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Cardiac Cell Regeneration in Zebrafish A Systematic Review Study.

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Article History

Abstract

Objective: This research aims to increase the level and quality of the information acquired from 90 previously conducted studies regarding zebrafish heart regeneration and to summarize the best and latest information as well as the methods gleaned from those studies, which will allow us to determine the best ways to rebuild cardiac tissue in zebrafish**.**
Methods: This study was conducted under the PRISMA guidelines. The search for primary research articles was conducted using PubMed, Web of science, and Mendeley. We used the latest update of Microsoft office Excel, Of the total 1158 results, 1066 were dropped according to the criteria for exclusion. The selected results included previously published and unpublished studies on cardiac cell regeneration in zebrafish from 2012 to 2022.
Results: 90 studies met the inclusion criteria. Out of these, 43 used the AR method, 36 used cryoinjury, and 16 used genetic amputation. All methods used were based on selected heart sections, not the whole heart. The primary evaluation technique used in the included studies was histology, either alone or in combination with other methods. Acid Fuchsin Orange G (AFOG), Masson's Trichrome (MT), Hematoxylin/Eosin (HE), immunofluorescence (IF), and in situ hybridization (ISH) were the main histological techniques employed to assess heart regrowth and regeneration.
Conclusion: This study may have a risk of bias due to the qualitative and quantitative data that was selected. Further research can help understand and utilize zebrafish regeneration genes in humans.

Keywords: Zebrafish , Cardiac Cell, Regeneration , Danio rerio ,Apex Resection.

Introduction

Unlike mammals, zebrafish display a highly regenerative potential in response to cellular injury. Hence, it has gained much attention as a viable model for the study of regeneration. Studies on zebrafish regeneration frequently concentrate on adult tissues such as the heart, brain, retina, caudal fin, and spinal cord. Extracellular matrix (ECM) and inflammatory mediators also play a significant role during cellular remodeling, even though progenitor cells are critical components in inducing regenerative responses (1), (2). By promoting cardiomyocyte proliferation, decreasing infarct size, and generally enhancing cardiac function, zebrafish ECM was able to drive the healing of the damaged myocardium. In vitro, the ECM of zebrafish was discovered to have a favorable impact on the development of human cardiac progenitor cells. Similarly, the ability of this ECM for in vitro expansion of the cardiomyocytes of newborn rats was also examined using their embryo, newborn, and adult cardiac ECM (3). In the investigations, histology was employed to distinguish between healthy myocardium and collagen-rich scar tissue. The latter is a sequela of myocardial infarction (MI) in mammals, and as a result, it is unable to functionally make up for the loss of cardiomyocytes (CM). However, despite its use in determining if apex resection (AR) zebrafish hearts have fibrosis, such histological stains do not demonstrate the ability of the heart to develop and regenerate. (4). To identify the markers of older zebrafish cardiomyocytes, transcriptional characterization in 7-month-old and 4-year-old zebrafish ventricles was used by differentiating gene expression analysis. Reuter revealed that 1,233 genes were identified as being differently adjusted to age (DEGs, padj 0.05). Furthermore, 745 genes were substantially upregulated in older fish, compared to younger fish, whereas 488 genes were downregulated sharply. The findings demonstrate an increase in immune cells, particularly macrophages, in the older zebrafish ventricle. These white blood cells not only intumesced in number but also changed in morphology and behavior as they aged (5). Adult regeneration hearts (3 dpi and 7 dpi) were found to be grouped with young hearts along PC3, supporting previous findings that wounded cardiomyocytes share several transcriptional similarities with fetal or young cardiomyocytes in zebrafish (6). Using Zebrafish single-resolution fate map in the subsection of the anterior lateral plate mesoderm (aLPM) at 18 hpf, the tissues do not organize themselves into separate regions. Although it gives rise to the commingled pericardial sac, peritoneum, pharyngeal arch, cardiac precursor, and the lineage tracing of individuals, the tissue is not organized into separate regions. The blastomeres in zebrafish show that cardiac precursors of the primary heart tube within the anterior lateral plate (aLPM) can migrate toward the midline to form the primary heart tube (PTH) (7). There are four stages to fine- tuning anesthesia in zebrafish: stage 1 describes the loss of equilibrium lasting more than 3 seconds in dorsal recumbency, and stage 2 is the absence of stage 3, which is defined as a loss of reflex to gentle touch; stage 4 is defined as a loss of reflex to tail pinching with forceps and tying. This is the stage where the zebrafish is ready for surgery. The AR model is still limited because it lacks ischemia-induced cell death or cell debris that should be cleaned, which is a characteristic of MI in humans (8). Adoptive transfer of macrophages from either adult mouse GFPtpz-collagen or collagen-tagged zebrafish donors promotes scar formation through cell-autonomous collagen synthesis. The bulk of the tagged collagen in zebrafish localizes close to the lesion, around the epicardium that lies over it, suggesting that there may be a difference between the collagen that is mostly laid down by macrophages and that which is more locally deposited. Myofibroblast Col4a3bpa and homologous Col4a1 are specifically targeted by macrophages in zebrafish, which greatly reduce scarring in cryoinjured hosts. The findings show that macrophages directly contribute to fibrosis during cardiac healing, in contrast to the existing concept of scarring in which collagen deposition is only attributed to the myofibroblast (9). The aim of this systematic review is to examine 90 studies regarding zebrafish regeneration after apex resection, cryoinjury, genetic ambition and to bring out the latest update on zebrafish regeneration.

Materials & Methods

This systematic review was guided in conformance with the PRISMA guidelines. However, since its aim was to make a systematical assessment of the existing literature regarding the methods used, the risk of bias was not evaluated. The systematical approach for literature is otherwise recommended for meta-analysis. Searches for primary research articles were conducted using three databases: Web of Science, PubMed, and Mendeley. In all search engines, the search keywords were “cardiac” or “heart,” “cell,” “regeneration,” and “zebrafish” or “Danio rerio.” These searches yielded a total of 1,158 results, 600 of which were either duplicated within a search or between the three search engines (Fig.1). There were no entries that were inapproachable or not in English. The remaining 90 unique abstracts were screened for inclusion as primary research articles in this study. We excluded any research about human regeneration, mouse regeneration, fish regeneration, or other organ regeneration, except that of heart or cardiac cells. Reports were excluded for the following reasons: 1)

did not discuss cardiac cell regeneration (n = 659), 2) did not use the listed methods (n = 60), 3) research is older than 10 years (n = 337), 4) research discusses other organs away from the heart (n = 8), after deep abstract screening from 94 studies 2 of studies were not included A study that investigated Optic tectum regeneration, another study which discussed the effect of LIM homeobox 9 and how it affects retinal development was not included. To retrieve available evidence related to the research objectives, both published and unpublished studies on cardiac cell regeneration in zebrafish from 2012 to 2022 were included. The entries included in the study investigated the structure and function of the cardiac cell regeneration pathway and the best procedure that can be used to achieve accurate information related to cardiac cell regeneration, careful consideration was taken regarding the inclusion of studies based on the model in question, which represented a rodent model of cardiac cell regeneration. For example, in the case of genetic models, studies were included to see if the gene in question was stimulating cell regeneration of the *Danio rerio* heart.

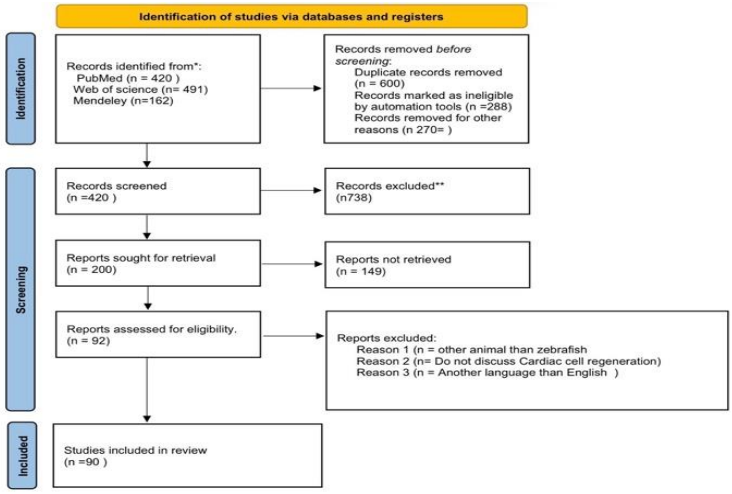

Figure 1: flowchart of the inclusion and exclusion criteria for the literature search. PRISMA guidelines are detailed in the approach**.**

Results:

The study used 90 papers as its main resource. A total of 420 studies from PubMed, 162 studies from Mendeley, and 576 studies from Web of Science were retrieved. Duplicates were identified, and the remaining 200 studies were screened according to the inclusion criteria based on title and abstracts (Table 2). Then, 90 papers were subjected to a second full-text screening. Of these, 43 research utilized apex resection (AR), 36 applied cryoinjury, and only 16 studies used genetic ablation; 18 studies used both apex resection and cryoinjury methods, and four studies used all three methods (Table 1).

Table 1. Studies conducted on zebrafish have been systematically categorized by injury type [10-90]

Apex resection	Cryoinjury	Genetic amputation	Both Apex resection and cryoinjury	AR, cryoinjury ، genetic amputation
1. Miklas 2020 2. Ditte 2021 3. Siomoes 2020 4. Mukherjee 2020 5. Peng 2021 6. Kaveh 2020 7. Lee 2020 8. Sun 2020 9. Hankoop 2021 10. Zhang 2020 11. Tahara 2021 12. Dyck 2020 13. Xie 2021 14. Bise 2020 15. Ye 2020 16. Jana Koth 2020 17. George 2020 18. Peng 2020 19. She 2020	1. Ruter 2020 2. Mukherhee 2021 3. Grivas 2021 4. Dicks 2020 5. Lee 2020 6. Paronobis 2021 7. Hankoop 2021 8. Peterson 2022 9. Dyck 2020 10. Bu hler 2021 11. Xie 2021 12. Bise 2020 13. Ye 2020 14. Del campo 2022 15. Koth 2020 16. Fukuda R 2020 17. George 2020 18. Feng X 2021	1. Sun 2022 2. Zhang 2020 3. Tahara 2021 4. Ryan 2020 5. Chen 2013 6. W.Mikals 2022 7. Dyck 2020 8. Xie 2021 9. Ye 2020 10. Bensimon-Brito 2020 11. George 2020 12. Feng X 2021 13. Peng 2020 14. Sharpe 2022 15. Chu 2020	1. Mukherjee2021 2. Lee 2020 3. Peterson 2020 4. Ye 2020 5. Koth 2020 6. George 2020 7. She 2021 8. Lowe 2021 9. Roshon 2020 10. Tahara 2016 11. Li 2021 12. Campo 2021 13. Wang 2022 14. Moyse's 2020 15. Melon 2019 16. Chiang's 2019 17. Stewart 2021 18. Harrison 2019	1. Ryan 2020 2. Dyck2020 3. Xie 2021 4. Peng 2020

20. Lowe 2021 21. Rochon 2020 22. Tahara 2016 23. Peng 2020 24. Li 2021 25. Campo 2021 26. Wang 2022 27. Moyes 2020 28. Wang 2013 29. Melon 2019 30. Chang's 2019 31. Xu 2019 32. Yin 2012 33. Huang's 2013 34. Harrison 2019 35. Campo 2021 36. Wang 2022 37. Brezitski 2021 38. Moyse's 2020 39. Melon 2019 40. Xu 2019 41. Wang 2013 42. Chiang's 2019 43. Cha`vez 2020	19. She 2020 20. Lowe 2021 21. Shrape 2020 22. Rochon 2020 23. Tahara 2016 24. Peng 2020 25. Iribarne 2021 26. Li 2021 27. Campo 2021 28. Wang 2022 29. Moys's 2020 30. Melon 2019 31. Chiang's 2019 32. Bakker's 2021 33. Iranzo 2018 34. Bednarek 2015 35. Harrison 2019 36. Francoeur 2021			

Investigators found that the majority of AR research used qualitative analysis of heart sections for evaluating viable myocardium, with 43 out of 92 studies using AR as an injury type, 36 using cryoinjury, and 16 using the genetic amputation approach (Belling, et al.,2020)

Table 2. Evaluation techniques for zebrafish heart regeneration and regrowth following genetic amputation, cryoinjury, and apex resection (AR).

Research 1st author.	Qualitative Histology.	Quantitative Histology
1. Ruter 2020	• 2	• 1
2. Miklas 2020	• 3	• -
3. Ditte 2021	• 1	• 2
4. Simons 2020	• 1	• 1
5. Mukherjee 2021	• 1	• 1
6. Hromowyk 2020	• 1	• 2
7. Peng 2021	• 1	• 1
8. Kaveh 2020	• 2	• 2
9. Grivas 2021	• 2	• 2
10. Lee 2020	• 2	• 0
11. sun 2022	• 1	• 2
12. Pronobis 2020	• 1	• 4
13. Ozhan 2015	• 1	• 0
14. Honkoop 2021	• 2	• -
15. Zhang 2020	• 1	• 4
16. Tahara 2021	• 2	• 2
17. Chen 2013	• 1	• 1
18. W.Mikals 2022+	• -	• 3
19. peterson 2022	• 3	• 0
20. Dyck 2020	• 1	• 0
21. Bu hler 2021	• 1	• 2
22. Li 2020	• 2	• 2
23. Xie 2021	• 3	• 2
24. Bise 2020	• 3	• 1
25. Ye 2020	• 2	• 3
26. Bergen 2022	• 1	• 2
27. Del Campo 2022	• 1	• 1
28. Jana Koth 2020	• 1	• 2
29. Kim, A.R. 2020		
30. Fukuda R 2020		

31. Bensimon-Brito 2020	• 1	• 0
32. George RM 2020	• 1	• 0
33. Feng X 2021	• 1	• 0
34. peng X 2020	• -	• 0
35. She 2020	• -	• 0
36. Lowe 2021		• -
37. Shrape 2022	• -	• -
38. Rochon 2020	• 2	• -
39. Yep 2021	• 1	• -
40. Tahara 2016	• -	• -
41. Peng 2020	• 2	• -
42. Chu 2022	• 2	• -
43. Iribarne 2021	• 1	• -
44. Li 2021	• -	• -
45. Campo 2021	• 3	• -
46. Wang 2022	• -	• 1
47. Bertozzi 2021	• -	• 2
48. Nunes 2022	• 2	• 1
49. Moyse's 2020	• -	• -
50. Melón 2019	• -	• -
51. Cao 2018	• 2	• 1
52. Xu 2019	• -	• -
53. Wang 2013	• -	• -
54. Chiangs 2013	• 2	• -
55. Jopling 2012	• 1	• -
56. Parente 2013	• 2	• 1
57. Bakker's 2021	• 1	• 3
58. Yin 2012	• -	• 3
59. Sánchez-Iranzo 2018	• 1	• -
60. Bednarek 2015	• -	• 1
61. Huang's 2013	• 2	• 1
62. N.Chávez 2020	• 2	• 1
63. Peng 2021	• 3	• 2
64. Bertozzi's 2020	• -	• 1
65. Harrison 2019	• 3	• 3
66. Francoeur 2021	• 1	• 1
	• 2	• 2
	• 1	• -
	• 2	• 3
	• 1	• -
	• -	• 1
		• 1
		• -
		• -
		• 1
		• 2

Cardiac Outgrowth: A Qualitative and Quantitative Analysis

In the analysis of cardiac outgrowth, a total of 56 out of 66 articles applied histology on segments to assess heart regrowth and regeneration after AR; cryoinjury and genetic amputation are illustrated in Table 2. Forty-two studies used quantitative methods for the detection of cardiac cell proliferation. Considering this, histopathology in general appears to be a recognized qualitative technique for assessing heart regeneration in zebrafish. Histology was performed by Acid Fuchsin Orange G (AFOG; 19 studies): “Orange G is used in the Papanicolaou stain to stain keratin. It is also a major component of the Alexander test for pollen staining. It is

often combined with other yellow dyes and used to stain erythrocytes in the trichrome method" (95). Masson's trichrome staining was used to visualize connective tissues, particularly collagen, in tissue sections. Collagen is dyed blue, nuclei are stained dark brown, muscle tissue is stained red, and the cytoplasm is stained pink in a typical Masson's trichrome technique (MT; 8 studies); this method uses two dyes—hematoxylin and eosin—that make it easier to see different parts of the cell under a microscope. Ribosomes, chromatin (genetic material inside the nucleus), and other structures are all visible in hematoxylin as a deep blue–purple dye. The cytoplasm, collagen, connective tissue, and other supporting and enclosing elements of the cell appear orange-pink-red in eosin. H and E staining offers crucial details on the pattern, shape, and structure of cells in a tissue sample and aids in the identification of various types of cells and tissues (96). In hematoxylin/eosin staining (HE; 9 studies), muscle and collagen differ from one another, as shown by AFOG and MT staining. In accordance with Poss et al., who measured the degree of fibrosis on heart tissue slices in 2D of the entire region of the ventricle to determine the extent of heart regeneration, the preference for AFOG staining could perhaps be explained by an enhanced sensitivity for collagen in zebrafish. Immunofluorescence (IF) is a type of immunohistochemistry technique that utilizes fluorophores to visualize various cellular antigens such as proteins (84). The localization of different cellular components within cells, tissues, and cellular spherical structures developed from 3D culture can all be recognized with this type of imaging. Immunofluorescence was used in multiple studies (39 studies). In situ hybridization (ISH) is a technique that allows for precise localization of a specific segment of nucleic acid within a histologic section. The fundamental premise of ISH is that nucleic acids can be detected using a reciprocal beachfront of the nucleic acid to which a reporter molecule is connected, provided it is stored adequately inside a histologic instance (17 studies). In situ hybridization (ISH) technique.

Table 3: The expression of proliferation markers was used to measure cardiac cell proliferation after AP, cryoinjury, and genetic amputation.

Research 1st author	Proliferation marker
1. Ruter 2020	1. 1233 gene up regulated.
2. Miklas 2020	2. mTOR
3. Ditte 2021	3. myh1 marker
4. Simons 2020	4. pcna
5. Mukherjee 2021	5. ccn2 a+ccn2b
6. Hromowyk 2020	6. mymk , pcna
7. Peng 2021	7. Pak2 / pS675-beta - catenin
8. Grivas 2021	8. mdka , BrdU,mdka cn105
9. Lee 2020	9. Pcna —-Mef2+
10. sun 2022	10. hapln 1+
11. Pronobis 2020	11. Pcna —-Mef2+ + Edu
12. Ozhan 2015	12. Wnt/beta- catenin
13. Honkoop 2021	13. ErbB2 +Pcna ,Nrg1/ErbB2., CaErbB2.
14. Zhang 2020	14. mvp- pcna
15. Tahara 2021	15. pcna _ mef2 -fgf
16. Chen 2013	16. mcherry, fluc,
17. W.Mikals 2022+	17. mTOR , PCNA, c-Myc , wnt/b catenin , edu
18. peterson 2022	18. leukocytes
19. Bu hler 2021	19. hdac1, if reduced it will cause reduce in regernrenation capstiy , pcna , Edu,
20. Li 2020	
21. Xie 2021	20. klf2a+Klf2B
22. Bise 2020	21. LC3-1, LC3-2, metaformin
23. Ye 2020	22. CreERT2-loxP
24. Bergen 2022	23. Δ113p53 promotes heart regeneration by increasing cardiomyocyte proliferation
25. Del Campo 2022	
26. Jana Koth 2020	24. mTOR ,
27. Kim, A.R. 2020	25. sp7 , entpd5a , col1a1a
28. Fukuda R 2020	26. epicardial cells
29. Bensimon-Brito 2020	27. Runx1
30. George RM 2020	28. 20(R)-ginsenoside Rh2 they refered to it as (CPP531)
31. Feng X 2021	29. Pdk3 and PDC
32. peng X 2020	30. TGF-b
33. She 2020	31. cNCC-derived cardiomyocytes
34. Lowe 2021	32. vegfc "he+/-
35. Shrape 2022	33. Wnt2bb and jnk1/creb1/c-jun

36. Rochon 2020	34. gridlock
37. Yep 2021	35. Neuregulin1, Cxcl12—Cxcr4, NOX/Duox, Aldh1a2
38. Tahara 2016	36. Ruvbl2
39. Peng 2020	37. mmp13a
40. Chu 2022	38. fili1a:GFP , flt1"enh:tdTomato .
41. Iribarne 2021	39. cmlc2 , CreER , Mef2, cTnT
42. Li 2021	40. wnt signaling , p21 , Dkks , sFrps , pcna , mef2 , Pak2 , pSer657 , beta catenin
43. Campo 2021	
44. Wang 2022	41. Sodium-calcium exchanger 1 (Ncx1)
45. Bertozzi 2021	42. Innate immune cells-Stem cells
46. Nunes 2022	43. endocardial Notch signaling pathway
47. Moyse's 2020	44. extracellular vesicles (EVs)
48. Melón 2019	45. Krt5-cytoskeleton-BMP4
49. Cao 2018	46. Wnt/β-catenin signaling
50. Xu 2019	47. FGF , BMP ,VEGF , IGF NRG-ERBB,kappa B
51. Wang 2013	48. mpeg1.1 (mpeg1) and csf1ra (c-fms)
52. Jopling 2012	49. sox10+, BrdU, MHC/mCherry
53. Parente 2013	50. Epicardium
54. Bakker's 2021	51. anti-inflammatory reagents (dexamethasone, MMP9/MMP13 inhibitor I) and prokinetic drugs (cisapride).
55. Yin 2012	
56. Sánchez-Iranzo 2018	52. Fibronectin
57. Bednarek 2015	53. Hypoxia
58. Huang's 2013	54. Hypoxia/Reoxygenation
59. N.Chávez 2020	55. Prrx1b + Nrg1
60. Peng 2021	56. miRNAs(miR-133)
61. Bertozzi's 2020	57. Tbx5a
62. Klaourakis's 2021	58. Telomerase
63. Helston 2021	59. Glucocorticoids
64. Harrison 2019	60. Autophagy + Rapamycin
65. Francoeur 2021	61. TGF-β/Smad3
	62. cardiac lymphatic vasculature
	63. Reactive oxygen species
	64. cardiac lymphatic vasculature, cxcr4a, prox1a+flt4+ lyve1b,mrc1a+ stab1+sFlt4
	65. Sox10

Cardiomyocyte Proliferation

Cardiomyocyte proliferation was utilized as a measurement for assessing heart regeneration in 66 of the 92 investigations, either alone or in conjunction with the previously mentioned approach. Consequently, the production of proliferating cell nuclear antigen (PCNA) (31 out of 66 studies) and phosphohistone-H3 (PHH3) (7 out of 66 studies) and the incorporation of 5-Bromo-20-deoxyuridine (BrdU) and 5-Ethynyl-2'-deoxyuridine (EdU) (15 out of 66 studies) have been regarded and employed to evaluate CM proliferation markers (Table 3) in combination with a cardiomyocyte marker (11 studies), myocyte enhancer factor 2 (Mef2c), myosin heavy chain 1 (MYH1), sarcomeric actin, or a transgene CM reporter (such as *cmlc2*::DsRed2.). Hyaluronic binding protein *hplan*1 is also listed in 1 out of 66 studies on cardiomyogenesis in heart morphogenesis and injury-induced regeneration; TGFbeta/BMP or Tbx are also activated during the regeneration pathway, and Wnt/bb/beta signaling pathways dampen cardiomyocytes proliferation during zebrafish heart regeneration process.

Evaluation of Heart Activity

The zebrafish heart has an electrical activity sequence and pumping function that is comparable to that determined by electrocardiography (ECG) and echocardiogram (ECHO) in humans, considering the only two chambers and the lack of a pulmonary system in zebrafish. However, given that parametric values differ between species,the data is best used for comparative measurements between groups like AR and cryoinjury and genetic amputation procedures in zebrafish. A total of four studies were found in our systematic review. A functional heart examination was the method used in the analysis of cardiac recovery following AR. ECG was utilized in four investigations, whereas echocardiography was used in two investigations. The four studies that used ECG measured changes in the R-R interval or the interval between each heartbeat to assess functional recovery following AR, cryoinjury, and genetic ambulation.

Table 4: Demonstrate the advantages and disadvantages of the different procedures that imply on zebrafish.[11-90]

Injury type	Advantage	Disadvantage
Apex resection	1-fast in recovery 2- old and common in literature 3-Injury to all cell types	1- No lymphatic regeneration 2-Less like human MI 3-Technically challenging 4-Variable injury size 5-Open chest model
Cryoinjury	1- Common in literature 2-Most like human MI 3-Injury to all cell types	1-Long recovery 2-Technically challenging 3-Open chest model
Genetic ambition	1-Cell-specific study 2-Non-invasive 3-Fast recovery 4-Technically simple.	1-Limited to single-cell type 2-Less like human

MI: myocardial infraction

The Immune Response

There are several elements that influence how zebrafish regenerate, and numerous studies have shown that diverse subpopulations of macrophages and a similar influx of neutrophils occur. In table 4 we demonstrate multiple recent studies showed that the acquired immune system, a separate Wt1 + mac innate immune system, is essential for zebrafish heart regeneration and that variations in the adaptive immune system may underlie variations in regenerative capacity. Investigating if these distinct macrophage cell states/subtypes seen in zebrafish could potentially be adapted in mammalian macrophages to possibly enhance human regeneration would be significant. Macrophage subpopulation with a pro-regenerative transcriptional profile was shown to originate, at least in part, from the hematopoietic niche. How zebrafish macrophage populations differ from mammalian macrophages is still unknown. One of the initial reactions to injury, aside from the natural immune response, is the inflammatory response. In addition to phagocytosing cellular debris at the site of injury, macrophages also participate in numerous approaches of cellular reactions afterward. For instance, they can guide collagen deposition, induce neo-angiogenesis, and start CM proliferation in order to control the fibrotic reaction. The significance of this cell population is demonstrated by the impairment of regeneration with a decrease in CM proliferation and an increase in scar formation in zebrafish upon general depletion or ablation of macrophage subsets. The discovery of proinflammatory macrophages expressing tumor necrosis factor α (TNF-α) at the earliest stages of cardiac insult has further clarified the function of macrophages. This finding is consistent with those of earlier research on the regeneration of the embryonic caudal fin. Additionally, during heart regeneration, pro-regenerative macrophages expressing Wilms tumor 1b (wt1b) exhibit particular recruitment dynamics and genetic markers (57). Additionally, osteopontin-positive macrophages have been linked to both the induction of a fibrotic response and the remission of fibrosis (59). Overall, for heart regeneration, a well-calibrated temporal and spatial management of inflammation is essential, lymphocytes are beneficial in the depletion of regulatory T cells (Tregs) during cardiac cryoinjury which leads to thinner myocardial walls, persistent collagenous scars, lowered CM proliferation, and macrophage polarization toward classical inflammatory phenotype. Timing: T lymphocytes begin mobilizing to the heart lesion on day one, increasing at 7 dpi and reducing by 14 dpi.

Discussion:

The current study aimed to investigate the various methods of inducing zebrafish heart injury and regenerating the heart tissue and evaluate the techniques performed to evaluate heart function, regrowth, and cardiac cell proliferation. This is crucial because zebrafish provide a chance to pinpoint the mechanisms behind heart regeneration, which might then be applied to non-regenerating mammalian cardiac cells to enhance cardiac recovery following MI. The objectives were to compare the advantages and disadvantages of different injury types, understand the immune response during heart regeneration, and provide insights into the histological evaluation techniques used in these studies. The methodology involved a systematic analysis of existing literature, including studies from PubMed, Mendeley, and Web of Science, to retrieve relevant articles based on the inclusion criteria. A total of 92 studies were incorporated into the analysis. The results showed that three main methods were used to induce heart injury in zebrafish: apex resection (AR), cryoinjury, and genetic

amputation. Among the 92 studies, 43 studies used AR, 36 used cryoinjuries, and 16 used genetic amputation. Additionally, four studies utilized all three methods. Most AR studies used qualitative histological analysis to evaluate viable myocardium, indicating its acceptance as a qualitative method for assessing heart regeneration in zebrafish. Histology was the primary evaluation technique used in the included studies, either separately or alongside other approaches. Acid Fuchsin Orange G (AFOG), Masson's trichrome (MT), hematoxylin/eosin (HE), immunofluorescence (IF), and in situ hybridization (ISH) were the main histological techniques employed to assess regeneration and regrowth of the heart. Proliferation markers such as PCNA, PHH3, BrdU, and EdU were used to measure the proliferation of cardiomyocytes. Furthermore, CM proliferation markers were often combined with cardiomyocyte-specific markers, such as Mef2c, MYH1, sarcomeric actin, or cmlc2: DsRed2, to assess cardiac cell proliferation accurately. The comparison of different injury types in zebrafish revealed unique advantages and disadvantages: AR was noted for its fast recovery and historical significance in the literature but lacked lymphatic regeneration and closely mimicked human myocardial infarction; cryoinjury, the opposite side, resembled human myocardial infarction the most but had a more extensive recovery interval and was technically challenging; and genetic amputation allowed for cell-specific studies and had a non-invasive approach with a fast recovery interval but was limited to single-cell type analysis and deviated from human myocardial characteristics. The immune response during zebrafish heart regeneration was found to involve different subpopulations of macrophages and neutrophils. The adaptive immune system, particularly the acquired immune system, was identified as critical for heart regeneration. Macrophages play a significant role in various cellular reactions, such as guiding collagen deposition, inducing neo-angiogenesis, and promoting CM proliferation. A well-calibrated temporal and spatial management of inflammation is crucial for successful heart regeneration. Immune cell depletion or delayed mobilization of neutrophils, monocytes/macrophages, and T lymphocytes inhibited heart regeneration and resulted in scar retention, reduced CM proliferation, and impaired angiogenesis. In conclusion, the findings from this systematic analysis shed light on the different methods used for inducing zebrafish cardiac injury and regeneration. Histology, in combination with proliferation markers, emerged as the primary evaluation technique for evaluating heart development and cardiac cell proliferation. Each injury type in zebrafish presented specific advantages and disadvantages, highlighting the need for careful consideration when selecting the appropriate method for a particular research question. Additionally, the immune response, particularly that of several immunological cell types, plays a crucial role in heart regeneration. These insights contribute to the understanding of zebrafish as a model organism for studying heart regeneration and may facilitate the development of potential therapeutic strategies for heart repair in humans. However, further research is required to explore the specific functions and interactions of immune cell subpopulations throughout the process of regeneration of the zebrafish heart and to elucidate their potential applications in mammalian models for regenerative medicine.

Conclusion

In conclusion, we have made significant contributions to the zebrafish domain cardiac cell regeneration. By consolidating and evaluating the available information and methodologies, we have improved the level and quality of knowledge in this area. Our findings have not only summarized the current state of research but have also highlighted the latest advancements. It should be noted that our study represents the first systematic review dedicated to zebrafish heart regeneration, including essential procedures for apex resection, cryoinjury, and genetic amputation. By filling this gap in literature, we have provided a valuable resource for researchers and scientists interested in this field. Our comprehensive analysis of previous studies has paved the way for identifying the most effective strategies and approaches for rebuilding cardiac tissue in zebrafish. Notably, the analysis revealed that most of the studies predominantly relied on the apex resection method.

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