https://doi.org/10.70882/88d2xa88

SCIENCE FORUM (JOURNAL OF PURE AND APPLIED SCIENCES) 26, (5 Special) (2026), 516 - 530

Science Forum (Journal of Pure and Applied Sciences)

Journal Homepage: https://atbuscienceforum.com.ng/index.php/jpas

Histological Mechanisms of Tissue Regeneration in Vertebrates: Comparative Insights from Fish, Amphibians, and Mammals; Article Review

Noora A. Farhan

Department of Pathological Analysis, College of Science , University of Wasit , Iraq.

Abstract

Tissue regeneration, the biological process in which in multicellular organisms damaged or lost parts are restored to a greater or less extent. The regenerative potential varies widely across vertebrate species and depends on the type of tissue, developmental stage, cellular plasticity, immune responses and organization of the extracellular matrix. The applications of histology to these processes are crucial, as they allow the investigation of cell growth, tissue reorganization, inflammatory reactions and the regeneration of normal tissue structure. In this review, the main mechanisms that drive tissue regeneration in fish, amphibians and in mammals are discussed, focusing on epithelial repair mechanisms, activation of progenitor cells, cell dedifferentiation, ECM remodeling and immune regulation. Organized and integrated interactions of the resident cells, signaling pathways and tissue environment are necessary for successful regeneration, as evidenced by established vertebrate models. Mammalian repair, on the other hand, is usually accompanied with longstanding inflammation and fibrotic scarring, with some organs being more regenerative than others. Recent studies have highlighted the significance of spatiotemporal controlled signaling in shaping outcomes of regeneration. Histological analysis, in combination with molecular and quantitative imaging techniques, can help to differentiate the differences between regenerative growth and scar-mediated repair. The knowledge of these mechanisms could assist in the further development of comparative zoological research and future studies in tissue biology and regenerative medicine.

Keywords

Animal Histology;
Tissue Regeneration;
Vertebrates;
Fish;
Amphibians;
Mammals;
Stem Cells;
Extracellular Matrix;
Wound Healing;
Fibrosis.

EISSN: 3156-5190 (Online)
ISSN: 1119-4618 (Print)
JPAS
Faculty of Science, ATBU Bauchi

Corresponding Author: Noora A. Farhan, Department of Pathological Analysis, College of Science , University of Wasit , Iraq.
Email: noora@uowasit.edu.iq © 2026 Faculty of Science, ATBU Bauchi. All rights reserved

1. Introduction

Tissue regeneration is a significant biological process in which an organism replaces damaged cells, restores tissue structure or regenerates complex anatomical structures. Regeneration is found to be different among the species and organs. In some vertebrates, they can regenerate a large part of a specific structure while in others they are mostly restoring injuries by wound closure and scar formation.

The comparative regeneration study offers a zoological perspective through which the evolutionary history, cellular organization and tissue specialization of the body to aid in the repair of damaged organs can be examined. Histologically, regeneration can be studied in relation to changes in epithelial continuity, cellular proliferation, distribution of inflammatory cells, changes in extracellular-matrix organization, and the reformation of tissue-specific structures. (Seifert et al., 2012; Tanaka, 2016; McCusker & Gardiner, 2011). (Alibardi, 2010; Seifert et al., 2012; Tanaka, 2016).

Regeneration should be separated from the normal healing of wound. Inflammatory responses, cell migration, proliferation and matrix remodeling are all crucial processes in both regeneration and remodeling, but proper organization of the tissues, and ideally their function, is essential for the successful regeneration. In contrast, fibrotic repair can repair a wound without regrowing the tissue pattern. (Goldman & Poss, 2020; Liao et al., 2026). (Gurtner et al., 2008; Eming et al., 2014; Goldman & Poss, 2020).

Comparative models such as fish, amphibians and mammals are of value because they allow for the comparison of different organs with different capacities to regenerate. Research on these organisms has helped in understanding of the activation of progenitor cells, cellular plasticity, positional information and tissue specific signaling. But, it's not a straightforward distinction between regenerator species and non-regenerator species. The capacity depends on the type of organ, the type of injury, age and experimental conditions. (Khyeam et al., 2021; Sammarco & McCusker, 2026; Liao et al., 2026).

In this review, the author brings together some of the key histological mechanisms of vertebrate tissue regeneration and consider how conventional microscopy can be coupled with quantitative and molecular techniques.

2. Review Objectives

The main aim of this narrative review is to understand the histological mechanisms of tissue regeneration in vertebrates and to correlate its expression in the fish, amphibians and mammals.

The objectives are to:

- To explain the histological phases of tissue repair and regeneration.
- To make comparisons of the regenerative processes of selected vertebrate models.
- To investigate the role of progenitor cells, immune reactions and extracellular-matrix remodeling.
- To consider how changes in animal tissues can be studied to see if they are regeneration.
- To determine constraints and priorities in future comparative histological work.

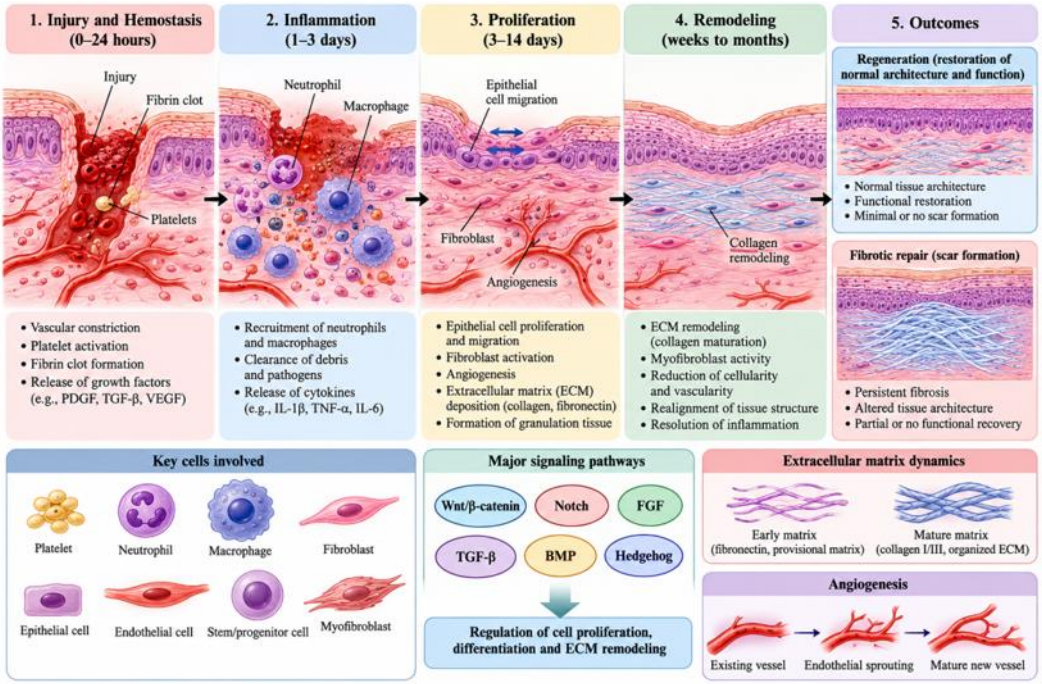


Figure 1. Conceptual stages of tissue repair and regeneration.

3. Histological Stages of Tissue Regeneration

3.1. Initial injury and wound closure

Immediate response to tissue injury is characterized by disruption of the integrity of the tissues, alteration of local blood flow, when present, and activation of protective mechanisms. Hemostasis is a factor in the provisional matrix formation in vascularized tissues. Then, there is sequential movement and proliferation of epithelial cells to reestablish barrier integrity. (Gurtner et al., 2008; Eming et al., 2014; Rodrigues et al., 2019).

The stage of the disease can vary in appearance on a microscopic level, depending on the type of tissue. Cellular migration might occur across the site of injury in surface epithelia, or edema, inflammatory-cell infiltration and disruption of the extracellular matrix may all occur in deeper tissues.

Regeneration is not successful when only wound closure is considered. Further testing is needed to determine if normal tissue structure has been restored or fibrosis has occurred. (Gurtner et al., 2008; Eming et al., 2014; Takeo et al., 2015).

3.2. Inflammation and cellular clearance

During inflammatory responses, damaged cells are eliminated and repair of the damaged tissue is promoted. Innate immune cells also clear debris, and produce mediators that control cell proliferation, vascular responses and matrix remodeling. (Godwin et al., 2013; Aurora et al., 2014; Behmoaras et al., 2025).

The healing process may be affected by factors, such as the length and type of inflammatory process. An overactive and/or prolonged inflammatory process can cause tissue damage and fibrosis, while a controlled inflammatory reaction can aid tissue repair. Such relationships are tissue specific and observations made in one organ should not be assumed to be representative of all vertebrates.

Histologically, the activity of inflammation can be assessed by the distribution and morphology of the inflammatory cells, the degree of tissue disruption and changes in the connective tissue surrounding the damaged tissue. Routine hematoxylin and eosin staining gives a good initial assessment, but may need to be supplemented with immunohistochemically methods to differentiate specific immune-cell types.

3.3. Progenitor-cell activation and cellular proliferation

The potential for regenerative growth relies on the presence of cells that are able to form the needed tissue components, and then they need to be activated. These cells can be, depending on the organ, resident stem cells, lineage-restricted progenitors or differentiated cells that exit the cell cycle. (Jopling et al., 2010; Kikuchi et al., 2010; Goldman & Poss, 2020).

The contribution of these populations varies tissue by tissue for different species. Therefore it is not possible to say generically that regeneration is the result of a single mechanism in cells. Cell-specific molecular analyses and experimental lineage tracing studies offer greater support for cellular origin than morphology.

The changes in cellular density, nuclear morphology, the amount of mitoses, and organization of tissues can be documented by a histological assessment. When used with validated protocols and appropriate controls, proliferation markers like Ki-67 or proliferating cell nuclear antigen (PCNA) may also be used for providing additional information. (Bancroft & Gamble, 2019; Kiernan, 2015; Schindelin et al., 2012).

3.4. Extracellular-matrix remodeling

The extracellular matrix supports cells, regulates cell attachment, migration, differentiation and cell signaling. Injury triggers matrix degradation, redistribution and resynthesis. These processes are balanced to determine the environment of the tissue whether restoration or fibrosis. (Mayorca-Guiliani et al., 2025).

A regenerative response will need matrix remodeling to allow for tissue reconstruction along with maintenance or restoration of proper structural relationships. However, too much collagen deposition and ongoing matrix accumulation can result in a scar and affect the mechanical and functional properties of the organ.

Changes in the distribution of connective tissues can be detected by conventional histology, while methods based on collagen sensitive stains and immunohistochemically methods offer more specific evaluations. If staining, acquisition and analysis of images are standardized, quantitative image analysis can be used to estimate the area of connective tissue.

3.5. Restoration of tissue architecture

At last, the organization of tissues is reconstructed. This can involve restoration of an epithelial layer, a muscle layer, vascular networks, glands, or any other special organ. (Poss, 2010; Tanaka, 2016; Goldman & Poss, 2020).

It should therefore be more than the closure of wounds or increased cell number. Histological evidence of regeneration should show the correct organization of the newly formed tissue, and identify it from a disorganized repair process or the presence of fibrosis.

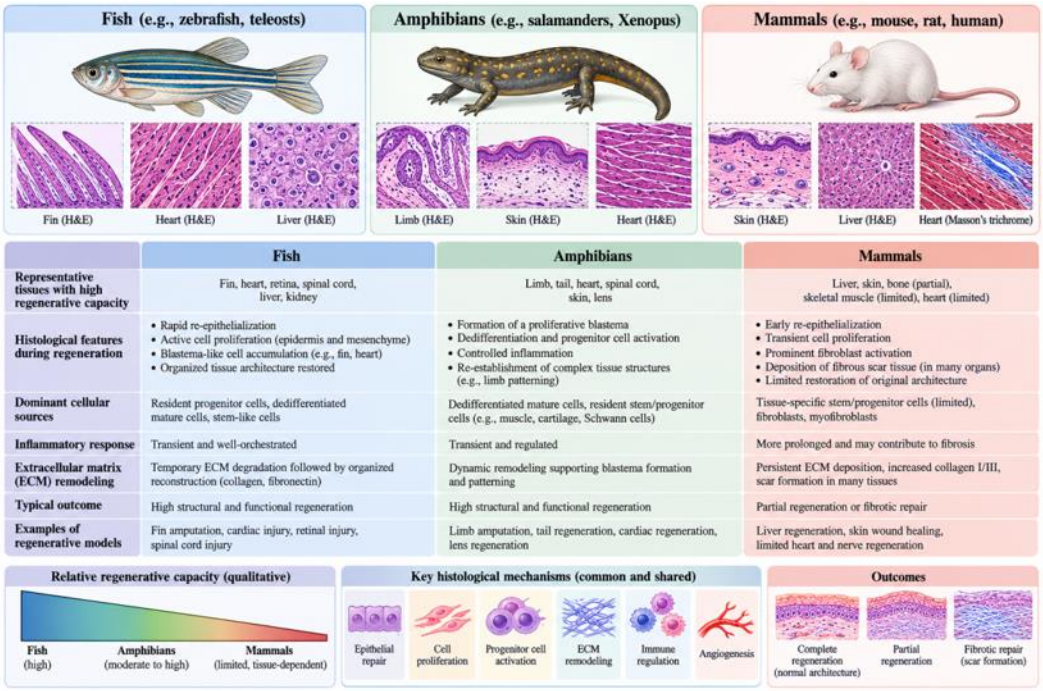


Figure 2. Qualitative comparative framework for histological assessment in fish, amphibians, and mammals.

4. Comparative Regeneration across Vertebrate Groups

4.1. Fish

A number of different fish species have emerged as models to study tissue regeneration. In particular, zebra fish have been used in research to investigate the regeneration of other structures and cardiac repair. They are easily accessible to experiment with and genetic tools are available for studies of cellular behavior and signaling during tissue restoration. (Poss et al., 2002; Gemberling et al., 2013; Kikuchi et al., 2010; Bertozzi et al., 2021). (Lebedeva et al., 2020). (Lebedeva et al., 2020; Yin & Poss, 2008; Gemberling et al., 2013).

In fish, regeneration can consist of an increase in the number of resident cells, alterations to cell-type of differentiated cells, and reorganization of tissue. Mechanisms depend on organ, and the type of injury. Therefore, care should be taken in extrapolating from zebrafish to other teleost.

Histological analysis can be used to look at how well the tissue is connected, the inflammatory response, cell growth, and reconstruction of the tissue. In cardiac studies, for instance, it is especially important to differentiate between the newly formed myocardium and the persistent fibrotic tissue. Other organs can be treated in a similar fashion, but it is necessary to have tissue-specific criteria. (Bertozzi et al., 2021; Bassat & Tanaka, 2021).

4.2. Amphibians

Amphibians, especially salamanders are established models for the study of appendage regeneration. After amputation, there is a specialized regenerative structure formed and subsequent growth and differentiation. (Bassat & Tanaka, 2021; Min & Whited, 2023; Kim & Whited, 2024). (McCusker & Gardiner, 2011; Tanaka, 2016; Knapp et al., 2013; Min & Whited, 2023). (McCusker & Gardiner, 2011; Tanaka, 2016; Min & Whited, 2023).

Regenerative response is characterized by interactions among resident tissues, progenitor populations, nerves and local signaling environments. There are differences in the contribution of individual cell populations depending on the tissue of origin and the model of regeneration.

The changes in epithelial organization, distribution of proliferating cells, remodeling of the extracellular matrix and progressive development of differentiated tissues can be identified through histological studies. Such observations can be useful in combination with lineage-tracing or molecular approaches to trace participating cells and their origins and fate.

Also the ability to regenerate is species-dependent, age-dependent and site-dependent, that is, it is different for different species, developmental stages and anatomical structures. It is therefore very important to be aware of the biological attributes of the model to be interpreted when comparing it to other models.

4.3. Mammals

Wound closure, inflammatory response, cell proliferation and deposition of extracellular-matrix are hallmarks of mammalian tissue repair. The relative proportion of regeneration versus fibrosis varies greatly between organs, however. (Liao et al., 2026; Behmoaras et al., 2025). (Porrello et al., 2011; Aurora et al., 2014; Liao et al., 2026).

There is considerable renewal capacity in certain tissues of mammals at normal life temperatures or following certain kinds of damage. In other tissues such as many contexts in the adult central nervous system and in the adult heart, there is more limited structural restoration after severe damage.

Fibrosis can both restore injury damaged tissue, but excessive scarring can negatively affect the tissue's function. Therefore, collagen-rich tissue or ongoing inflammation or a mix of cells may be a sign of repair instead of regeneration.

The amount of structural restoration from wound closure is important and requires histological methods to determine. In mammalian models the use of conventional stains can be complemented by collagen-specific stains, immunohistochemistry and molecular assays for fibrosis, cellular proliferation and tissue-specific differentiation.

4.4. Comparative interpretation

It is more useful to think of the differences between fish, amphibians and mammals as variations in mechanisms of regeneration, rather than as biological differences. The local extracellular matrix, immune regulation, cellular plasticity, development stage and tissue identity all contribute to the regenerative outcomes.

These differences may be documented on a common ground by comparative histology. However, there are the need for standardization of the definition of severity of injury, sampling time, tissue orientation and outcome measures in comparisons.

Table 1. Comparative histological features of regeneration in selected vertebrate models

Model group	Representative context	Histological features to assess	Interpretive caution
Fish (e.g., zebrafish)	Cardiac and appendage regeneration	Tissue continuity; cell proliferation; provisional matrix and remodeling; residual fibrosis	Capacity varies by organ, injury model, and species (Brezitski et al., 2021; Bertozzi et al., 2021).
Amphibians (e.g., salamanders)	Limb regeneration and blastema formation	Wound epidermis; blastema cellularity; proliferating progenitors; reinnervation and redifferentiation	Morphology alone cannot determine cell lineage; lineage-tracing evidence is needed (Bassat & Tanaka, 2021; Min & Whited, 2023; Kim & Whited, 2024).
Mammals	Skin, cardiac, and other organ repair	Inflammatory infiltrate; collagen distribution; tissue-specific cell renewal; restoration versus scar formation	Outcomes vary by species, age, organ, and injury; fibrosis can coexist with partial regeneration (Behmoaras et al., 2025; Liao et al., 2026).

5. Cellular and Molecular Regulation of Regeneration

5.1. Signaling pathways

Interacting signaling networks, instead of a single pathway, are responsible for regenerative responses. Other signaling pathways such as Wnt, fibroblast growth factor (FGF), transforming growth factor-beta (TGF-β) and Notch have been studied in various regenerative scenarios. (Goldman & Poss, 2020; Khyeam et al., 2021; Bangru et al., 2026). (Stoick-Cooper et al., 2007; Zhao et al., 2014; Bangru et al., 2026).

Along these pathways they can affect cell proliferation and differentiation, migration and matrix remodeling. Their functions are dependent on the tissue, the time and the cell, as well as interactions with other signaling systems. A pathway that was found to be involved in regeneration in one model can be involved in fibrosis or in changes in differentiation in another model.

The spatial and temporal control of signals that regulate regeneration has recently been stressed. The presence of a particular signal is important but so is the timing, location, and duration of signals.

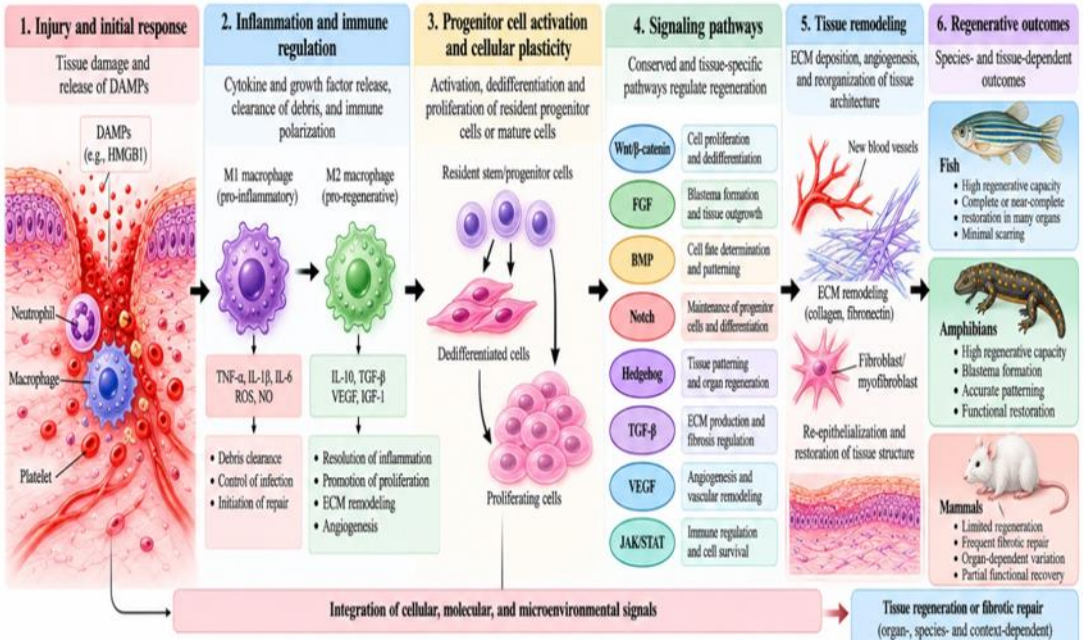


Figure 3. Conceptual overview of cellular and molecular signaling pathways involved in tissue regeneration in vertebrates.

5.2. Stem cells and cellular plasticity

Multiple organs in vertebrates have renewal by stem cells and tissue specific progenitors. They are active in local niches that give them structural, biochemical and cellular signals. (Jopling et al., 2010; Rinkevich et al., 2011; Seifert et al., 2012).

In some models of regeneration, differentiated cells also play a role in a change in cell state and a renewed proliferation. There are, however, different biological processes that include dedifferentiation, trans differentiation and stem-cell mediated regeneration and should not be confused.

The only way to determine the origin of new cells would be the original tissue type that they have assumed a new appearance. The best conclusion is one that is supported by evidence from lineage tracing and/or cell markers or complementary molecular evidence.

5.3. Immune regulation

Removal of cellular debris, secretion of signaling molecules and interaction with the progenitor cells and fibroblasts can all help to regulate regeneration mediated by immune cells. The effect of immune activation will differ depending on the level, the duration and the cell type(s) of the activation. (Behmoaras et al., 2025; Bangru et al., 2026). (Godwin et al., 2013; Aurora et al., 2014; Behmoaras et al., 2025).

An uncontrolled inflammatory response can promote ongoing injury to tissues and fibrosis, while an appropriate inflammatory response can help to repair tissues. The mechanisms discussed highlight the need to consider the immune response in the context of its anatomical and temporal localization. (Godwin et al., 2013; Aurora et al., 2014; Wynn & Ramalingam, 2012; Behmoaras et al., 2025).

5.4. Fibrosis & Regenerative Failure

Fibrosis is defined by an abnormal and/or chronic deposition of the extracellular-matrix components, especially collagen. It can occur when the process of injury, inflammation and matrix remodeling are not regulated. (Wynn & Ramalingam, 2012; Eming et al., 2014; Mayorca-Guiliani et al., 2025).

Histologically, fibrosis can manifest as increased areas of connective tissues, distorted tissue margins and disruption of normal tissue patterns. The diagnosis of fibrotic changes, however, should be based on other staining, if necessary, with molecular markers in support.

There is not a "one or the other" relationship between regeneration and fibrosis. A partial restoration of the tissue can take place and some areas may have some scarring. Therefore, quantitative methods would be more preferred than the classification based on the presence or absence of fibrosis.

Table 2. Histological endpoints and complementary methods for regeneration studies

Research question	**Histological endpoint**	**Complementary method / caveat**
Has the wound closed?	Epithelial continuity and wound-edge morphology	Serial sections and consistent sampling time; closure alone is not regeneration.
Are cells proliferating?	Mitotic figures and spatial distribution of proliferating cells	Ki-67/PCNA immunostaining with species-validated reagents and controls.
Is fibrosis present?	Collagen-rich area and disruption of normal architecture	Masson's trichrome or picrosirius red; standardize staining and image thresholds.
Has tissue identity been restored?	Organization of tissue-specific structures and differentiated cell types	Combine morphology with lineage tracing or validated molecular markers.
Are comparisons reproducible?	Predefined morphometric measurements across biological replicates	Calibrated imaging, blinded scoring, and animal—not field—as the biological unit.

Table 3. Comparative summary of selected published studies on tissue regeneration in vertebrates

Author (year)	Animal species	Tissue/organ	Main reported finding	Source / DOI
Poss et al. (2002)	Zebrafish (Danio rerio)	Heart	Adult zebrafish restored resected ventricular tissue; new myocardium replaced the initial wound region over time.	Science 298:2188–2190. https://doi.org/10.1126/science.1077857
Jopling et al. (2010)	Zebrafish (Danio rerio)	Heart	Lineage tracing showed that regenerated cardiomyocytes arose mainly from pre-existing cardiomyocytes through dedifferentiation and proliferation.	Nature 464:606–609. https://doi.org/10.1038/nature08899
Kikuchi et al. (2010)	Zebrafish (Danio rerio)	Heart	Genetic lineage tracing identified gata4-positive cardiomyocytes as a major source of regenerated myocardium.	Nature 464:601–605. https://doi.org/10.1038/nature08804
Godwin et al. (2013)	Axolotl (Ambystoma mexicanum)	Limb	Macrophage depletion impaired limb regeneration, showing that macrophages contribute to a regenerative tissue environment.	PNAS 110:9415–9420. https://doi.org/10.1073/pnas.1300290110
Porrello et al. (2011)	Neonatal mouse (Mus musculus)	Heart	One-day-old neonatal mice showed a transient regenerative response after resection; this capacity was largely lost by postnatal day 7.	Science 331:1078–1080. https://doi.org/10.1126/science.1200708
Gemberling et al. (2013) — Review	Zebrafish (Danio rerio)	Multiple tissues	Review synthesized evidence for regeneration in heart, fin, retina, brain, spinal cord, and other	Trends Genet. 29:611–620. https://doi.org/10.1016/j.tig.2013.07.003

Author (year)	Animal species	Tissue/organ	Main reported finding	Source / DOI
			tissues; not a single original experiment.	
Gurtner et al. (2008) — Review	Mammals / human wound-healing literature	Skin and wounds	Review described cellular and molecular processes in wound repair and the common role of scar formation in adult mammalian healing.	Nature 453:314–321. https://doi.org/10.1038/nature07039

6. Histological Techniques for Investigating Regeneration

6.1. Hematoxylin and eosin staining

Hematoxylin and eosin (H&E) stain is still the basic tool used for the evaluation of tissue architecture. It allows analysis of the morphology of cells, continuity of the epithelium, inflammatory infiltrate, necrosis, and overall structure. (Bancroft & Gamble, 2019; Kiernan, 2015).

Its main benefit is to be easily accessible and applicable. H&E, however, is not able to identify all cell types or molecular mechanisms and can be subject to certain biases due to tissue processing, section orientation, and experience of the observer.

6.2. Special stains

Special stains can help to bring out the color of certain components of tissues. One of the most popular stains used with Masson trichrome is for collagen-rich connective tissue from other tissues. Others stains can be chosen based on the tissue and question of the study.

Use of special stains should be with validated protocols and appropriate controls. When comparing two numbers, it is necessary to stain the same number of cells and have the same acquisition conditions.

6.3. Immunohistochemistry

Immunohistochemistry (IHC) is a technique that allows the detection and localization of specific proteins in tissue sections. Cellular proliferation, populations of immune cells, extracellular-matrix components or tissue-specific differentiation can be studied as markers depending on the biological question.

The selection of markers should be justified based on the species, tissue and antibody validation. The presence of positive immunohistochemically signals does not alone indicate the presence of a cell that has regenerated a specific tissue structure.

6.4. Digital histomorphometry

Various quantitative data such as tissue thickness, cellular density, collagen positive area and other pre-defined morphological data can be obtained by digital image analysis. It also aids in the documentation of the procedure used for measuring and assess repeatability of the observer. (Bancroft & Gamble, 2019; Schindelin et al., 2012).

The use of reliable histomorphometry requires calibration of images, consistency in the direction of the sections taken, prespecified sampling rules and selection of the biological experimental unit. More than one microscopic field from a single animal is not necessarily considered as independent biological replicates.

6.5. Integration with molecular methods

Changes seen in the transcriptomes and proteomes of plants as well as other molecular techniques can detect changes that are hard to deduce from morphology. These methods, when used in conjunction with histology, can also be used to connect the molecular signals to their spatial tissue context.

There are advanced methods that can be used to enhance the mechanistic interpretation but they must be carefully validated, statistically analyzed and have proper sampling. Well-designed conventional histology could be a good first step and then moving on to more complex techniques, if resources in the lab are limited.

6.6. Integrative interpretation of histological evidence

A well-developed understanding of regeneration needs to be based on three elements: morphology, timing, and mechanism. The regeneration of a wound is not complete enough by itself; the matrix of the newly formed tissue must also be investigated for organization, differentiation and persistence of matrix associated with scar. Highly regenerative models, such as salamanders and zebrafish, have a temporally coordinated response of cell proliferation and tissue patterning, however, regenerated cells are not always fully differentiated and can't always develop into normal tissue architecture (Bassat & Tanaka, 2021; Bertozzi et al., 2021). Macrophages and matrix accumulation may be responsible for determining whether or not repair is successful or whether it leads towards fibrosis in mammalian tissues (Behmoaras et al., 2025; Mayorca-Guiliani et al., 2025). Histological studies should therefore have a primary endpoint that is defined beforehand and include representative micrographs together with quantitative measurement(s) and if mechanistic claims are included, a validated molecular or lineage based evidence.

6.7. A synthesis of evidence in selected model systems

Zebrafish research demonstrates that the adult cardiomyocyte and other resident cell populations can play a role in cardiac repair, although the reparative tissue may consist of undifferentiated cells or be fibrotic (Poss et al., 2002; Jopling et al., 2010; Kikuchi et al., 2010; Bertozzi et al., 2021). The blastema formation, cell plasticity and need for a temporal coordinated wound environment are highlighted by comparative work in salamanders (McCusker & Gardiner, 2011; Tanaka, 2016; Bassat & Tanaka, 2021). The results of regeneration are not as straightforward as the regeneration/scar dichotomy suggests; there are important exceptions in neonatal cardiac regeneration and examples of specialized regeneration in adult mammals (Porrello et al., 2011; Aurora et al., 2014; Liao et al., 2026). The results of these findings justify the choice of using organ-specific histological endpoints instead of general species-level endpoint products.

7. Limitations of Current Evidence

The species, the tissue type of plant material, the injury model, the sampling interval, the staining method and the definition of the regeneration outcome vary between comparative regeneration studies. The differences make it complicated to directly compare studies. (Khyeam et al., 2021; Goldman & Poss, 2020; Liao et al., 2026).

Variability in tissue processing, section orientation, fixation quality and observer differences may also affect the histological interpretation. Furthermore, the lineage of regenerated cells can often be determined by morphology alone, and the causal role of a signaling pathway cannot be determined by it. (Goldman & Poss, 2020; Kim & Whited, 2024).

A further drawback is that results in highly regenerative model species might not directly apply to mammals or to other species of the same vertebrate class. It is important to remember that experimental results may have different implications for different species.

Standardized histological endpoints, clear descriptions of sampling procedure, quantitative

image analysis and combination of the morphological results with validated molecular methods would be useful for future studies.

8. Future Research Directions

Standardization of measurement of regenerative outcomes across species and tissues should be prioritized for future studies. This would make comparisons more accurate and make it easier to understand the differences in the regen.

The combination of traditional histology, with digital image analysis and molecular profiling, provides new possibilities to study the relationships between cellular behaviors, tissue architecture and signaling activity. Spatially resolved methods could also help to define the differences and similarities in the regenerating processes occurring at the wound edge, the regenerating area and the surrounding intact tissue.

Studies of the comparative research should also take into account the impact of age, developmental stage, tissue identification and injury severity. These factors can impact the ratio of repair to fibrosis.

Last but not least, it is important to note that in zoological labs with limited resources, accessible histological techniques are still important. The use of a standardized H&E staining, clearly defined morphometric measurements and clear documentation of images can give reproducible baseline evidence, and help inform future mechanistic studies.

9. Conclusion

In vertebrates, the process of tissue regeneration is a complex one that requires coordinated responses of cells, regulation of immune system, remodeling of the extracellular matrix, and tissue-specific signaling. Complementary models of fish, amphibians, and mammals will be used to elucidate structural restoration mechanisms or the mechanisms that promote fibrotic repair mechanisms. Histology is an important tool in comparative research on regeneration, as it provides a record of the structure and status of tissues during the regeneration process. However, in cases where the interpretation of the mechanism requires associated morphological evidence, it should be interpreted in conjunction with the appropriate quantitative and molecular evidence. Standardizing histological, morphometric and molecular methods to integrate them into the same study will enhance the value of comparative studies and better inform the understanding of the biological mechanisms that govern the regenerative outcomes from one species to another in vertebrates.

References

Al-Shammai, M., Alanazi, J. S., Nazel, S., Alanazi, A., Hejazi, A., Achira, M., Alosaimi, A., Alhamidi, A., Alsaigh, D., & Aldahan, M. (2024). Cardiac cell regeneration in zebrafish: A systematic review study. International Journal of Medicine in Developing Countries, 8(11), 3169–3180. https://doi.org/10.24911/IJMDC.51-1724172304

Alibardi, L. (2010). Morphological and cellular aspects of tail and limb regeneration in lizards. Advances in Anatomy, Embryology and Cell Biology, 207, 1–109. https://doi.org/10.1007/978-3-642-03733-7

Appendage Regeneration in Vertebrates: What Makes This Possible? (2021). Cells, 10(4), 869. https://doi.org/10.3390/cells10040869

Aurora, A. B., Porrello, E. R., Tan, W., Mahmoud, A. I., Hill, J. A., Bassel-Duby, R., Sadek, H. A., & Olson, E. N. (2014). Macrophages are required for neonatal heart regeneration. Journal of Clinical Investigation, 124(3), 1382–1392. https://doi.org/10.1172/JCI72181

Bancroft, J. D., & Gamble, M. (Eds.). (2019). Theory and Practice of Histological Techniques (8th ed.). Elsevier.

Bangru, S., Diegmiller, R., Di Talia, S., & Poss, K. D. (2026). Signal control during tissue regeneration in adult animals. Nature Reviews Molecular Cell Biology, 27, 316–335. https://doi.org/10.1038/s41580-025-00917-1

Bassat, E., & Tanaka, E. M. (2021). The cellular and signaling dynamics of salamander limb regeneration. Current

Opinion in Cell Biology, 73, 117–123. https://doi.org/10.1016/j.ceb.2021.07.010

Behmoaras, J., Mulder, K., Ginhoux, F., et al. (2025). The spatial and temporal activation of macrophages during fibrosis. Nature Reviews Immunology, 25, 816–830. https://doi.org/10.1038/s41577-025-01186-x

Bertozzi, A., Wu, C.-C., Nguyen, P. D., Dalvoy Vasudevarao, M., Mulaw, M. A., Koopman, C. D., de Boer, T. P., Bakkers, J., & Weidinger, G. (2021). Is zebrafish heart regeneration "complete"? Lineage-restricted cardiomyocytes proliferate to pre-injury numbers but some fail to differentiate in fibrotic hearts. Developmental Biology, 471, 106–118. https://doi.org/10.1016/j.ydbio.2020.12.004

Calve, S., Odelberg, S. J., & Simon, H.-G. (2010). A transient extracellular matrix directs regenerative potential in the zebrafish ear. Developmental Biology, 344(1), 271–285. https://doi.org/10.1016/j.ydbio.2010.04.027

Eming, S. A., Martin, P., & Tomic-Canic, M. (2014). Wound repair and regeneration: Mechanisms, signaling, and translation. Science Translational Medicine, 6(265), 265sr6. https://doi.org/10.1126/scitranslmed.3009337

Gemberling, M., Bailey, T. J., Hyde, D. R., & Poss, K. D. (2013). The zebrafish as a model for complex tissue regeneration. Trends in Genetics, 29(11), 611–620. https://doi.org/10.1016/j.tig.2013.07.003

Godwin, J. W., Pinto, A. R., & Rosenthal, N. A. (2013). Macrophages are required for adult salamander limb regeneration. Proceedings of the National Academy of Sciences, 110(23), 9415–9420. https://doi.org/10.1073/pnas.1300290110

Goldman, J. A., & Poss, K. D. (2020). Gene regulatory programmes of tissue regeneration. Nature Reviews Genetics, 21, 511–525. https://doi.org/10.1038/s41576-020-0239-7

Gurtner, G. C., Werner, S., Barrandon, Y., & Longaker, M. T. (2008). Wound repair and regeneration. Nature, 453, 314–321. https://doi.org/10.1038/nature07039

Jamwal, V. S., Masselink, W., Crawford, K., & Murawala, P. (2026). Non-canonical muscle stem cells and other lessons from regenerative vertebrates. npj Regenerative Medicine, 11, Article 20. https://doi.org/10.1038/s41536-026-00468-9

Jopling, C., Sleep, E., Raya, M., Martí, M., Raya, A., & Belmonte, J. C. I. (2010). Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. Nature, 464, 606–609. https://doi.org/10.1038/nature08899

Khyeam, S., et al. (2021). Genetic, epigenetic, and post-transcriptional basis of divergent tissue regenerative capacities among vertebrates. Advanced Genetics, 2(2), e10042. https://doi.org/10.1002/ggn2.10042

Kiernan, J. A. (2015). Histological and Histochemical Methods: Theory and Practice (5th ed.). Scion Publishing.

Kikuchi, K., Holdway, J. E., Werdich, A. A., Anderson, R. M., Fang, Y., Egnaczyk, G. F., Evans, T., MacRae, C. A., Stainier, D. Y. R., & Poss, K. D. (2010). Primary contribution to zebrafish heart regeneration by gata4+ cardiomyocytes. Nature, 464, 601–605. https://doi.org/10.1038/nature08804

Lange, C., & Brand, M. (2020). Vertebrate brain regeneration—a community effort of fate-restricted precursor cell types. Current Opinion in Genetics & Development, 64, 101–108. https://doi.org/10.1016/j.gde.2020.06.014

Lebedeva, L., Zhumabayeva, B., Gebauer, T., Kisselev, I., & Aitasheva, Z. (2020). Zebrafish (Danio rerio) as a model for understanding the process of caudal fin regeneration. Zebrafish, 17(6), 359–372. https://doi.org/10.1089/zeb.2020.1926

Liao, K., Babal, Y. K., & Lewandowski, S. A. (2026). Mammalian models of adult tissue regeneration. Annual Review of Animal Biosciences, 14, 297–317. https://doi.org/10.1146/annurev-animal-030424-071428

Mayorca-Guiliani, A. E., Leeming, D. J., Henriksen, K., Mortensen, J. H., Nielsen, S. H., Anstee, Q. M., Sanyal, A. J., Karsdal, M. A., & Schuppan, D. (2025). ECM formation and degradation during fibrosis, repair, and regeneration. npj Metabolic Health and Disease, 3, Article 25. https://doi.org/10.1038/s44324-025-00063-4

McCusker, C., & Gardiner, D. M. (2011). The axolotl model for regeneration and aging research: A mini-review. Gerontology, 57(6), 565–571. https://doi.org/10.1159/000323761

Min, S., & Whited, J. L. (2023). Limb blastema formation: How much do we know at a genetic and epigenetic level? Journal of Biological Chemistry, 299(2), Article 102858. https://doi.org/10.1016/j.jbc.2022.102858

Porrello, E. R., Mahmoud, A. I., Simpson, E., Hill, J. A., Richardson, J. A., Olson, E. N., & Sadek, H. A. (2011). Transient regenerative potential of the neonatal mouse heart. Science, 331(6020), 1078–1080. https://doi.org/10.1126/science.1200708

Poss, K. D. (2010). Advances in understanding tissue regenerative capacity and mechanisms in animals. Nature Reviews Genetics, 11, 710–722. https://doi.org/10.1038/nrg287

Poss, K. D., Wilson, L. G., & Keating, M. T. (2002). Heart regeneration in zebrafish. Science, 298(5601), 2188–2190. https://doi.org/10.1126/science.1077857

Pérez-Montes, C., Lavado-Fernández, E., Martínez-Rojo, I., Santos-Ledo, A., & García-Macia, M. (2026). Eat, heal, repeat: How zebrafish master regeneration. International Review of Cell and Molecular Biology, 404, 81–134. https://doi.org/10.1016/bs.ircmb.2025.10.008

Rinkevich, Y., Lindau, P., Ueno, H., Longaker, M. T., & Weissman, I. L. (2011). Germ-layer and lineage-restricted stem/progenitors regenerate the mouse digit tip. Nature, 476, 409–413. https://doi.org/10.1038/nature10346

Rodrigues, M., Kosaric, N., Bonham, C. A., & Gurtner, G. C. (2019). Wound healing: A cellular perspective. Physiological Reviews, 99(1), 665–706. https://doi.org/10.1152/physrev.00067.2017

Sammarco, M., & McCusker, C. (2026). Limb regeneration from amphibians to mammals. Development, 153(19). https://doi.org/10.1242/dev.205585

Schindelin, J., Arganda-Carreras, I., Frise, E., et al. (2012). Fiji: An open-source platform for biological-image analysis. Nature Methods, 9, 676–682. https://doi.org/10.1038/nmeth.2019

Seifert, A. W., Monaghan, J. R., Voss, S. R., & Maden, M. (2012). Skin regeneration in adult axolotls: A blueprint for scar-free healing in vertebrates. PLoS ONE, 7(4), e32875. https://doi.org/10.1371/journal.pone.0032875

Takeo, M., Lee, W., & Ito, M. (2015). Wound healing and skin regeneration. Cold Spring Harbor Perspectives in Medicine, 5(1), a023267. https://doi.org/10.1101/cshperspect.a023267

Tanaka, E. M. (2016). The molecular and cellular choreography of appendage regeneration. Cell, 165(7), 1598–1608. https://doi.org/10.1016/j.cell.2016.05.038

Wynn, T. A., & Ramalingam, T. R. (2012). Mechanisms of fibrosis: Therapeutic translation for fibrotic disease. Nature Medicine, 18, 1028–1040. https://doi.org/10.1038/nm.2807

Yin, V. P., & Poss, K. D. (2008). New regulators of the regenerative response in zebrafish. Nature Chemical Biology, 4, 262–265. https://doi.org/10.1038/nchembio.87

Zhao, L., Borikova, A. L., Ben-Yair, R., et al. (2014). Notch signaling regulates cardiomyocyte proliferation during zebrafish heart regeneration. Proceedings of the National Academy of Sciences, 111(40), 1403–1412. https://doi.org/10.1073/pnas.1407462111