Drug Discovery and Molecular Docking (DDMD)
ISSN: 3107-6688 | Vol. 01, Issue 03, Sep-Dec. 2025 | pp. 26-43

Single-Cell Transcriptomics to Map Cellular Heterogeneity in Tissue Regeneration Models

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Abstract:

Single-cell transcriptomics has also revolutionized regenerative biology by offering a resolution never before achieved into both the cellular and molecular pathways mediating tissue repair in different animal models. Through profiling of thousands of individual cells throughout the regenerative process, this technology has revealed widespread cell heterogeneity, lineage-biased progenitor subclusters, active dedifferentiation processes, and pivotal immune interactions between niche that have been lost by bulk estimates. Zebrafish, axolotl, planarian, and mammalian tissue studies demonstrating conservation and species-specificity of stem-cell plasticity, immune modulation and main signaling pathways, Wnt, Fgf, Notch and Tgf- beta, provide insights into conserved and species-specific mechanisms of these developmental changes. Recent methodological progress such as optimised dissociation-based assays, single-nucleus RNA sequencing and the ability to compute lineage relations and find uncommon regenerative cell states have facilitated the reconstitution of lineages and the recovery of rare regenerative cell states. In spite of issues associated with dissociation artifacts, the loss of spatial context, and the variation between studies, the single-cell technologies still transform the knowledge of the regeneration and promise a lucrative translational outlook on generating regenerative therapies that are more specific. This is a review that compiles the recent evidence, analyzes the methodology strengths and weaknesses, and reports the future prospects in bringing together multi-omics, spatial mapping, lineage tracing and cross-species comparisons to complement the mechanistic aspect and hastens the applications of regenerative medicine.

Keywords: Single-Cell RNA Sequencing, Tissue Regeneration, Stem-Cell Plasticity, Immune Regulation, Lineage Trajectories, Spatial Transcriptomics, Multi-Omics, Regenerative Medicine.

Received: Sep, 18,2025 **Revised: Oct. 20,2025** **Accepted: Nov. 29,2025** **Published: Dec. 10, 2025**

DOI: https://doi.org/10.64474/3107-6688.Vol1.Issue3.3

https://ddmd.nknpub.com/1/issue/archive

1. Introduction

The single-cell transcriptomics has become one of the most revolutionary tools in the contemporary biology and it is providing unprecedented opportunities into cellular and molecular processes that underlie tissue regeneration[1]. Animal regeneration of the whole body, like the amazing whole-body regeneration of planarians, and smaller-scale but still functionally important regeneration in mammals, relies on an immensely well-coordinated interaction between stem cells, immune cells, components of the niche and signaling pathways. In the past, studies of regeneration have depended on bulk gene-expression methods and histological analysis that obscured heterogeneity of cells and resulted in a lack of access to rare or transient cell states. In comparison, single-cell RNA sequencing (scRNA-seq) allows the exact profiling of thousands of single cells, including the variety of cell identities and dynamic changes necessary to enable successful tissue repair[2]. This new technology has created opportunities in the study of how various organisms coordinate complex regenerative programs and the reason why regenerative abilities differ so radically among species.

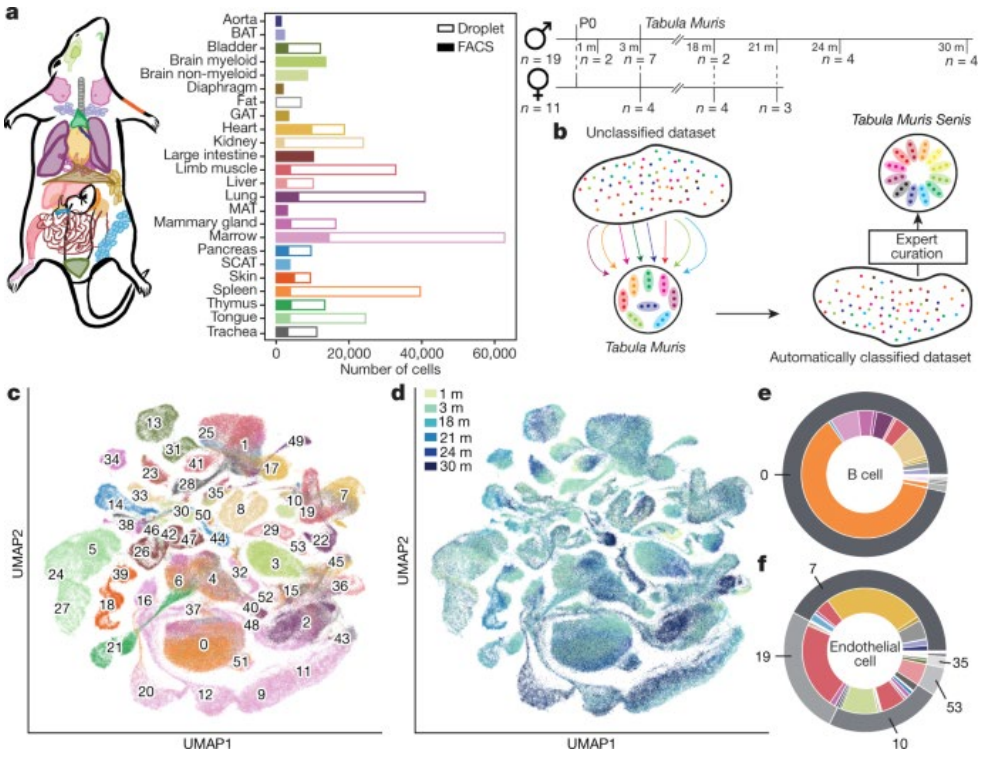

Figure 1: Single-Cell Transcriptomics[3]

The recent years have seen the use of scRNA-seq on animal regeneration models revealing a surprising amount of cellular diversity and plasticity previously unknown. The research on zebrafish caudal fins, axolotl limbs, planarian tissues and mammalian organs like liver, skin

and skeletal muscle has revealed new populations of progenitors, new functions of immune cells and complex interactions in the microenvironment that ultimately determine regenerative outcomes. These findings have not only enhanced our basic conception of regeneration but also upset some old follies concerning limitation of lineage, dedifferentiation ability and the effect of immune and stromal cells[4]. With the fast growth of the field, there exists an increasing need to review the emerging information and to assess how single-cell technologies are transforming regeneration biology in various animal systems.

1.1 Background and Context

Tissue regeneration is a biological process that is variable across the animal species. Whole body parts are replaceable by highly regenerative organisms e.g. planarians, hydra, and axolotls, whereas in mammals' regenerative ability is usually restricted to individual organs. Conventional methods were deprived of the solution to separate the interlocked cellular networks to this variability[5]. Single-cell transcriptomics addresses these shortcomings by undertaking the study of single cells in high resolution, making it possible to describe cell types, transitional conditions, signaling pathways, and lineage relationships. Its use in a wide variety of regeneration models has given more than ever before the detail about the formation of blastemas, the response of stem cells to injury, and the role of immune and stromal cells in regenerative success.

1.2 Objectives of the Review

The main aim of the review is to summarize the increasing number of studies applying single-cell transcriptomics to map cellular heterogeneity in animal tissue regeneration models. Particularly, this review will set out to:

- To map cellular heterogeneity during tissue regeneration using single-cell transcriptomics across diverse animal models.
- To identify regeneration-specific stem/progenitor states, immune cell dynamics, and niche interactions revealed through scRNA-seq.
- To evaluate and compare methodologies—including dissociation techniques, scRNA/snRNA-seq platforms, and computational tools—used for profiling regenerating tissues.
- To analyze conserved and species-specific signaling pathways and regeneration mechanisms uncovered through single-cell datasets.
- To highlight current limitations and propose future research directions integrating multi-omics, spatial transcriptomics, and lineage-tracing approaches to advance regenerative biology.

1.3 Importance of the Topic

The analysis of the molecular logic that enables specific organisms to regenerate lost tissues with precision has only been unraveled via an understanding of regeneration at the single-cell

level. These findings have far-reaching implications to both evolutionary and developmental biology and to the translational arena, including regenerative medicine, tissue engineering, and disease modeling[6]. Through unearthings of the cellular diversity and dynamic shifts that spearhead tissue repair, single-cell transcriptomics holds the possibility of discovering pro-regenerative pathways, revealing concealed cellular competencies in mammals, and informing future directions on how to enhance the healing results. With the ongoing and continuous improvements of resolution, accuracy, and capacity of integrating ideas, single-cell-based regenerative studies can change the potential of understanding and controlling tissue repair in all organisms.

1. INSIGHTS, METHODS, AND EVALUATION OF SINGLE-CELL TRANSCRIPTOMICS IN REGENERATIVE BIOLOGY

Single-cell transcriptomics has shown the nature of several regenerative cell populations and lineage state in various animal models including zebrafish, axolotl, planarian and mice which has identified key progenitors, immune modulators, and signals pathways that occur when tissue is injured[7]. Such insights have been backed by sophisticated means of dissociation editing, single nucleus sequencing, and the use of computational software that determines cell states and interactions at a very fine scale. Although the methodology facilitates the identification of rare cell types and regeneration-specific markers, it faces dissociation artifact, loss of contextical information, is costly, and differs across studies[8].

2.1 Summary of Key Research Studies

- **Zebrafish Caudal Fin Regeneration:** Wide scRNA-seq analysis of caudal fin regeneration in zebrafish has revealed very diverse populations of cells in blastema formation. Profiling has also revealed several types of osteoblasts, such as pre-osteoblasts, mature osteoblasts, and osteolineage cells of regeneration. It was demonstrated that fibroblasts are categorized into different subtypes that are involved in the remodelling of the extra cellular matrix and positional signalling. One of the major findings is the discovery of lineage-restricted progenitors which retain tissue identity, which is in contrast with lineage-plastic cells which can acquire several fates during regeneration[9].
- **Axolotl Limb Regeneration:** The axolotl limb regeneration has been studied in single cell transcriptomic profiling and reported the dynamic cellular changes of epidermal, mesenchymal and immune-derived populations during blastema formation. There is a transcriptional signature in the wound epidermis that is rich in growth factors which direct underlying mesenchymal cells. The dedifferentiated fibroblasts that were presumed to be the same were found to be various subpopulations with certain positional identities preserved by HoxA, HoxD, Meis1, and Gata transcription factors. The positional memory cues enable regeneration to reproduce original limb structure.

- **Planarian Whole-body Regeneratio:** High-resolution single cell transcriptomics has been used in planarian regeneration to generate some of the most detailed cellular atlases in regenerative biology. Over 20 different neoblast stem-cell populations are described and each of these has a specific and determined lineage, including muscle, neural, epidermal, digestive and excretory. The hierarchical architecture of regeneration has been illuminated by these studies that demonstrated that pluripotent neoblasts migrate into lineage-activated conditions through the mediation of the chromatin modifier Smed-egr-5 and by the mitogen-activated ERK and JNK signaling pathways[10].
- **Mouse Liver Regeneration (Partial Hepatectomy Model):** Single cell transcriptomics on the mouse liver regeneration model have shown highly heterogeneous hepatocyte populations as well as zonations changes in dynamics as the cell changes its metabolic and proliferative state. Hepatocytes around central and portal areas have particular regenerative phenotypes and aberrant progenitor like hepatocyte-state resultantly occur during regeneration. The immune response is coordinated by kupffer cells, monocyte-derived macrophage and innate lymphoid cells, and adaptive T-cell subsets control cell proliferation and tissue remodeling of hepatocytes.
- **Mouse Skeletal Muscle Injury (Satellite Cell Activation):** scRNA-seq of regenerating skeletal muscle has revealed the events in colonizing quiescent satellite cells into activated myoblasts, proliferating progenitors, and differentiating myocytes. These studies have found intermediate precursors with Myf5, MyoD and Myogenin expression patterns. Such so called non-essential fibro-adipogenic progenitors (FAPs) were found to serve as a major regulator of the regenerative niche, producing extracellular matrix protein and cytokine which helps maintain myogenesis.
- **Skin Wound Healing in Mice:** Profiling of single-cells of mouse skin following injury has resulted in the detailed mapping of the wound epidermal cells such as the migratory keratinocytes, activated stem cells, and wound-edge-specific populations. There is also a significant heterogeneity in dermal fibroblasts which are subdivided into those focused on ECM deposition, inflammation modulation and hair follicle regeneration. The angiogenesis, re-epithelialization, and ECM turnover are regulated stages of macrophage progression between pro-inflammatory and pro-regenerative phenotypes[11].

2.2 Methodologies and Their Findings

- **Sample Preparation Approaches:** In regeneration studies, single-cell transcriptomics is dependent on optimized tissue dissociations protocols based on each animal model. Repulsory dissociation of the zebrafish fins or axolotl limbs enables the survival of delicate blastema, and cold-active enzymes are commonly utilized to reduce the transcriptional stress reactions. Where mechanical dissociation is difficult, such as in tissues such as mouse muscle and liver, single-nucleus RNA sequencing (snRNA-seq) is more likely to be preferred to minimize cell-type bias and to sample rare or large cell

types such as hepatocytes. High-throughput droplet-based systems such as 10x Genomics Chromium can profile tens of thousands of cells at a time point, and are necessary to monitor a cellular transition in a dynamic fashion[12].

- **Computational Tools and Analyses:** The scRNA-seq datasets related to regeneration contain some advanced computational frameworks. The clustering methods like Seurat, Scanpy single out cell groups and states of transcription with high fidelity. Monocle, RNA Velocity and PAGA are phenotype inference tools that can be used to reconstruct developmental transitions, demonstrating the progression of progenitor cells through regeneration-specific intermediate states. Prediction of ligand-receptor interactions is possible using CellPhoneDB and NicheNet, and the point of contact between immune cells and niche fibroblasts with regenerating stem or progenitor populations can be characterized.
- **Overall Findings and Significance:** Scientific progress in methodologies has allowed scientists to discover new regenerative cell types, transcriptional reprogramming events, and niche interactions signaling that previously were not available. The strategies, along with their contribution to a more profound mechanistic insight, do form the standardized frameworks to compare regenerative mechanisms between the most differentiated species[13].

2.3 Critical Evaluation

- **Strengths**

Single-cell transcriptomics offers some important advantages that render it an effective method of tissue regeneration research on animal models. The most impressive feature about it is that it can produce high-resolution cellular images, where researchers can differentiate various cell states, which could not be identified in bulk (ones that were not previously observed by bulk measurements)[14]. High granularity also allows recognition and definition of rare progenitor, stem, or immune cell groups which are important in the process of regeneration development and progression. The other significant strength is the ability to map lineage trajectories, aiding in the tracking of how individual cells change their quiescence to an activation, differentiation, or dedifferentiation state in case of regeneration[15].

- **Weaknesses**

However, in spite of its benefits, single-cell transcriptomics also possesses a number of drawbacks, which should be taken into account when interpreting the findings. One of the biggest challenges is that the dissociation artifacts are introduced since enzymatic and mechanical dissociation of tissues may change transcriptional profiles, which may also produce artificial cell states or conceal natural ones[16]. The other limitation is the loss of spatial information by nature as the dissociated cells lose their tissue context and can only be used to learn about spatial patterning and cell-cell interactions with spatial transcriptomic methods. It

is also resource-intensive, as the required resources to execute the technique are high and this may limit the size of samples or time resolution in large-scale regeneration experiments.

2. THEMATIC INSIGHTS INTO REGENERATION REVEALED BY SINGLE-CELL TRANSCRIPTOMICS

The transcriptomics of individual cells has revised the concept of regeneration through the mapping of the plasticities of stem cells, immune systems, and remodelling of niches across species[17]. The state of the art has developed that planarian neoblasts have several lineage-biased subclusters, axolotl fibroblasts experience reversible dedifferentiation, and mammalian satellite cells traverse clear intermediate states during muscle repair. The immune cells are demonstrated to play a leading role in the regulation of regeneration, including macrophages that alternate between pro-regenerative and inflammatory phenotypes, neutrophils that trigger initial reactions, and T cells that control proliferation of hepatocytes. Other microenvironmental factors that are also important as highlighted by these studies include ECM-producing fibroblasts, fibro-adipogenic progenitors and endothelial cells which modulate and maintain the regenerative tissue architecture[18].

Similar findings in parallel evidence of conserved signaling pathways between stem cells, immune cells, and components of their niche, such as Wnt/ -catenin, Fgf, Notch, Tgf-b/BMP, and Hedgehog, have indicated that regeneration is was coordinated by co-ordinated paracrine signaling[19]. The comparative cross-species studies have shown that only more regenerative organisms such as planarians and axolotls possess elevated cellular plasticity and quicker recovery of inflammation compared to mammals, but the organisms have shared fundamental transcriptional modules, upon which regeneration depends. These findings combine to indicate the variety and the evolutionary consistency of regenerative processes across models of animals[20].

3.1 Stem Cell Activation and Plasticity

Study of transcriptomics in solitary cells has contributed greatly to explain the behavior and plasticity of stem-cells in regeneration[21]. Research indicates that planarian neoblasts are heterogeneous in that they have several lineage-biased subclusters, each of which is set up to different tissue fates. Fibroblasts in axolotl exhibit a striking ability at reversible dedifferentiation and change to progenitor-like states so as to regrow tissues. Both conserved and species-specific regeneration processes in mammals Skeletal muscle In mammals, especially skeletal muscle, the satellite cells undergo discrete, well-defined intermediate forms then differentiate into mature muscle fibers[22].

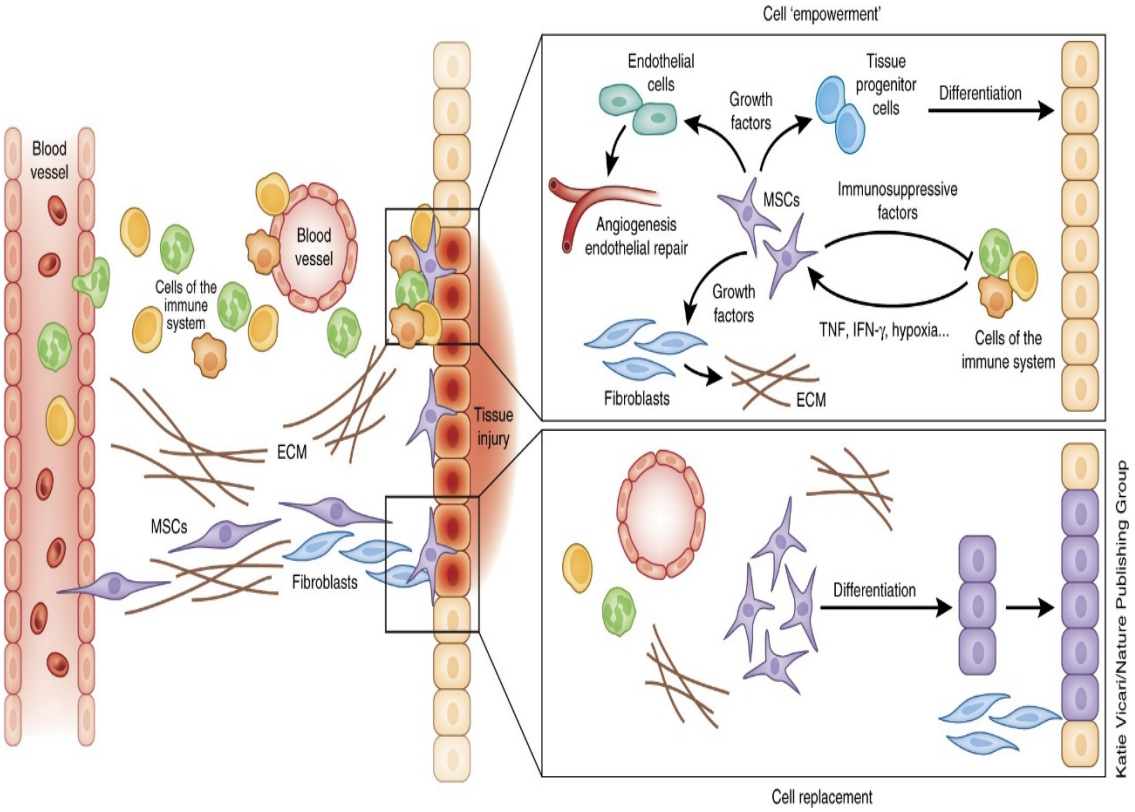

Figure 2: Stem Cell Activation and Plasticity[23]

3.2 Immune Regulation in Regeneration

scRNA-seq has defined the immune system dynamic and critical role in the process of regeneration. Macrophages become most flexible controllers that change to pro-inflammatory or pro-regenerative states according to the stage of injury. Neutrophils set early inflammatory events in models such as zebrafish and axolotl, upon which the subsequent events of repair are then activated. T cells regulate hepatocyte proliferation and tissue remodeling in the process of mammalian liver regeneration, and this illustrates how organ-stirred immune responses can influence regeneration[24].

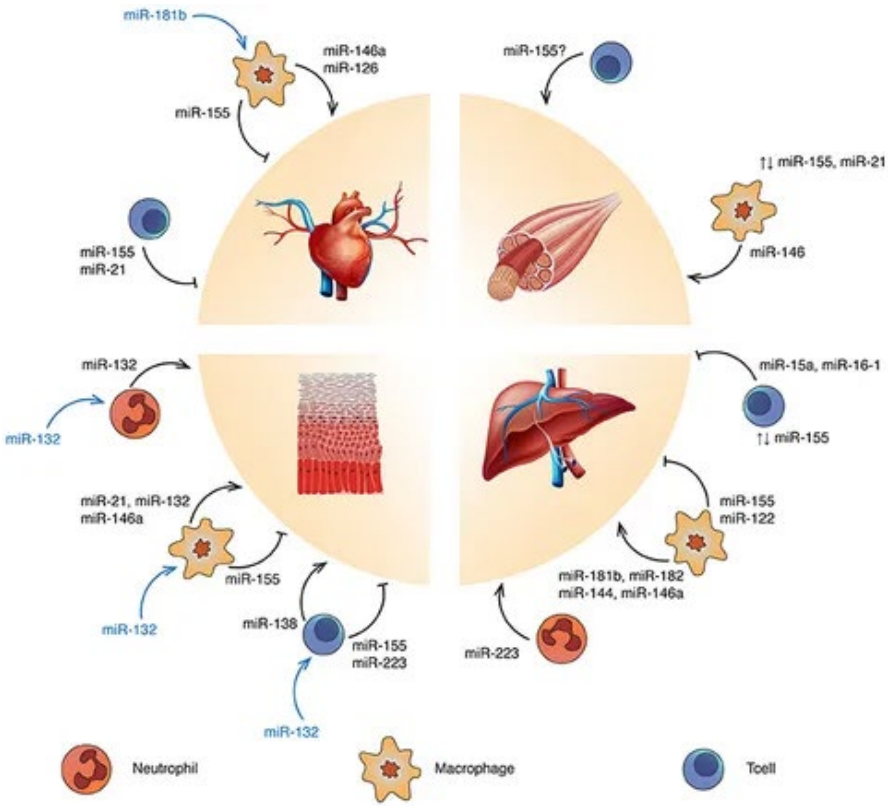

Figure 3: Immune Regulation[25]

3.3 Microenvironment and Niche Remodeling

Regeneration requires niche elements that have been discovered in animal cells that are analyzed in a singular manner. Fibroblasts of zebrafish and planarians that produce ECM facilitate the development of positional cues and structure scaffolds on new tissues. The fibro-adipogenic progenitors FAPs play a fundamental role in muscular skeletal regulation by releasing ECM molecules and cytokines that activate and differentiate satellite-cells in muscular vectors. Moreover, endothelial remodeling in regeneration of the skin and limbs is also a major characteristic whereby the regenerating tissues should have sufficient vascularity that guides them.

3.4 Signaling Pathways Orchestrating Regeneration

The essential regenerative pathways exhibit tightly controlled, cell type specific dynamics in tissue repair. Repeatedly activated signaling cascades such as Wnt/ 2-catenin, Fgf, Notch, Tgf-b/BMP and Hedgehog and through scRNA-seq ligand receptor mapping has demonstrated the role of these pathways in these regeneration models and demonstrates the molecular logic underlying regeneration across a variety of organisms.

3.5 Comparative Regeneration Across Species

Comparisons of scRNA-seq of cross-species reveal the basics of regeneration and common principles. Planarians and axolotls are highly regenerative living organisms and display cellular plasticity and extend the dedifferentiation capacity beyond that of mammals. There is also a significant difference in the inflammation healing process with fish having high transition to pro-regenerative immune conditions in contrast with slower mammalian reactions. Evolutionary distance notwithstanding, a large number of stem-cell transcriptional modules are conserved highlighting common ancestral processes of tissue repair[26].

3. FUTURE PERSPECTIVES AND EMERGING TECHNOLOGIES

Single-cell transcriptomics is in continuous development, and provides additional opportunities to comprehend tissue regeneration on their more molecular and spatial scales. A key area of future research would be to combine multi-omic strategies, i.e. single-cell epigenomics, proteomics, metabolomics, and ATAC-seq to build more detailed maps of regenerative action. The methods can be used to uncover how cell-fate choices in regeneration depend on chromatin accessibility, binding of transcription factors and networks of signaling proteins. As an illustration, combining scRNA-seq with single-cell ATAC-seq would help explain how regulatory components mediate dedifferentiation in axolotl fibroblasts or planarian neoblast prime. Equally, single-cell proteomics can help reveal post-transcriptional pathways governing immune remodeling in mammals or blastema development in zebrafish. The multi-omics datasets are becoming increasingly more important and computational models using machine learning and deep learning will become more crucial to integrate and interpret these layers of information complexity[27].

Spatially resolved transcriptomics is another improvement that breaks one of the largest limitations of scRNA-seq, the loss of spatial organization. New methods like Slide-seq, MERFISH, and seqFISH, and Visium spatial transcriptomics, allow the researcher to visualize the patterns of gene expression directly in regenerating tissues. Positional cues, niche boundaries and cell-cell communication of animal regeneration models can be mapped with even greater amounts of precision by applying these tools. An example might be the arrangement of fibroblast subtypes around axolotl blastemas, or changes in endothelial networks in mouse skin healing, or the formation of stem-cell niches by planarian regeneration. Integrating spatial data with single-cell trajectories will allow reconstructing tissue architecture dynamically as it regenerates and connect the molecular states to their physical environment[28].

The technologies of lineage tracing and genetic barcoding also are promising to answer long-standing questions regarding the cell-fate plasticity and identity transitions. The use of CRISPR-based system of lineage tracing, inducible barcoding and transgenic reporter lines are not only on the rise to trace cells in time on fish, amphibians and mammalian systems. Together with scRNA-seq, these methods can give a clear answer to the question of whether regeneration is dependent on lineage-restricted progenitors, multipotent stem cells or committing differentiated mature cells- questions that are still controversial across species. In addition,

transcriptional data in combination with live-imaging methods, including light-sheet microscopy, can be utilized to study real-time cellular events during regeneration, like migration, proliferation, and dedifferentiation. Such a multimodal functional tracking-transcriptomics combination can construct a high-resolution dynamic atlas of regeneration processes[29].

Lastly, research into regeneration in the future will be informed by the development of cross-species comparative models and computer simulation models. New algorithms will be necessary, as regenerative datasets continue to expand such as zebrafish, axolotl, planarian and mammal cell types, cellular pathways, and evolutionary phenotypes. Machine-learning tools that can compare transcriptional states between different species can aid in determining ancestral regenerative modules and understanding the reason behind the regeneration efficiency of different species. These findings might be used someday to guide bioengineering and regenerative medicine activities, including the creation of pro-regenerative gene circuits, immune response regulation, or artificial niches proficient in inducing tissue healing in mammals. The combination of these emerging technologies will not only expand the knowledge about nature on how to regenerate itself but also open the possibilities to translate major principles into therapeutic interventions in the process of repairing and regenerating tissues[30].

Table 1: Summary of Single-Cell Transcriptomics Studies on Cellular Heterogeneity and Tissue Regeneration[31]

Author(s) & Year	Study Focus	Focus Area	Methodology	Key Findings
Tepe et al. (2018)[32]	Single-cell RNA-seq of mouse olfactory bulb	Neuronal and glial heterogeneity, adult-born neurons	scRNA-seq	Revealed diverse neuronal and glial populations; identified activity-dependent transcriptional signatures; provided insights into olfactory neurogenesis and functional integration
Theocharidis et al. (2022)[33]	Single-cell transcriptomics of diabetic foot ulcers	Impaired wound healing, chronic inflammation	scRNA-seq	Identified fibroblast, immune, and endothelial subpopulations with

				altered gene expression; revealed mechanisms of chronic inflammation, fibrosis, and impaired tissue regeneration
Tower et al. (2022)[34]	Mapping regenerative and fibrotic healing after musculoskeletal injury	Regeneration vs fibrosis	scRNA-seq	Identified distinct cell states for regeneration and fibrosis; highlighted key transcriptional regulators and signaling pathways; provided potential therapeutic targets
Wang et al. (2021)[35]	Single-cell transcriptome atlas of human MSCs	Cellular heterogeneity, stem cell potential	scRNA-seq	Demonstrated functional MSC subpopulations with variable proliferation, differentiation, and immunomodulatory potential; emphasized importance of heterogeneity in therapeutic design
Wu et al. (2021)[36]	Single-cell transcriptomic analysis of pluripotent stem cell chondrogenesis	Chondrogenic differentiation, cartilage formation	scRNA-seq	Identified transitional progenitor states, regulatory transcription factors, and gene expression programs guiding chondrogenesis; provided molecular framework for cartilage tissue engineering

4. DISCUSSION

A single-cell transcriptomics study demonstrates that regeneration is dependent on varied states of stem-cell, dynamic dedifferentiation and synchronized immune-niche contacts, which present new regenerative therapy targets. Nevertheless, there are gaps because there is a spatial and temporal resolution, and there is a requirement to test it functionally. The use of multi-omics, spatial mapping, and cross-species comparison in future efforts should be combined to establish a stronger, more translational view of the working mechanisms of regenerative processes[37].

5.1 Interpretation and Synthesis of Findings

Transcriptomics on individual cells has dramatically transformed the concept of tissue regeneration by demonstrating the remarkable variety and flexibility of the cell states in a variety of animal models. The literature reviewed shows that previously thought to be rather uniform, stem cells, in fact, comprise many lineage-biased subclusters, such as in planarian neoblasts or mammalian satellite cells. Equally, reversible dedifferentiation in axolotl fibroblasts provides powerful indicators to support that regeneration is an activity of dynamic reprogramming as opposed to fixed lineage limits. Some additional insights into the immune-system indicate the presence of a macrophage, neutrophil, and T-cell subsets that orchestrate injury responses in a manner that has never been discovered before. These findings have led to the integration of successful regeneration suggesting that stem or progenitor cells carry out the process of a successful regeneration in concert with immune modulators, fibroblastic niches, vascular systems, and preserved signaling pathways. These findings, taken together, point to the conclusion that regeneration is a multi-layered, complicated biological program that is determined by the intrinsic and extrinsic cell states[38].

5.2 Implications for Regenerative Biology and Medicine

The knowledge extracted in the study of single-cell transcriptomics has wide-ranging applications to fundamental biological knowledge and regenerative biomedical use. The definition of regeneration-specific progenitor populations, including those of the blastema fibroblast subsets or short-lived hepatocyte intermediate, gives some possible cellular objectives to be manipulated therapeutically. The elucidation of immune cell functions offers novel opportunities of controlling inflammation to foster a pro-restorative effect in human tissues, in which chronic inflammation usually detersiorates recovery. Moreover, these conserved signaling pathways (WNT, Notch, Fgf) provide molecular points of entry to tissue reconstruction or to improve endogenous repair processes. The comparative view focusing on demonstrating why certain species regenerate effectively and others fail to regenerate gives a basis to find out the so-called regenerative switches that may be turned on in mammals. These results, in general, reinforce the idea that natural regenerative approaches, when translated to clinical practice in the repair of organs, degenerative diseases, and recovery of trauma.

5.3 Current Gaps and Limitations in Understanding

In spite of great improvements, there are still significant gaps in the field. Majority of scRNA-seq studies involve snapshots of regeneration and not time-course of regeneration, which remains unclear about whether cell states are transient and whether the rapid transient signaling events happen or not. The fact that tissue patterning, positional memory and microenvironmental organization cannot be understood due to the loss of spatial information during cell dissociation is a major limitation to understanding tissue patterning. Species methodological variability changes of protocols used to dissociate, sequencing platform, and computational pipeline have been an impediment to cross-study comparisons and reproducibility. Also, inferred lineage relationships based on transcriptional traces are not directly validated genetically, but are likeliest. There is an abundance of remarkable species with interesting regenerative capabilities that do not have high-quality genomic references that could be examined further about regulatory networks. Lastly, a large part of the work is descriptive, not mechanistic, suggesting that the inferred pathways, ligand receptor interactions, and proposed regulatory factors have to be experimentally verified[39].

5.4 Future Directions and Research Opportunities

In spite of great improvements, there are still significant gaps in the field. Majority of scRNA-seq studies involve snapshots of regeneration and not time-course of regeneration, which remains unclear about whether cell states are transient and whether the rapid transient signaling events happen or not. The fact that tissue patterning, positional memory and microenvironmental organization cannot be understood due to the loss of spatial information during cell dissociation is a major limitation to understanding tissue patterning. Species methodological variability changes of protocols used to dissociate, sequencing platform, and computational pipeline have been an impediment to cross-study comparisons and reproducibility. Also, inferred lineage relationships based on transcriptional traces are not directly validated genetically, but are likeliest. There is an abundance of remarkable species with interesting regenerative capabilities that do not have high-quality genomic references that could be examined further about regulatory networks. Lastly, a large part of the work is descriptive, not mechanistic, suggesting that the inferred pathways, ligand receptor interactions, and proposed regulatory factors have to be experimentally verified[40].

5. CONCLUSION

The analysis of tissue regeneration using single-cell transcriptomics has revolutionized the topic as it has revealed astonishing cell heterogeneity, plasticity, and dynamic regulatory programs that mediate repair in a wide range of animal models. It has been possible to resolve rare progenitor states, map immune-niche interactions, and track lineage transitions with unprecedented precision using this technology, allowing a comparative study of regenerative strategies of highly regenerative species such as planarians and xenoplasms with more restricted mammalian systems. These experiences underscore the idea of regeneration as a multilayered process that entails stimulation of the stem-cells, dedifferentiation, immune

regulation, extralaminar matrix reorganization and conserved signal transduction. In spite of associated issues with dissociation artifacts, loss of spatial information and inconsistent methodologies, single-cell techniques continue to challenge the limits of regenerative biology. While the field is currently moving towards the incorporation of spatial transcriptomics, multi-omics, lineage tracing, and computational modeling, these developments will not only provide further insight into the mechanistic details, but will speed up the application of such to therapeutic innovations to fix human tissues and regenerate them in vivo.

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