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TISSUE REPAIR AND EPIMORPHIC REGENERATION: AN OVERVIEW

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Abstract

Purpose of the Review—This manuscript discusses wound healing as a component of epimorphic regeneration and the role of the immune system in this process.

Recent findings—Epimorphic regeneration involves formation of a blastema, a mass of undifferentiated cells capable of giving rise to the regenerated tissues. The apical epithelial cap plays an important role in blastemal formation.

Summary—True regeneration is rarely observed in mammals. With the exception of transgenic strains, tissue repair in mammals usually leads to non-functional fibrotic tissue formation. In contrast, a number of lower order species including planarians, salamanders, and reptiles, have the ability to overcome the burden of scarring and tissue loss through complex adaptations that allow them to regenerate various anatomic structures through epimorphic regeneration. Blastemal cells have been suggested to originate via various mechanisms including de-differentiation, transdifferentiation, migration of pre-existing adult stem cell niches, and combinations of these.

Keywords

“wound healing”; “super-healing organisms”; blastema; regeneration; “regenerative medicine”; “epimorphic regeneration”

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Compliance with Ethics Guidelines

Conflict of Interest

The authors declare that they have no conflict of interest.

Human and Animal Rights:

All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

1. Introduction: Mammalian Tissue Repair

A few privileged adult tissues including the liver¹, the bone marrow², and the epithelium of the gut³ have the ability to completely recover after mild to moderate injury. Unfortunately, this remarkable ability seems to be limited to tissues with high proliferative capacity and abundant stem cell reserve populations⁴ and therefore, most adult mammalian tissues do not have the ability to regenerate. Instead, the default response to tissue injury typically results in scar tissue formation in the form dense collagenous tissue deposition (i.e. fibrosis).

The physiologic response to tissue injury has been extensively studied in a variety of settings including epithelial disruption⁵, myocardial ischemia⁶, bone fracture⁷, and hepatic injury⁸, among many others, and while the specific features of the tissue repair process depend primarily on the tissue type and mechanism of injury, the overall objectives remain the same: restoration and maintenance of homeostasis, and to the extent that is possible, recovery and preservation of tissue function.

Perhaps the most extensively studied model for tissue repair is the wound healing response following acute traumatic injury⁹. The wound healing response rapidly achieves tissue repair through a series of complex and well-established phases that include hemostasis, inflammation, cell migration/proliferation, angiogenesis, granulation tissue formation, and tissue remodeling. This well-organized and rapid response has clear evolutionary advantages including the prevention of exsanguination following acute injury, activation of the immune system for neutralization of pathogens that may be present at the wound site, rapid modification of the extracellular matrix to promote cellular infiltration and angiogenesis, and restoration of epithelial barriers to maintain electrolyte balance and prevent further exposure to pathogens and dehydration.

However, despite the clear benefits of a prompt and rapid wound healing response, the ability to quickly restore and maintain homeostasis following tissue damage comes at both metabolic and functional costs¹⁰. Although tissue remodeling is a highly organized and effective means of wound healing, it nonetheless fails to faithfully replicate the events of tissue growth and differentiation during development. Compared to organogenesis, scar tissue formation is a rapid process of tissue repair that prioritizes wound closure over structural restoration with site-appropriate cell types, and as a result, fibrotic scars lack the strength, flexibility, and functionality of original tissues¹¹.

The degree to which scar tissue will form - and hence, the overall long-term burden the organism will sustain from injury, depends on several factors including the magnitude and mechanism of injury (e.g. trauma vs chronic exposure vs acute ischemia), the complexity and type of tissue involved (e.g. brain tissue vs epidermis), the presence and abundance of a nearby stem cell reserve populations (e.g. intestinal crypts vs myocardium), the presence of pathology at the injury site¹² (e.g. healthy tissue vs contaminated wounds vs an ischemic wound bed), and the developmental stage¹³ and species of the organism, among many others.

In short, adult mammalian tissue repair is a complex multi-factorial process that in the vast majority of cases results in a combination of scar tissue formation and compensatory native

cell proliferation. True mammalian regeneration is rare and with the exception of lab-generated transgenic species, it only occurs in a few privileged tissues particularly during the early stages of life¹³. While the default tissue repair process observed in most adult mammals may have many evolutionary advantages, it fails to replicate the events that characterize embryonic development, and therefore, an injury of sufficient magnitude—even if successfully repaired, may result in permanent disability and/or death.

2. Beyond the Wound Healing Response

In contrast to most mammals, a number of lower order species have the ability to regenerate various anatomic structures through complex adaptations that allow them to overcome the burden of scarring and tissue loss. The ability to regenerate tissues has been observed in most animal phyla¹⁴, and not surprisingly, a wide variety of regenerative adaptations exists across species. The phylogenetic distribution of these adaptations has led some evolutionary biologists to hypothesize that regenerative ability has emerged and disappeared multiple times across evolutionary history¹⁵. Furthermore, given the complexity of these adaptations and shared biochemical and cellular pathways with various developmental processes, these mechanisms have been suggested to have evolved as epiphenomenons of embryonic development, rather than as independent processes themselves¹⁴.

Regenerative capacity varies widely across species. While certain species of cnidarians¹⁶, planarians¹⁷, and echinoderms¹⁸ have the ability to regenerate an entire organism from a small fragment of original tissue, higher order species exhibit progressively more restricted capabilities. Zebrafish, for example, can regenerate their fins and a number of their internal organs^{19,20}. Similarly, urodeles such as newts and salamanders can also regenerate appendages^{21,22}, the lens²³, and to a lesser extent, neural^{24–26} and cardiac²⁷ tissue. In turn, some lizard species have a more restricted regenerative potential, and while they can also regenerate their tail, the regenerated appendage is not a perfect replica of its original counterpart^{28,29}. Birds and mammals comprise the end of the regenerative spectrum, and with the exception of embryos and transgenic species, possess very limited regenerative capacity (Table 1). A widespread hypothesis to explain this extensive range of regenerative potential among animals and why regenerative potential is not conserved in mammals is that higher order species have evolved the ability to mount strong inflammatory and adaptive immune responses that promote fibrosis at the cost of regeneration^{30,31}.

The diversity in regenerative capacity among animal species is mirrored by the diversity of tissue repair mechanisms that exists in nature. Regeneration mechanisms can be broadly classified into two distinct categories: morphallaxis and epimorphosis³². Morphallaxis is observed among lower order species and it entails the complete reorganization of the remaining tissues to give rise to an overall smaller but complete organism. Morphallaxis is beyond the scope of this article but has been reviewed by Thomas C. G. Bosch¹⁶ and Agata et. al³³. Epimorphosis, on the other hand, refers to the regeneration of missing tissue through cell proliferation at the injury site without affecting the structure of the rest of the organism. This type of regeneration is observed in higher order species and it relies on the formation of a blastema^{34–36} - a mass of undifferentiated cells at the injury site capable of giving rise to the differentiated tissues of the regenerated structure. (Table 1)

3. Epimorphic Regeneration

The term regeneration has been used to describe multiple processes that result in the replacement of missing tissues with various degrees of fidelity. In this article, the term regeneration is used to describe processes that recapitulate the events of development following tissue loss at any point during the post-embryonic period. Hence, most examples of true regeneration in vertebrate species are classified as epimorphic regeneration³².

Epimorphic regeneration has been extensively studied in zebra fish^{30,46,47}, newts²¹, salamanders^{21,22}, and reptiles^{28,29,48}, among other species. This process consists of multiple complex and overlapping steps beginning with the early stages of the wound healing response and culminating with the regeneration of identical or nearly identical scar-free replicas of the original tissue (Figure 1). Although differences exist between the exact mechanisms of epimorphic regeneration in different species, there is also significant overlap in these processes. Therefore, general principles derived from these commonalities can be used to describe this phenomenon using salamanders and reptiles as examples - Whereas salamanders are capable of perfect tail regeneration, reptiles are unable to regenerate a perfect replica of the original tail. However, as amniotes, reptiles are the closest relatives to mammals that possess naturally enhanced healing ability, and therefore, both salamanders and reptiles are considered valuable models for tissue regeneration and repair. (Figure 1)

The regenerative response begins immediately after injury. Following amputation or autotomy, the open wound at the tail stump in both species undergoes hemostasis and quick re-epithelialization. These early stages of the regenerative process are nearly identical to the initial stages of the wound healing response in non-regenerating organisms. However, organisms capable of regeneration possess unique adaptations that enable them to progress rapidly through these stages and continue with the regenerative process. In the lizard, these adaptations involve the presence of fracture planes positioned along the entire length of the tail²⁸ - Fracture planes act as evenly spaced pre-formed breaks located distally to equally distributed vascular sphincters in the caudal arteries (Figure 1C). After tail loss, these sphincters contract to limit the passage of blood, helping partially decrease the degree of hemorrhage while simultaneously facilitating hemostasis⁴⁸.

Salamanders, on the other hand, lack fracture planes and vascular sphincters, but they can nonetheless complete the re-epithelialization process in just a few days as a result of additional adaptations including the presence of a specialized immune system and the ability to slowly synthesize a transitional extracellular matrix (ECM) at the wound site⁴⁹. Similar adaptations are seen in regeneration-capable mammals such as the spiny mouse - During tissue repair, this species synthesizes a modified, porous, collagen III-rich ECM that contributes to their ability to complete the re-epithelialization process in just 3 days⁴⁰. Similarly, other healer species such as rabbits⁵⁰, laboratory-generated transgenic strains (e.g. C57BL6/SJ, SJL)⁵¹, and mammalian fetuses⁵² possess comparable re-epithelialization capabilities. In contrast, non-healer adult mammalian species can take twice as long to re-epithelialize skin wounds of equivalent size⁵³.

As the re-epithelialization process progresses over the tail stump, soft tissues retract into the wound bed and the diameter of the wound begins to decrease (Figure 1D). A specialized multi-layered signaling epithelium known as the apical epithelial cap (AEC) begins to form^{54,55} (Figure 1F). The AEC is an important structure that plays a key role during the regenerative process as it provides necessary molecular cues for regeneration. Early studies demonstrated that the AEC - particularly the basal layer, exhibits robust secretory activity⁵⁶⁻⁵⁸, producing many of the factors needed by the underlying blastema. In fact, germinal experiments in salamanders have shown that blastemal formation will not occur without a functioning AEC^{59,60}. The AEC establishes critical stimulatory gradients for regeneration including differential expression of fibronectin⁶¹, nerve signaling⁶², and other signals⁶³ that allow modulation of proximo-distal patterning⁶⁴ and directionality⁵⁹. The exact mechanism of AEC function, particularly in tail bud formation, are not entirely understood. However, this structure has been suggested to function similarly to the apical ectodermal ridge (AER) of the developing amniote - as they are both located at distalmost tip of the growing structure and participate in similar processes⁶¹.

Molecular signals originating from regenerating neural tissues, particularly the spinal cord and the brachial nerve have been identified as required mediators of the regenerative process. Deflection of the brachial nerve into distant skin wounds has been shown to promote ectopic blastema formation⁶⁵, and independent studies have shown that without proper innervation, regeneration will not occur - as denervated limbs lose the ability regenerate⁶⁶. Interestingly, nerve dependence of regenerative processes is conserved throughout phylogeny⁶⁷, and therefore this feature has been suggested to be one of the primary determinants for the loss of regenerative ability among mammals, as innervation density is lower in most vertebrate appendages than it is in salamander species^{66,68}.

4. The Blastema

The blastema is considered the central component of the regenerative process in epimorphosis because it is the primary source of differentiated tissues for the regenerating appendage^{35,69,70}. Following AEC formation in lizards and salamanders, cells localized distally to the injured spinal cord migrate and accumulate directly underneath the AEC resulting in blastema formation⁷¹⁻⁷⁴. In some lizards, the blastema can be identified as early as one week after injury⁵⁵. Blastema formation is also observed in other regenerating species including mammals, particularly in the regenerating digit tip^{75,76} and regenerating epidermis in mice⁴⁰. Interestingly, although human fetuses have enhanced regenerative capacity compared to adults, it is currently unknown if a blastema or a blastema-like structure plays a role in regeneration of human tissues.

Not surprisingly, the origin of blastemal cells has been a topic of particular interest and debate among researchers⁷⁷. Initially considered to be composed of a homogenous cell population⁷⁸, blastema cells are now known to be composed of a heterogeneous cell population that originates via various mechanisms in different species including de-differentiation (Figure 1G), transdifferentiation (Figure 1H), migration from pre-existing adult stem cell niches (Figure 1I), and combinations of these.

De-differentiation involves a differentiated cell reverting back to a previous, less differentiated state that is able to proliferate and partially replace lost tissues while remaining within the same lineage⁷⁹. This concept is not new. The contribution of de-differentiated cells to regenerating tissues was first described by Elizabeth D. Hay based on electron microscopy studies⁸⁰. The concept was further developed by lineage tracing experiments using triploid axolotl donor tissue implanted into a diploid host⁸¹. More recent studies have shown that zebrafish are capable of remarkable cardiac regeneration - even after loss of more than 20% of ventricular mass⁴⁶. During cardiac regeneration, differentiated cardiomyocytes must revert to a less differentiated state and disassemble the sarcomere contractile apparatus. This is a required step because the sarcomere occupies a significant portion of the cytoplasm and physically prevents cytokinesis from occurring. These cellular changes are also associated with gene expression changes that correspond with patterns of de-differentiation and re-differentiation^{46,47}. Other examples of dedifferentiation during regeneration include Schwann cells during nerve damage^{82,83}, and multiple tissues during limb regeneration in urodeles⁸⁴.

Transdifferentiation involves lineage switching among terminally differentiated cells, allowing cells to differentiate into different tissue types. Transdifferentiation was first observed in the lens of the newt²³. During these experiments, when the lens was removed, differentiated pigmented epithelial cells in the iris were observed to undergo transdifferentiation to switch lineages and replace the missing lens. In order for this process to occur, epithelial cells must de-differentiate, re-enter the cell cycle, proliferate, and re-differentiate into mature lens cells. Recent studies have revealed that during this process, cancer and apoptosis- related genes are upregulated⁸⁵ and epigenetic modifications also take place⁸⁶.

Pre-existing adult stem cell niches also play an important role in regeneration. For example, in platyhelminths and acoels, blastemas form exclusively from existing stem cells¹⁴. In some lizards, blastemal cells have been proposed to originate either from pre-existing adult progenitor cells that get recruited following injury⁸⁷⁻⁸⁹, from dedifferentiated cells, and from a combination of both^{72,90,91}. The origin of blastemal cells in mammals has not been completely established.

Despite the identification of these clearly distinct processes of blastemal formation, recent evidence suggests that there is heterogeneity with respect to the origin of blastemal cells even within closely related species. In a recent study²¹ two salamander species were investigated: *Notophthalmos viridescens* (newts) and *Ambystoma mexicanum* (axolotl). In this study, dedifferentiation was found to be an important component of limb regeneration in the newt but not in the axolotl. In the newt, myofibers fragment and give rise to rapidly proliferating, PAX7- mononuclear cells. These cells accumulate in the blastema and proceed to become the muscle in the new limb. In contrast, myofibers in the axolotl do not give rise to proliferating cells, nor do they contribute to newly regenerated muscle tissue. Instead, regenerated muscle originates from resident PAX7+ cells. The heterogeneity with respect to blastemal cell origin even among closely related species highlights the diversity of tissue repair mechanisms that exists in nature.

5. Conclusion: Regeneration and Wound Healing

The ability to regenerate tissues offers clear benefits beyond those offered by the oftentimes scar-producing wound healing response. While mild soft tissue injuries in a clinical setting may lead to aesthetically unpleasant scars⁹², more severe injuries may result in extensive scar tissue formation that can lead to lack of tissue functionality⁹³, contracture⁹⁴, discomfort, and pain⁹⁵. These complications can in turn lead to limited mobility and a significant increase in morbidity and mortality depending on the extent and anatomic location of the injury. In the case of internal organ damage, scar tissue formation may result from a variety of etiologies and may lead to more severe and potentially life-threatening complications: Ventricular remodeling following myocardial infarction oftentimes leads to heart failure and ventricular wall rupture⁶, esophageal scarring in the form of stricture formation is the typical outcome of therapeutic esophageal mucosal resection⁹⁶, and pulmonary fibrosis due to autoimmune disease may lead to decreased gas exchange⁹⁷.

A number of lower order species have the ability to regenerate various anatomic structures including internal organs through complex adaptations that allow them to overcome the burden of scarring and tissue loss. However, despite the advantages offered by tissue regeneration over the wound healing response, regenerative potential seems to have been significantly restricted or completely lost in many animal lineages throughout evolutionary history^{14,15}. A number of diverse hypotheses have been proposed to explain the loss of regeneration capabilities among animal clades including the loss of selective advantage due to decreased loss frequency resulting in regeneration becoming an ecologically-irrelevant trait, changes in the functional importance of loss structures across evolutionary history, and break down of pleiotropic interactions between regenerative and developmental processes¹⁵.

Regeneration is nonetheless prevalent in nature, and by studying these processes and adaptations, researches are starting to understand the cellular and molecular mechanism that may one day be translatable into the clinic. The initial stages of the wound healing response including hemostasis, immune system activation, and re-epithelialization of the wound site are common to both scarring and regeneration. Hence, the traditionally described wound healing response may be seen as the initial steps of the regenerative process in regenerative species. In most mammals, however, the regenerative process seems to stall during the early stages of wound healing and divert towards the scarification process. It has been hypothesized that the immune system is involved in the switch between regeneration and fibrotic scar formation because human fetuses - which can heal without scarring, have immature immune systems⁹⁸. Incidentally, transgenic mammals that have been shown to overcome these obstacles exhibit the formation of a blastema-like structure, indicating that the induction of signaling centers that include a blastema and an AEC under the right immunologic conditions and neural stimulus may enhance regenerative capabilities in other species. Through mechanistic and genomic studies of the key elements and pathways that effect regeneration in lower evolutionary species, we hope to one-day bridge the gap between wound healing and regeneration.

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Figure 1. Stages of Epimorphic Regeneration

The initial stages of epimorphic regeneration share many features with the wound healing response. Regeneration-capable species such as some lizards have unique adaptations including the ability to autotomize (A), the presence of pre-formed fracture planes (B, white dotted line in C), and vascular sphincters (red arrowheads in C) to facilitate clot formation (yellow asterisk in C), hemostasis and a rapid wound healing response (D and E). Once re-epithelialization occurs, the AEC forms and provides necessary molecular signals for blastema formation (black arrows in F). Blastema formation occurs via three methods: Dedifferentiation (G), transdifferentiation (H), adult stem cell recruitment (I), and combinations of these. The blastema gives rise to the differentiated tissues of the regenerating appendage. Whereas salamanders are capable of perfect tail regeneration, lizards can only regenerate an imperfect replica of the original appendage.

Table 1
Selected List of Regeneration-Capable Organisms

Regenerative potential varies widely among animal species.

Species	Regenerative Potential	Resources
<i>Platyhelminthes</i>		
Planarians	Extensive – whole body	Reddien et. al. ³⁷
<i>Urodeles</i>		
Axolotl	Limb and tail regeneration	Kragl et. al. ²²
Newts	Limb and tail regeneration	Sandoval-Guzman et. al. ²¹
<i>Fish</i>		
Zebra Fish	Fin and organ regeneration	
<i>Reptiles</i>		
Mourning Gecko	Tail regeneration	Lozito et. al. ^{28,29}
Anolis	Tail regeneration	
<i>Birds</i>		
Hen/chick	Hair cell and cochlear regeneration	Stone et. al. ³⁸
<i>Mammals</i>		
MRL Mouse	Ear punch healing Ventricular repair	Clark et. al. ³⁹
Spiny Mouse	Skin regeneration Ear punch healing	Seifert et. al. ⁴⁰
p21 ^{-/-} Mouse	Ear punch healing	Bedelbaeva et. al. ⁴¹
Lin28 Mouse	Lin28a reactivation promotes hair regrowth and ear/digit regeneration.	Shy-Chang et. al. ⁴²
Rabbit	Ear punch healing	Goss and Grimes ⁴³ Javanmard et. al. ⁴⁴
Deer	Antler regeneration	Price et. al. ⁴⁵