However, deep-sea species have developed special adaptations to overcome these challenges [10], but the adaptability of shallow-water fish is still remains unclear.

At the cellular level, HHP alters cytoskeletal dynamics, induces nuclear deformation, and disrupts extracellular matrix (ECM) remodeling, potentially impairing mechanotransduction pathways such as YAP/TAZ signaling [11]. Concurrently, HHP induces metabolic and redox perturbations, including mitochondrial dysfunction, ROS overproduction, and activation of the Nrf2/Keap1 antioxidant pathway [12]. These stressors may intersect with cardiac regeneration processes by dysregulating inflammation resolution, angiogenesis, and oxidative stress responses, ultimately compromising myocardial repair. So, this research expect that under some certain-pressure conditions, the heart regeneration of zebrafish will be inhibited. However, under certain pressures, it may promote the proliferation of heart cells.

2. Materials and methods

2.1. Zebrafish Hypoxia-Reoxygenation (H/R) model

To establish a hypoxia-reoxygenation (H/R) model in adult zebrafish to simulate ischemia-reperfusion injury in mammals and observe cardiomyocyte damage and regenerative capacity [13].

2.2. Experimental materials

In Figure 1, the patterns of some of the experimental materials are showed.

1) Adult zebrafish (Danio rerio), aged 6–12 months [14]
2) Deionized water and a controlled aquarium system (maintained at 28°C)
3) Medical-grade oxygen and nitrogen gas cylinders (for oxygen level adjustment) [15]

DOI: 10.54254/2753-8818/2025.LD31507

4) Dissolved oxygen (DO) probe or oxygen meter (to monitor oxygen levels) [16]
Immunostaining reagents, including antibodies for:
5) TUNEL assay (to detect apoptosis) [17]
6) pHH3 (phospho-Histone H3, for mitosis detection) [18]
7) PCNA (Proliferating Cell Nuclear Antigen, for cell proliferation) [19]
8) Masson's trichrome staining kit (to assess fibrosis and collagen deposition) [20]
9) High-resolution cardiac imaging system (for functional analysis like FAC) [21]
10) Cryostat and microscopy equipment
11) PreSenS
12) Tissue processing materials, such as fixatives, embedding media, slides, and coverslips
13) Argon

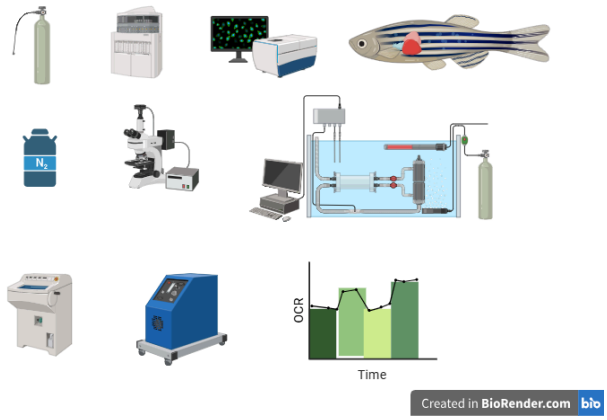

Figure 1. Illustrations of some experimental materials

3. Experimental groups

Heart-damaged zebrafish
Heart-undamaged zebrafish

4. Procedure

4.1. Hypoxia induction

Acclimate zebrafish in 5 L tanks (28°C). This temperature is suitable for the growth and reproduction of zebrafish.

Reduce O_2 to 0.4 mg/L (5% of normoxia) by bubbling 95% Ar/5% CO_2 for 15 min. This can simulate severe ischemia and induce damage to cardiac muscle cells.

Using argon to prevent nitrogen from interfering with dissolved oxygen measurement and ensure stability of the anoxic environment.

Monitor pH (7.5 ± 0.1) and O_2 levels in real-time to prevent acidosis.

4.2. Reoxygenation

Transfer fish to normoxic water (6.5 mg/L O_2). Simulated blood flow reperfusion, triggering inflammation or oxidative inflammation.

DOI: 10.54254/2753-8818/2025.LD31507

Observe recovery for 10 min (stunned behavior resolution). Make sure the fish wakes up from its coma.

4.3. Sample collection

Sample at these key time points:

2 hours Detection of early markers of oxidative stress.

6 hours Peak inflammatory response

18 hours Peak apoptosis (TUNEL assay)

3- and 7-days Proliferation peak (pHH3, PCNA staining)

30 days Assessment of functional recovery and fibrosis (Masson stain)

4.4. Immunohistochemistry (IHC) sample preparation

Sample Collection: Use proper surgical or biopsy tools to obtain tissue specimens.

Formaldehyde Fixation: Immerse Sample (less than 5 mm^3) in 4% paraformaldehyde (PFA) solution for 4 - 6 hours at room temperature.

PBS Rinsing: After fixation, wash the samples with phosphate - buffered saline (PBS) multiple times (usually 3 times, 5 - 10 minutes each). This step removes excess fixative, reducing the risk of non - specific reactions in later steps.

Freezing-OCT Compound Embedding: Place the fixed sample in a cryomold and pour optimal cutting temperature (OCT) compound over it to completely cover the sample. Ensure the sample is properly positioned in the mold.

Storage: Store the frozen tissue blocks at - 80°C until ready for sectioning. Label the blocks clearly with relevant information such as sample ID, date of freezing, and the type of tissue.

Sectioning with Cryostat: Pre - cooling the Cryostat: Set the cryostat temperature to an appropriate level (usually around - 20°C to - 25°C, but it may vary depending on the tissue type). Pre - cool the cryostat for at least 30 minutes before starting sectioning.

Mounting the Block: Mount the frozen tissue block onto the cryostat chuck using OCT compound. Make sure the block is firmly attached and properly oriented.

Sectioning: Cut the tissue into thin sections, typically 5 - 10 μm thick. Use a sharp blade and adjust the sectioning speed according to the tissue characteristics. Carefully transfer the sections onto pre - cleaned, adhesive - coated glass slides.

Antigen Retrieval (if necessary): Unmask the antigenic sites that may be masked during fixation or freezing.

Heat - induced Antigen Retrieval: Boil the tissue sections in a citrate buffer (pH 6.0) or EDTA buffer (pH 8.0) using a pressure cooker, microwave, or water bath for 10 - 20 minutes. Then, let the sections cool down slowly in the buffer.

Antibody Staining: Dilute the antibody according to the manufacturer's instructions or based on prior optimization experiments. Apply the diluted primary antibody to the tissue sections and incubate at 4°C overnight or at room temperature for 1 - 2 hours, depending on the antibody and the target antigen.

4.5. Immunohistochemistry (IHC) imaging

Sample Mounting: Cover the sample sections with a coverslip using a mounting medium. Make sure the coverslip is properly aligned and there is no air bubbles trapped under it. Excess mounting

DOI: 10.54254/2753-8818/2025.LD31507

medium can be removed using a paper towel.

Microscopy: Take photographs of the stained tissue sections using a microscope.

5. Results and discussions (expected)

5.1. Zebrafish Hypoxia-Reoxygenation (H/R) model

High Pressure Promotes Regeneration: The myocardial regeneration area and proliferation activity in the moderate high-pressure (2 atm) group were significantly higher than those in the normal pressure group. Explanation: High pressure may promote the proliferation of cardiac progenitor cells by activating mechanical-sensitive pathways (such as YAP/TAZ).

High-pressure Inhibition of Regeneration: In the high-pressure (3 atm) group, scar formation increased and regeneration was delayed. Explanation: Excessively high pressure may cause cellular stress or apoptosis, thereby inhibiting regeneration.

No significant effect: There was no significant difference in the regeneration efficiency between the high-pressure group and the normal-pressure group. Explanation: Zebrafish heart regeneration is not sensitive to pressure changes, or the pressure range has not reached the threshold.

5.2. Immunohistochemistry (IHC) experiment

Proliferation Dynamics: pHH3+ cardiomyocytes clustered near injury border (3d peak).

Co-localization of YAP/TAZ nuclear translocation with proliferation zones.

Fibrosis vs. Regeneration: Inverse correlation between collagen deposition (Masson's trichrome) and PCNA+ cells.

Oxidative Stress Markers: Nitrotyrosine signal in H/R vs. controls (2h), resolving by 24h.

Interpretation: Implicates YAP/TAZ mechanotransduction in pressure-dependent regeneration.

Suggests ROS scavengers (e.g., NAC) may mitigate H/R damage.

6. Discussion

This research can provide a theoretical basis for cardiac repair under high-pressure conditions and may inspire new regenerative medicine strategies, such as promoting myocardial regeneration by regulating mechanical force signals. Simulate the impact of diving or pressure injury on the repair of the human heart.

Evolutionary Perspective: Deep-sea zebrafish adaptations vs. laboratory strains.

Translational Relevance: Simulating diving/barotrauma effects on human cardiac repair.

7. Conclusion

The adult zebrafish heart, after transient hypoxia and reoxygenation, exhibits acute oxidative stress, cell death, and inflammation, but shows full functional recovery without fibrosis. This model provides a powerful system to study scar-free cardiac regeneration.

References

[1] Kikuchi K, Poss KD. Cardiac regenerative capacity and mechanisms. Annu Rev Cell Dev Biol. 2012; 28: 719-41. doi: 10.1146/annurev-cellbio-101011-155739. PMID: 23057748; PMCID: PMC3586268.

[2] Elhelaly WM, Lam NT, Hamza M, Xia S, Sadek HA. Redox Regulation of Heart Regeneration: An Evolutionary Tradeoff. Front Cell Dev Biol. 2016 Dec 15; 4: 137. doi: 10.3389/fcell.2016.00137. PMID: 28018900; PMCID:

DOI: 10.54254/2753-8818/2025.LD31507

PMC5157008.
[3] Holdway JE, Werdich AA, Anderson RM, Fang Y, Egnaczyk GF, Evans T, Macrae CA, Stainier DY, Poss KD. Primary contribution to zebrafish heart regeneration by gata4(+) cardiomyocytes. Nature. 2010 Mar 25; 464(7288): 601-5. doi: 10.1038/nature08804. PMID: 20336144; PMCID: PMC3040215.
[4] Kalivas J. Comment on: Kikuchi et al. (Int J Dermatol 2010; 49: 346-348). Int J Dermatol. 2011 Jan; 50(1): 121; author reply 121-2. doi: 10.1111/j.1365-4632.2010.04687.x. PMID: 21182515.
[5] Lee MC, Choi BS, Kim MS, Yoon DS, Park JC, Kim S, Lee JS. An improved genome assembly and annotation of the Antarctic copepod Tigriopus kingsejongensis and comparison of fatty acid metabolism between T. kingsejongensis and the temperate copepod T. japonicus. Comp Biochem Physiol Part D Genomics Proteomics. 2020 Sep; 35: 100703. doi: 10.1016/j.cbd.2020.100703. Epub 2020 Jun 11. PMID: 32563028.
[6] Comparative Biochemistry and Physiology Part D: Genomics and Proteomicsgene, 2020
[7] 1997Chen, J. W., & Zhang, H. B. (2017). A research progress of physiological effects of marine animals by hydrostatic pressure and temperature. Marine Bulletin, 36(6), 601-610.
[8] 2019Réhault S, Brillard-Bourdet M, Bourgeois L, Frenette G, Juliano L, Gauthier F, Moreau T. Design of new and sensitive fluorogenic substrates for human kallikrein hK3 (prostate-specific antigen) derived from semenogelin sequences. Biochim Biophys Acta. 2002 Apr 1; 1596(1): 55-62. doi: 10.1016/s0167-4838(02)00204-2. PMID: 11983421.
[9] Yayanos AA, Pollard EC. A study of the effects of hydrostatic pressure on macromolecular synthesis in Escherichia coli. Biophys J. 1969 Dec; 9(12): 1464-82. doi: 10.1016/S0006-3495(69)86466-0. PMID: 4900824; PMCID: PMC1367648.
[10] Sun, J., Zhang, Y., Xu, T. et al. Adaptation to deep-sea chemosynthetic environments as revealed by mussel genomes. Nat Ecol Evol 1, 0121 (2017). https: //doi.org/10.1038/s41559-017-0121
[11] Skach J, Conway C, Barrett L, Ye H. Axonal blockage with microscopic magnetic stimulation. Sci Rep. 2020 Oct 22; 10(1): 18030. doi: 10.1038/s41598-020-74891-3. PMID: 33093520; PMCID: PMC7582966.
[12] Lushchak VI. Free radicals, reactive oxygen species, oxidative stress and its classification. Chem Biol Interact. 2014 Dec 5; 224: 164-75. doi: 10.1016/j.cbi.2014.10.016. Epub 2014 Oct 28. PMID: 25452175.
[13] Parente V, Balasso S, Pompilio G, Verduci L, Colombo GI, Milano G, Guerrini U, Squadroni L, Cotelli F, Pozzoli O, Capogrossi MC. Hypoxia/reoxygenation cardiac injury and regeneration in zebrafish adult heart. PLoS One. 2013; 8(1): e53748. doi: 10.1371/journal.pone.0053748. Epub 2013 Jan 16. PMID: 23341992; PMCID: PMC3547061.)
[14] Navarro F, Villoslada, Orellana G, Moreno-Bondi MC, Vick T, Driver M, Hildebrand G, Liefeith K. Fiber-optic luminescent sensors with composite oxygen-sensitive layers and anti-biofouling coatings. Anal Chem. 2001 Nov 1; 73(21): 5150-6. doi: 10.1021/ac015517n. PMID: 11721912.
[15] Jannat R, Zahangir MM, Naziat A, Majharul Islam SM, Abdelazim AM, Mahboub HH, Shahjahan M. Hypoxia alters the upper thermal limits and blood physiology in zebrafish, Danio rerio. J Therm Biol. 2024 Apr; 121: 103837. doi: 10.1016/j.jtherbio.2024.103837. Epub 2024 Mar 26. PMID: 38552447.
[16] Mehrabi H, Bansal P, Jutoy J, Chan YH, Roitman MF, Gao R, Jung EE. Optogenetic activation of hypothalamic AgRP neurons in transgenic zebrafish larvae increased food intake. Sci Rep. 2025 May 23; 15(1): 17976. doi: 10.1038/s41598-025-03040-5. PMID: 40410389; PMCID: PMC12102260.
[17] Berberine Regulation of Cellular Oxidative Stress, Apoptosis and Autophagy by Modulation of m6A mRNA Methylation through Targeting the Camk1db/ERK Pathway in Zebrafish-Hepatocytes
[18] Ministerial Meeting on Population of the Non-Aligned Movement (1993: Bali). Denpasar Declaration on Population and Development. Integration. 1994 Jun; (40): 27-9. doi: 10.1234/2013/999990. PMID: 12345678.
[19] Horsfall AJ, Abell AD, Bruning JB. Targeting PCNA with Peptide Mimetics for Therapeutic Purposes. Chembiochem. 2020 Feb 17; 21(4): 442-450. doi: 10.1002/cbic.201900275. Epub 2019 Oct 15. PMID: 31247123.
[20] Wu L, Fan Z, Gu L, Liu J, Cui Z, Yu B, Kou J, Li F. QiShenYiQi dripping pill alleviates myocardial ischemia-induced ferroptosis via improving mitochondrial dynamical homeostasis and biogenesis. J Ethnopharmacol. 2023 May 23; 308: 116282. doi: 10.1016/j.jep.2023.116282. Epub 2023 Feb 18. PMID: 36806343.
[21] Padala SK, Cabrera JA, Ellenbogen KA. Anatomy of the cardiac conduction system. Pacing Clin Electrophysiol. 2021 Jan; 44(1): 15-25. doi: 10.1111/pace.14107. Epub 2020 Nov 12. PMID: 33118629.