INTRODUCTION

Regeneration: Remarkable Restoration Reign

In the dynamic journey from a single cell to a highly complex, articulate living system, multicellular organisms have learnt to repair their respective physical system in an orderly fashion (Morgan, 1901; Brockes et al., 2001; Kowald et al., 2020). Organ repair provides the living forms a chance to suit the surrounding requirements and thrive to the best of their potential. It operates through a broad spectrum of mechanisms and at variable intensities (Suzuki and Mittler, 2012).

Throughout the animal kingdom, the capacity to regenerate a lost body part is deeply rooted, though in a scattered fashion. The basic understanding of the variable modus operandi comes from the enormous history of regeneration studies (Goss, 1991). Trembley explained the potential to regenerate lost body parts, as exhibited by some 'less complicated' animals such as hydra in the 18th century (Galliot, 2012). Although, what still remains a burning question is the exact differential mechanism, which allows a group of animals to regrow the lost body parts, while few others have not received this magical quality (including humans). Instead, the latter ones learn to live in a physically compromised state and if unsuccessful, the individual perishes. As per up-to-date knowledge, regeneration of the injured or lost tissue is drafted through four major modes (Alvarado and Tsonis, 2006; Gilbert, 2014).

a. Morphallaxis: Rearrangement of pre-existing tissues/cells
b. Stem-cell mediated regeneration: Reconstruction through resident stem-cell
c. Epimorphosis: De-differentiation, proliferation and re-differentiation
d. Compensatory Regeneration: Hypertrophy and hyperplasia

Any of these four routes converge to heal and repair the lost tissue, ideally by replacing the original part (Poss, 2010; Tanaka and Reddien, 2011). Based on the degree and intensity of repair observed, Bely and Nyberg (2010) have classified the multifaceted event of regeneration into five subtypes.

a. Whole-body regeneration: Eg. Hydra and Planaria (Chera et al., 2009; An et al., 2018)
b. Structural regeneration: Eg. Axolotl and Salamander appendages; Teleost fish tail (Tanaka et al., 2016; Gonzalez-Rosa et al., 2017; Patel et al., 2019)

c. Organ regeneration: Eg. Tail regeneration in lizards; heart regeneration in zebrafish; lens regeneration in newts (Sousounis et al., 2015; Gonzalez et al., 2017)
d. Tissue regeneration: Eg. Gut lining regeneration in Drosophila (Belacortu and Paricio 2011; Worley et al., 2012)
e. Cellular regeneration: Eg. Axon regeneration in *C. elegans* (Ghosh-Roy and Chisholm, 2010; Basu et al., 2017)

Organisms display either solo or combinations of the repair mechanisms, while every lineage deals with the concurrent healing process differentially, resulting in contrasting outcomes. Scientists believe that disparate wound healing methods employed at the variable injury sites determine the differential repair outcome (Clark, 1989).

Post-injury repair mechanism is meticulously categorised in three major sub-events based on their roles in the healing process. They are mentioned below in the chronology of their occurrence.

a. Wound Healing
b. Recruitment of stem cells
c. Redevelopment

Present endeavour deals closely with the primary response post-injury and the first step towards regenerative repair - Wound healing. Road taken by signalling cascades regulating wound repair determines the fate of this complex process. Either the injury site harbours an apt microenvironment for reconstructing the lost part or repairs it with a permanent scar (Flanangan, 2000; Beldon, 2010). We try to decode these underlying nuances of wound healing and the related signalling pathways, which coordinate the differential response. This project's futuristic approach is designed to address the ultimate question, - if early evolved and simpler animals can compose an intricate event of regeneration, why can we not?

Invertebrates elicit unmatched potential of wound repair, wherein organisms like planaria undergo whole body regeneration. On the contrary, well-advanced groups such as vertebrates show only limited levels of restorative behaviour. In order to explore the underlying routes of regeneration in competent clans or its absence in the groups that cannot perform this task, one needs to dissect this mega event of wound healing meticulously.

Figure I illustrate the scattered and variable regenerative potential across the animal kingdom, which encompasses both invertebrate and vertebrate pattern of organ regrowth. Further section discusses these regenerative incidences in detail.

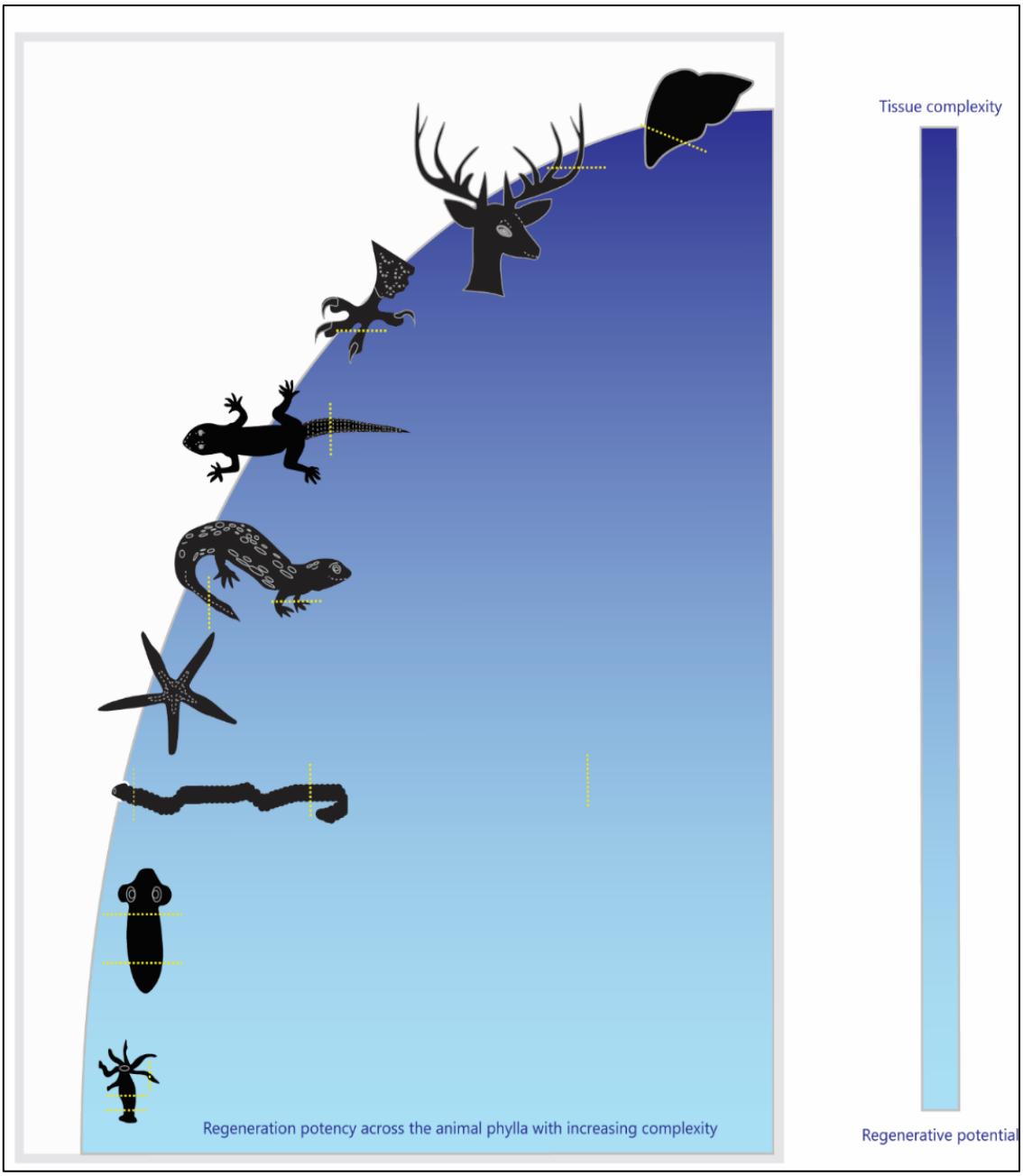

Figure I: Variable regeneration potential across the animal kingdom

Iconic Invertebrates: Archetypes of Amends

As emphasized by many researchers, invertebrates possess unusual potentials of cellular and tissue repair. The basal metazoans Porifera (sponges) can undergo whole-body regeneration. In sycon (calcareous sponge), an injury is often followed by the formation of a 'regenerative membrane,' which reforms the lost plane of the body, whether longitudinal or transverse. Thus, structure and function are entirely regained through perfect regeneration, as shown in figure II (Ereskovsky et al., 2021)

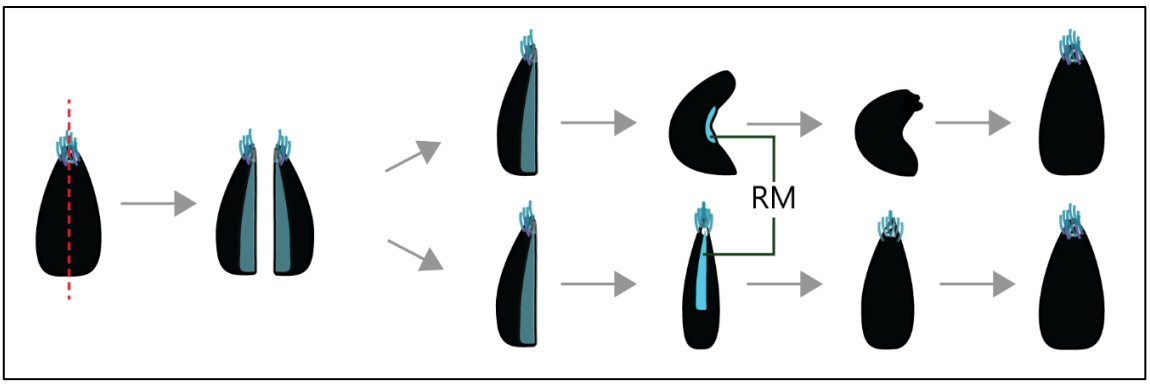

Figure II: Perfect regeneration in Sycon: RM - Regenerative membrane (adopted from Ereskovsky et al., 2021)

Next in line appears one of the most studied animal phyla, Cnidaria, whose reparative prodigy has been well-established for centuries. Since the last few years, studies on model organisms such as *Nematostella vectensis* (starlet sea anemone) have unravelled the critical role of organised wound healing in achieving successful and perfect restoration (DuBuc et al., 2014).

Platyhelminthes are yet another stalwart of regeneration, which can reconstruct the entire body from as small as 1/279th part (Morgan, 1901). The virtuous positional control genes laced in the body wall muscles are responsible for this excellent healing quality along with many other signalling wonders (Witchley et al., 2013). Molecular studies on freshwater planarians - *Dugesia japonica* and *Schmidtea mediterranea* have brought out occult insights on wound repair mechanisms deployed here. From discovering neoblasts and blastema formation to decoding around 240 genes involved in the events such as cell proliferation, maintenance and differentiation, planaria have provided a wealth of information to researchers (Alvarado and Tsonis, 2006). Despite the widely spread restoring potential observed, not all invertebrates are 'immortal' enough to reform their lost body parts endlessly. *Caenorhabditis elegans,* for instance, is an extensively studied nematode model to address homeostasis and senescence

related queries (Nagy et al., 2014; Good and Hawle, 2020). Although *C. elegans* has stringently maintained cell numbers that restrict its regenerative capacities, it can modulate the cell maintenance pathways to increase its life span by ten folds, thus proving the excellent wound healing proficiencies (Coffman et al., 2016).

Another phylum in a row is of annelids. Again, not all members of this group can regenerate, while some syllids (polychaete worms) can reconstruct as many as 31 anterior segments (Hyman, 1940). As per the recent reports of Chen and co-workers (2020), freshwater annelid *Aeolosoma viride* can reconstruct both anterior and posterior ends by 120 hours post-amputation (hpa). Here, the attributed mechanism responsible for the regenerative outcome is epimorphosis, which is rigorously conserved in some groups of higher vertebrates as well (Fernando et al., 2011; McCusker et al., 2015). Epimorphosis will be discussed in detail in the following parts of the Introduction. Meanwhile, it is important to note that immediate response to any form of injury is once again a well-programmed wound repair.

Moreover, astounding observations are made by Treaster and colleagues, who reported extensive proteostasis causing exceptional longevity of as many as 500 years in clam *Arctica islandica* (Treaster et al., 2014). Although shell regeneration in molluscans has been demonstrated for half a century now (Wagge and Mittler, 1953), nerve and muscle repair mechanisms got ascertained recently. Here again, the regenerative path is paved by quick and refined wound healing events (Zullo et al., 2017; Imperadore and Fiorito, 2018). The largest phylum of the animal kingdom, Arthropoda, is no different but poses one of the most successful groups on the planet, as organisms of this group can moult their exoskeleton and regenerate lost appendages (Figure III). Yet again, epimorphosis and meticulous wound healing provide a helpful microenvironment to the regenerating tissue in arthropods (Pellet et al., 2019; Tworzydlo and Bilinsky, 2019).

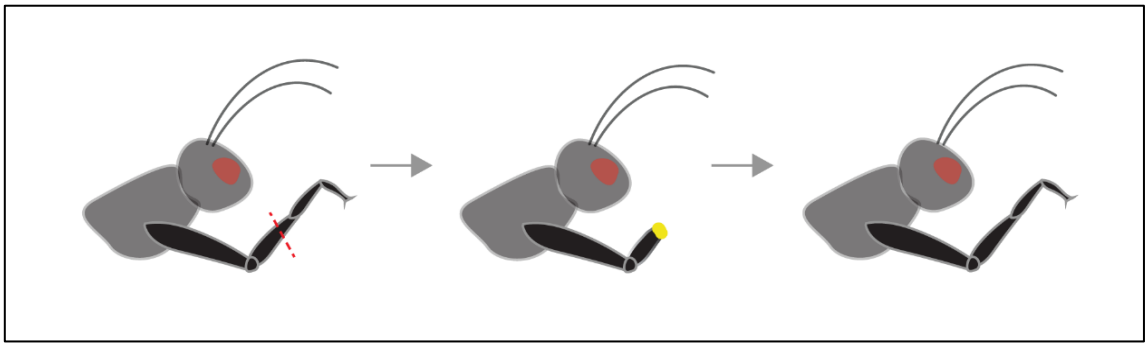

Figure III: Epimorphosis in arthropod appendages

Marching towards the sequentially complex group of organisms, the Echinoderm model of regeneration, such as *Coscinasterias muricate* (starfish) exhibits a combination of morphallaxis, transdifferentiation and epimorphosis while regrowing its autotomised arm (Byrne, 2020). At every instance, it seems that invertebrates have the benefit of restoration, although their status in the phylogenetic tree stays below the groups who essentially lack this feature, e.g., mammals. Nevertheless, there occurs a vast arena of pro-regenerative vertebrates such as Pisces (Teleost fishes) and Amphibians (Axolotls and Salamanders), which have been extensively studied globally. Scientists have used these animal models to unravel the mysteries behind regeneration, their triggers and signalling nuances.

Vivacious Vertebrates: Restorative with Restrictions

Vertebrates portray a striking disparity in the pattern of restoration and do not follow any particular flowchart for the same, instead, it seems to be developing sporadically (Gawriluk et al., 2016; Iismaa et al., 2018). Ranging from the paleontological records of Agnatha to the modern jawed vertebrates, regenerative potentials are found present in the members of vertebrates (Nogueira et al., 2016). What stays unexplained is the bias in the healing mechanisms, which do not visibly fit the otherwise ascending pyramid of the complexity of vertebrates (Alvarado, 2000; Bryant et al., 2004; Tanaka, 2003). Present time molecular biology advancements have brought to light various hidden cellular signalling that could contribute to this variable differentiation, primarily through entities such as conserved regenerative elements or injury-responsive enhancers (Wang et al., 2020). It is now believed that particular injury responsive gene expression can be induced to cause wound healing (Kang et al., 2016).

Furthermore, amongst the gnathostomes, teleost fishes such as *Danio rerio* and *Poecelia latipinna* have been extensively used to study dermal and fin regeneration (Akimenko et al., 2003; Rajaram et al., 2016). Moving up in hierarchy, arrive the forerunners of regeneration, Amphibians (Figure IV). The restorative potential of Urodeles is robust enough to reform their lost appendages and many other vitals numerous times (Roy and Levesque, 2006; Bely, 2010). On the other hand, the regenerative capacities of anurans are confined to the larval stage and do not show up in adults (Dent, 1962; Muneoka et al., 1986a).

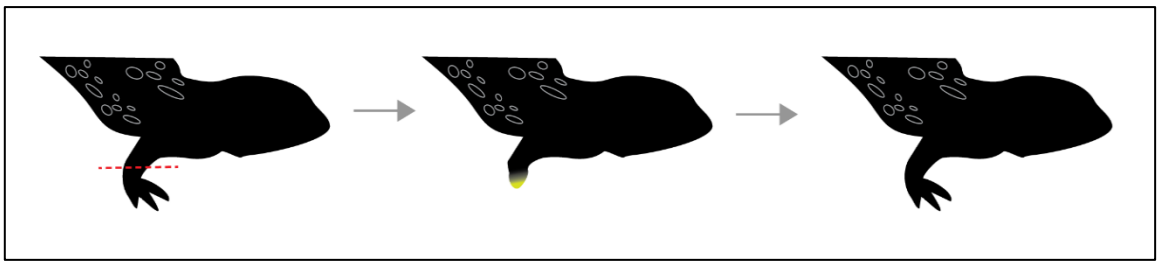

Figure IV: Epimorphosis in urodele amphibian limb

Following the line-up, reptiles show relatively restricted restoration, wherein not all organs can be regrown. Organisms belonging to all the four orders - Testudines (Tortoises and Turtles), Crocodilia (Crocodiles and Alligators), Sphenodontia (Tuatara) and Squamata (Snakes and Lizards) of reptiles show limited regenerative competency via epimorphosis (Bryant, 1967; Avery, 1994; Vervoort, 2011; Li et al., 2015). One notch above in hierarchy resides the aves, with limited tissue restorative abilities and only a few models have been studied so far. The best studied are the feathers and hair cell regrowth in chicks, along with studies of beak and claw regeneration, which by and large involve the epimorphic route (Alvarado and Tsonis, 2006).

Appearing last in the race of regenerative proficiency, mammals practically lack this excellent epimorphic potential. Compensatory regeneration of the human liver through hypertrophy and hyperplasia; stem cell mediated repair of muscles and skin are a few examples of the limited restorative ability of mammals. Amongst the outstanding examples of epimorphosis, African spiny mice (*Acomys* spp.) can regrow digit tips (Seifert et al., 2012; Simkin et al., 2015; Maden and Varholick; 2020). Also, during embryonic stages, repair and regeneration are more frequent in mammals compared to adult life (Satoh et al., 2015; Gawriluk et al., 2016).

What sieves out of all these skilful investigations over the years is that epimorphosis is the epicentre of refined regeneration. Be it the primitive cnidarian forms or the significantly evolved and complex higher vertebrates, epimorphosis reconstructs both simple and complex tissues to restore the structure and functional ability of the lost organ (Agata et al., 2007; Sallin et al., 2015).

Epimorphosis: Emending Eloquently

The term epimorphosis has been used since the early 1900s, while its definition remained debatable over a century in order to be categorised as epimorphosis, the regenerative pattern follows a specific route. Firstly, the tissues undergoing the massive reformation involve a process of de-differentiation of well-defined cells (Reddien and Alvarado, 2004). Next, these de-differentiated cells aggregate in a pool of progenitors called 'blastema,' which reacts to all wound site cues and moulds into complex histological features (Muneoka et al., 1986a; Agata et al., 2007; Kierdorf et al., 2007). In its nuances, epimorphosis holds the technical details of regeneration machinery, governed and guided by wound repair. Wound healing is a cardinal step directing the course and chronology of repair events, construing to regeneration (Roy and Gardiner, 2002).

Wound healing: Floors the Fate

Physical trauma or insult caused by external or internal factors is defined as a wound. Intensity, degree and time taken to heal the wound are the factors considered to categorise it as either Acute or Chronic (Shaw and Martin, 2009). Immediately after injury, when internal tissues are exposed to the external environment, the condition is termed as an 'acute wound'. At the same time, it turns to a chronic wound if it does not get healed within a certain amount of time (Visha and Karunagaran, 2019). Blood cells, dynamic extracellular matrix (ECM) and constantly altering signalling leads to successful wound healing (Trent and Kirsner, 2003; Troxler et al., 2006; Metcalfe and Ferguson, 2008). The process of wound repair is organised by a synchronised set of events, Inflammation, Proliferation and Remodelling. Each sub-event's ratio, proportion, and time span constructs either pro-regenerative or scar-making microenvironment (Murawala et al., 2012).

Events of Wound Healing

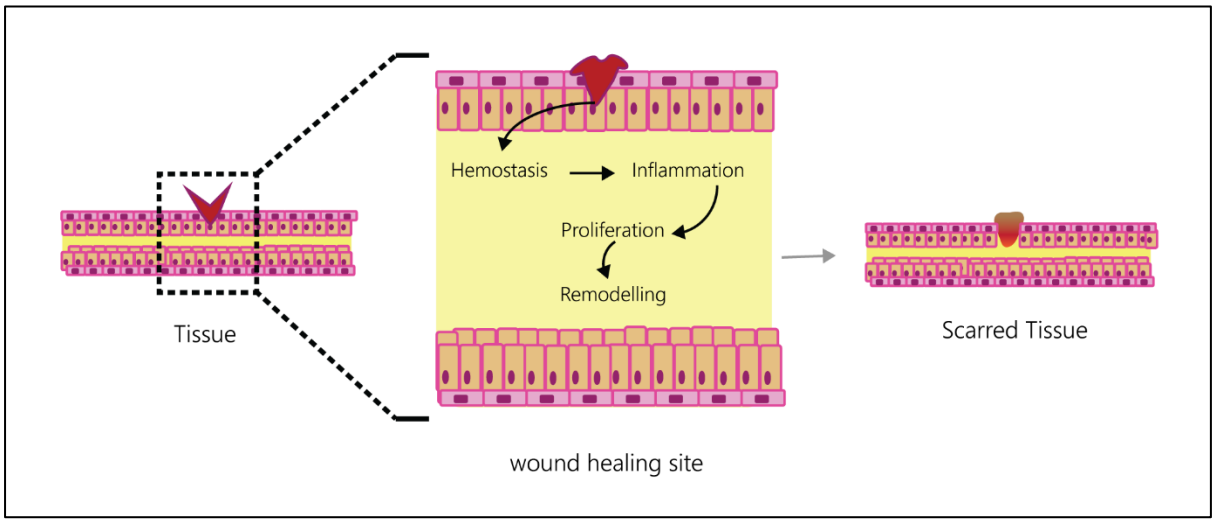

Figure V: Proceedings at the wound healing site

The first step towards repair, irrespective of its origin or plausible outcome, is hemostasis, which registers the trauma of injury and prepares the wounded site for the following course of inflammation (Shaw and Martin, 2009). Inflammation is a consolidated product of many cellular and molecular annexed pathways participating in systemic immunity, a cardinal step to combat any pathogenic influx (Takeo et al., 2015). During this cellular warfare, the healing front is developed for the next step in line, i.e., 'Cell proliferation' and following 'Remodelling' as illustrated in figure V. It is important to note that, inflammation is more like a double-edged sword as claimed by a large number of researchers. It has both constructive and destructive sides, which are revealed depending on its involvement at the wound site (Mescher et al., 2017). This work further studies the correlation of inflammation with wound healing and the underlying signalling pathways leading to either regenerative or mere scarred repair of wounds. However, following or rather in coordination with inflammation, cell proliferation takes over the reparative reign. Cells surrounding the wounded region undergo rapid proliferation along with migration (Gurtner et al., 2008; Barrientos, 2008; Gonzalez et al., 2015). Wilkins-Port and co-workers (2007) have explained EMT events for premalignant keratinocytes through their *in-vivo* experiments. These coherent cellular events lead to wound closure, barricading entry of external microbial agents (Martin, 1997; Turksen, 2018). Cell proliferation and resulting clusters lead to blastema formation in pro-regenerative organisms, which then leads to tissue remake (Kumar et al., 2007; Stocum, 2017; Tworzydlo and Bilinsky, 2019).

If the wound micro-niche does not support blastema formation at this juncture, the repair machinery reroutes to form a collagen-rich scar with minimal remodelling, skipping de-

differentiation entirely. (Judith et al., 2013; Godwin and Rosenthal, 2014). Mammals, especially humans, present apt illustrations as they live with such redundant scars for their entire life post-injury (Nathan, 2002; Rosique et al., 2015). Of all cellular processes managing this repair hotspot, inflammation is the first to initiate, almost immediately after injury and thus makes way for all the following events (Filbin, 2006; Eming et al., 2007; Kyritsis et al., 2012; Karin and Clevers, 2016).

Inflammation: Imperative, Influential yet Infamous

Inflammation and wound healing are both friends and foes. Inflammation is a pivotal participant of wound healing while equally harmful for the same process if it persists (Nathan 2002; Filbin, 2006; Reinke and Sorg, 2012). Early in times, around 40AD, Celsus described the physiological event of inflammation and meticulously categorised it into sub-events- popularly known as rubor, calor, dolor and tumor (redness, heat, pain and swelling). Presently, after centuries of research, inflammation has been studied rigorously and found to be involved in the manifestation of a wide spectrum of trauma, infection and disease pathophysiology (Lee et al., 2002; Szpaderska et al., 2003). The medical definition of inflammation encompasses its early onset as a defence response against any trauma encountered by the system. It is a process designed to meet the emergencies with all forces diverted to chase and clear any foreign entity attempting to invade the body (Singer and Clark, 1989; Noursharg and Alon, 2014). It is a syndicate of devastating events that, if not resolved timely, can blast the healthy tissues around its zone of activity, both locally and systemically. The catastrophic effects of prolonged inflammation have been reported to cause massive damage to the organs in rheumatoid arthritis, granulomatosis, asthma, fibrosis cirrhosis, etc. (Williams and Maier, 1992; Arroyo et al., 2014; Shah et al., 2017). In order to understand any event as dubious as inflammation, it becomes mandatory to dissect its underlying objectives carefully. The variable, tissue-specific operation of inflammation is regulated by a set of molecules, thereby governing the process and determining the repair site's fate.

Mediators of Inflammation

Being the composite outcome of a large number of molecular events, numerous accomplices modulate its pathway. Primarily they are either cells of immunity or secretory molecules released by these cells (Henry and Garner, 2003).

1. Cellular components of Inflammation

Hematopoietic stem cells (HSCs) originating from the bone marrow are made to reach all tissues of the living system, where they are specified as either myelo-erythroid progenitors or lymphoid progenitors (Kawamoto, 2006). Both these progenitors are responsible for forming the huge consortia of immune cells localised at the peripheral vasculature around the tissues or are present in circulation. These cells create the microenvironment that determines the level of inflammation locally and the resultant effect on the healing process.

A. Myeloid cells

Originating from the bone marrow, progenitors are present in the tissue periphery and based on surrounding cues such as stem cell factor (SCF) and Interleukin-3 (IL-3), they get differentiated into promyelocytes. Further, based on specific ligand-receptor activity, cells with peculiar features are formed. Of all the functions these cells perform, their contribution towards inflammation is discussed in the following section (Yamashita and Passegué, 2019).

Neutrophils

Promyelocytes and surrounding stromal cells release granulocyte-colony-stimulating factor (G-CSF), which forms mature neutrophils. These cells are responsible for the primary combat with the pathogenic components entering the tissue. Their immediate action is responsible for the spiked inflammation in tissue micro-niche, making it hostile for the microbes (Soehnlein et al., 2017).

Macrophages

Under simultaneous stimulation of the multipotent progenitors with granulocyte-macrophage colony stimulating factor (GM-CSF) and macrophage-colony stimulating factor (M-CSF), followed by only M-CSF exposure, macrophages are formed of HSCs. These cells are responsible

for removing cell debris and other waste material from the healing site, thus reducing the levels of inflammation there (Gupta et al., 2014).

Eosinophils

Similar to neutrophils, eosinophils are formed as a response to GM-CSF, along with IL-3 and IL-5. They too possess proteolytic granules, helpful in the removal of foreign entities. Eosinophil release cytotoxins and other cytokines such as IL-4, IL-10, etc to contribute to tissue remodelling (Geering et al., 2013).

Basophils

Basophils are another cell type from the clan that contributes to the removal of pathogens and participates in the rise of inflammation. They release IL-4, histamines and other regulators of allergy at the healing site (Schwartz et al., 2016).

Platelets

Of all the cells working at the healing site, platelets recruit leukocytes by altering membrane permeability and promoting chemotaxis. Their contribution to tissue repair has recently been discovered along with their long-known roles in blood coagulation (Nurden, 2011).

Erythrocytes

Under heightened inflammation, local Nitric oxide (NO) titre increases due to phagocytosis and heavy fibrinogen activity. Erythrocytes are responsible for scavenging the increasing levels of NO, thus keeping a check on inflammation (Saldanha and Silva-Herdade, 2017).

B. Lymphoid cells

Lymphoid cells originate from the hematopoietic cell lineage, similar to the myeloid counterparts, via common lymphoid precursor (CLP) (Blom and Spits, 2006). They are responsible for managing inflammation and the entire immune response post-injury through the cytotoxins and cytokines they release (Artis and Spits, 2015).

Natural Killer cells

The intricate process of differentiating between self and non-self-components is carried out by Natural Killer cells (NK cells), as they necessarily kill the foreign entities through cytokine and cytotoxin release. A diverse variety of cell surface receptors occurs, which on binding amplifies in a complex signalling cascade, making NK cells efficient mediators of inflammation (Vivier et al., 2008).

T cells and B cells

Both T and B lymphocytes are found in their small, resting stage until they collide with the pathogens or extraneous factors. Once exposed, many cytokines are released under immediate and urgent response, alarming the entire system and recruiting all cells of combat at the wound healing site (Mantovani et al., 2005).

Dendritic cells

These are the unique cell types functioning in the niche of wound healing as they identify and clashes with the non-self-molecules while developing tolerance for self-entities. On exposure to pathogens, these cells release large volumes of Interferon-γ (IFN-γ), thus making the environment harsh for microbes. They also release cytokines to steer T cell action on the repair front (Segura and Amigorena, 2013).

2. Cytokines

This entire cell conglomerate drives inflammation and resultantly governs the tissue repair post-injury. The signalling pathways active for ensuing inflammation are a function of receptor-ligand interaction, which is achieved by releasing a wide spectrum of cytokines. Cytokines relay the immune signals, amplify them, and generate the signals for effector tissues to function accordingly (Cameron and Kelvin, 2000).

Cytokines are the blanket term for secretions such as monokines (released by macrophages), lymphokines (released by lymphocytes) and so on. Another crucial subdivision of cytokines is the family of ILs, which are the effector secretory molecules causing modulations in inflammation status (Judith et al., 2013).

All these groups of mediators function in coherence with each other to govern the signalling cascades of inflammation and based on their action and activity, they are categorised as:

i. Proinflammatory mediators
ii. Antiinflammatory mediators

As per their respective functions, molecules are either called proinflammatory, which promote and aid inflammation, while others who lead to its resolution are called antiinflammatory. It is equally noteworthy that the cellular players once contributing to promote inflammation can further evolve to abate it in context specific fashion (Rosique et al., 2015; Ellis et al., 2018). Cells of immunity such as neutrophils and monocytes derived macrophages, lymphoid cells (B lymphocytes and T lymphocytes), released cytokines, neuropeptides, leukotrienes, prostaglandins (PGs), proteases, hydrolases and Reactive Oxygen Species (ROS) - all are the major proinflammatory mediators, introduced to the healing site depending on the stage of repair (Chow et al., 2005; Lawrence, 2009). Neutrophils are the first cluster of cells rushed to a trauma site. Early on the repair front, they function in accordance with interleukins released locally and remove away the cell debris. Damage associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs) bind to the neutrophil surface receptors and activate them. These signals are further amplified by the tissue resident cells such as macrophages and fibroblasts, which release higher titres of chemo-attractants to bring more neutrophils, eventually increasing inflammation (Farrera and Fadeel, 2013). Chemokines belonging to the C-X-C motif chemokine ligand 8 (CXCL8) family, such as CXCL5, 6 and 7, play a significant role in hiking local inflammation. They develop a chemical gradient around the injured tissue, thus attracting more neutrophils (McDonald et al., 2010; Ellis et al., 2018).

Neutrophils and chemokines produced by them are crucial for recruiting other inflammatory agents such as macrophages, T cells, and interleukins. Neutrophils also recruit cell proliferation and angiogenic factors like vascular endothelial growth factor (VEGF), membrane cofactor protein-1 (MCP-1) and epidermal growth factor (EGF) (Engelhardt et al., 1998; Theilgaard et al., 2004). The entire regime is swiftly performed and the resultant apoptotic neutrophils are cleared off by none other than macrophages. This cell clearance primarily works as a stop signal for tissue-specific inflammation (Ferrante and Leibovich, 2012). The latest research in this domain has brought up a new theory, where neutrophils leave the site of healing back into circulation through reverse trans endothelial migration (r-TEM), causing a reduction in local

inflammation (Ellis et al., 2018). Macrophages, which remove neutrophils from the wound environment through efferocytosis, further aid cell proliferation, migration and EMT processes.

As monocyte lineage derivatives, macrophages present in the tissue environment undergo activation induced by danger signals or chemokines released by neutrophils and call out for the monocytes present in surrounding vasculature (Silva and Correia-Neves, 2012; Chazaud, 2014). These cells are called classically activated M1 macrophages, posing heightened inflammatory action and releasing a barrage of interleukins such as IL-1β (Zhang et al., 2016), IL-6 (Schaefer et al., 2017) and tumor necrosis factor-α (TNF-α) (Kroner et al., 2014) all contributing to a massive spike in inflammation (Eming et al., 2014). Once the cell debris and other potentially harmful entities have been checked, the same macrophages are 'alternatively activated' into their antiinflammatory forms, named as M2 type (Yunna et al., 2020). Once the cells have been modified, they release many interleukins, this time, nonetheless, they are antiinflammatory and facilitate the reduction of local inflammation. Along with antiinflammatory interleukins such as IL-4 and IL-10, VEGF, transforming growth factor- β (TGF-β) are also released in the microenvironment (Mantovani et al., 2002; Jetten et al., 2014). This drastic shift of events prepares the cells of surrounding tissue to undergo both genotypic and phenotypic changes, thus driving them towards the regenerative route (Figure VI).

Otherwise, the microenvironment remains arrested in the catastrophic state and does not allow any growth or healing (Mia et al., 2014; Da Silva et al., 2015). Based on all characters attributed to this process, inflammation is either acute/reparative inflammation or chronic inflammation (Ryan and Majno, 1977; Rankin, 2004).

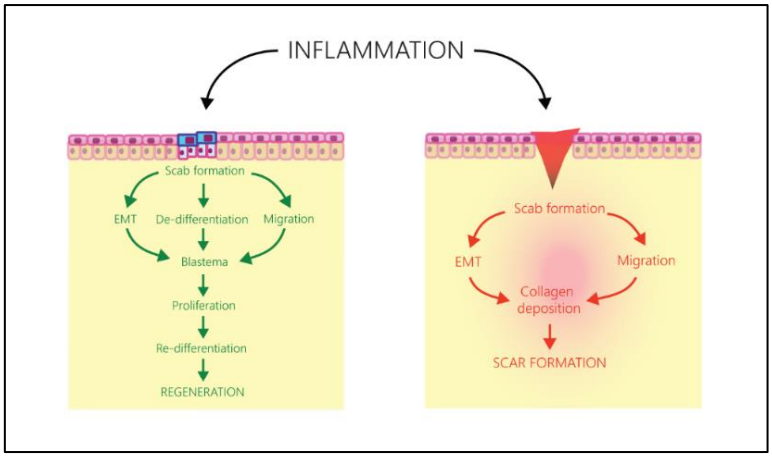

Figure VI: Dual role of inflammation

Acute/Reparative Inflammation

Acute inflammation is triggered as an immediate early response to any injury or infection, a process initiated to combat the pathogenic influx, clear the debris and initiate the repair machinery (Karin and Clevers, 2016) (Figure VII). Intensified blast of immune system drives acute inflammation, which is predominantly short lived and immensely effective (Ryan and Majno, 1977; Ariel and Serhan, 2007). Timely resolved inflammation clears out the foreign invaders (microbes, dead cells, and physical irritants), making way for the regenerative molecules to control the microenvironment (Kyritsis et al., 2012).

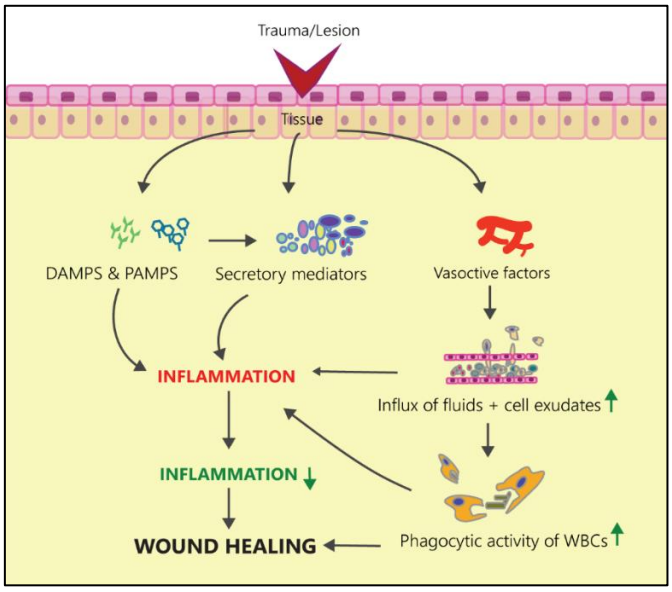

Figure VII: Wound healing effect under acute inflammation

Predominantly, exposure to foreign substances is recognised as PAMPs and DAMPs, which engage pattern recognition receptors (PRRs) and simultaneously start multiple inflammatory pathways, through upregulation of transcription factors like nuclear factor-κB (NF-κB), activator protein-1(AP-1) and signal transducer and activator of transcription (STAT) (Kyritsis et al., 2012; Han et al., 2015). These same molecules, on the other side initiate the wound repair mechanisms as found in model organisms ranging from fly to mammals (Gillitzer and Goebeler, 2001; Eming et al., 2014; Ellis et al., 2018). Reactive oxygen species (ROS) is produced at high titres during this cellular warfare and links cell proliferation with inflammation through activation of janus kinases (JNKs), which further stimulate Fos and interleukin pathways (Jung et al., 2010; Ohyama et al., 2018). Crucial events of cell migration are primed coherently by this signalling, opening gateways for wound closure (Seki et al., 2014). Parallelly, this release stimulates the localised stem cells to undergo cell proliferation, which would culminate into successful wound repair (Jia et al., 2018).

Chronic Inflammation

Prolonged stay of unresolved inflammation maintains a harsh, intolerable wound environment and, in the process, endangers the healthy tissues around (Figure VIII). This unresolved bout of immune response is termed as 'Chronic inflammation' (Tidball, 2011; Oishi and Manabe, 2018).

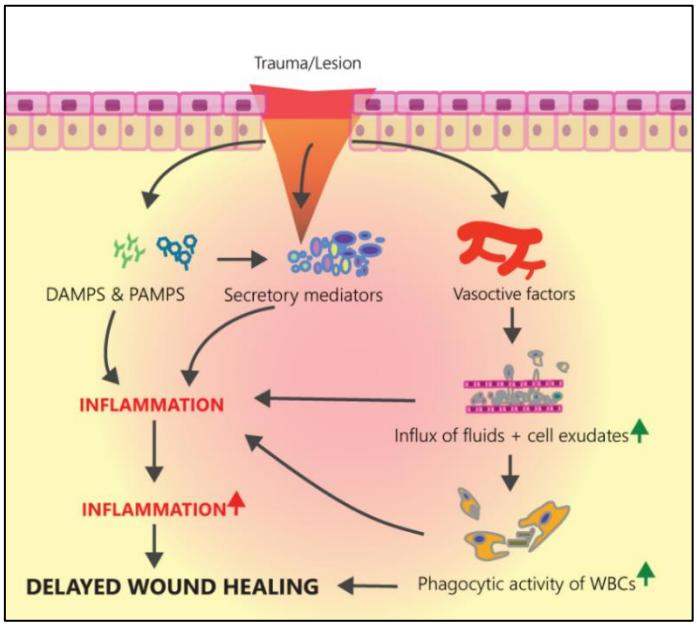

Figure VIII: Wound healing under chronic inflammation

Tissue resident mast cells react to the pathogenic activity and release exudates such as histamines, eicosanoids, proteases, TNFs and other chemokines, thus killing the foreign cells. Only when stop signals are not generated or ignored, amplified and evident tissue damage is observed under the blazing immune system blasting away the healthy cell population. (Mescher et al., 2013; Heneka et al., 2013). For instance, the first cell cluster recruited at the wound site are neutrophils which set up neutrophil extracellular traps (NET) to target the microbes by killing or immobilising them (Fuchs et al., 2007). The same NET can hinder routine wound healing as found in the chronic wound sites in diabetic patients (Josefs et al., 2020). Matrix metalloproteinases (MMPs) released by NET harms the ECM in healthy tissue on prolonged stay as found in cystic fibrosis and diabetic foot ulcers (Fadini et al., 2016; Kumar et al., 2018). The cells and cytokines such as macrophages, TNFs etc, are deployed to clear away the necrotic waste and neutrophils, induce ROS and target the healthy cells under prolonged inflammation (Behnen et al., 2017).

Convoluted Connections: Inflammation and Wound healing

Massive research and enormous resources have been utilised to decode the correspondence of these two events and the molecular switches that prompt these processes (Redd et al., 2004; Mercandetti and Cohen, 2017). Is it just the time duration of inflammation, or a specific type of molecular environment, which decides the pattern of inflammation? Does the increase or decrease of inflammatory response, depend on the same signalling cascade? What steers the microenvironment to either continue with high levels of inflammation or allows its gradual reduction? All these questions can be addressed by reviewing the epicentre itself, i.e., the wound site. The primordial contribution on this account, arrives from the specific mediators leading to either a hike in inflammation or its abatement (Henry and Garner, 2003; Alexopoulou et al., 2007).

Cyclooxygenases: Cardinal and Cogent

Owing to tissue injury, along with interleukins and cells of the immune system, a troop of eicosanoids is activated parallelly. These are among the primary mediators of inflammation, supporting both its initial rise and gradual resolution (Khanapure et al., 2007). Proinflammatory mediators released during the rigorous cellular events post-injury also induce phospholipases production, which in turn catalyses the release of membrane-associated arachidonic acid

(Langenbach et al., 1995). Injury to the tissue also induces the cyclooxygenase (COX) family of enzymes, which acts on the available arachidonic acid and converts it into active prostaglandins, thus named prostaglandin H synthase (Ricciotti and FitzGerald, 2011). Research of over 50 years since their discovery, prostaglandins have been established as major inflammatory mediators, primarily contributing to its rise (Ricciotti and FitzGerald, 2011; Harris et al., 2002; Dennis and Norris, 2015). Animal models lacking early availability of prostaglandins have elicited compromised immune responses (Tilley et al., 2001; Liang et al., 2005). There occur to be three members in the cyclooxygenase family, COX-1, COX-2 and COX-3 (Simmons et al., 2004). The last one being a spliced gene variant of the first isoform (Willoughby et al., 2000; Simmons et al., 2004). COX-1 is a proven organiser of tissue homeostasis and its absence or inhibition causes a multitude of developmental anomalies. COX-2, on the other hand, functions predominantly under inflammatory milieu (Kuwano et al., 2004; Seibert et al., 2014). It is a cardinal regulator of inflammation and its expression profile can be altered via drugs such as non-steroidal antiinflammatory drugs (NSAIDs) (Lee et al., 2009). Being closely linked to inflammation, its transcriptional and proteomic levels directly affect the wound healing process positively and negatively (Wilgus et al., 2004; Romana-Souza et al., 2016) as observed in many models studied for understanding regeneration. Zhang and colleagues (2018) have shown the periodic changes in the status of COX-2 transcription during wound healing in lipopolysaccharides (LPS) treated mice lung cells. The present study is one such venture envisaged to account for the roles of COX-2 derived inflammation in differential wound healing. COX-2 biology and its impact on wound healing have been discussed at great lengths in the coming chapters. Meanwhile, the project's focal point was to observe the congruence in COX-2 based inflammation and wound healing and how the former governs the differential repair outcome in the same model organism.

In order to study the influence of inflammation on wound healing, one needs to choose the track of signalling cascade to be followed. As discussed earlier, many pathways are triggered immediately after injury, but we selected the COX pathway for our investigation to understand the manifestation and orchestration of inflammation. Our lab has extensively studied and reinforced the cardinal role of COX and PGs in orchestrating Wingless-INT (Wnt), TGF-β, Fibroblast growth factors (FGFs) etc using *Poecilia latipinna* and *Hemidactylus flaviviridis* (Sharma and Suresh, 2008; Buch et al., 2017; 2018). Unravelling the molecular interplay of COX and inflammation has been well documented for decades (Mahajan and Sharma, 2005;

Lu et al., 2017). However, we are keen to understand the regulation of inflammation through COX pathway involving effector prostanoids.

As stated earlier in this section and observed by researchers, reptiles have reslatively limited regenerative potential, only confined to some body parts (Clause and Capaldi, 2006; Maden, 2018). For an instance, the lizard - *H. flaviviridis* is an excellent model, exhibiting organ-specific regeneration, only in its tail. This limitation makes it an alluring model to study the differential wound healing in the same system. All other popular study models have exquisite regeneration capability, uniform and unbiased. Although they have helped discover the fundaments of regeneration, amphibian systems cannot explain the practical loss of epimorphosis, as observed in humans. Hence, exploring lizards comes in handy, rather becomes inevitable, to address the perplexing inability of mammals to regenerate. Only the tail undergoes super healing in lizards, while all other appendages form thick, permanent scar - like a wound healing routine found in mammals. This restricted, biased and differential wound healing approach leads to contrasting repair outcomes in tail and limb, wherein the former organ regrows like original appendage before injury, while the latter one settles with a perpetual scar. We used this quality of lizards to study differential wound healing in appendages and the pertaining status of COX-2 derived inflammation in the course of repair.

Hemidactylus flaviviridis: A Sterling exemplary of Epimorphosis

The lizard *Hemidactylus flaviviridis* belongs to the Gekkonidae family, possessing regenerative potentials, albeit with certain limitations. These animals have been astonishingly successful and are found thriving pan-Asia, along with many countries of Africa. They are popularly known as the yellow-bellied geckos or Northern House Geckos. Staying loyal to the name, these reptiles have learnt to thrive around man, unknowingly scaring most of the human race. Lizards are one of the most feared living creatures, without any major fault of their own. Herpetophobia is a psychological condition that sums up all hardwired fear of reptiles, especially snakes and lizards. Evolutionarily, fearing snakes has helped human survival, though being afraid of their other relatives comes naturally to us. House lizards, on the other hand, have overcome significant anthropological pressures to flourish around humans.

Lizards have used their inbuilt competence of regenerating the lost tail as an aid of survival. Biologically, Northern House Geckos can regrow their tail if lost in combatting predators and

to gain social acceptance. The tissue architecture is ingeniously designed to enable this conscious effort of salvage. There happen to be predetermined 'planes' present on geckos' tail, which represent the organ's internal segmentation. These are known as 'Fracture Planes', which are broken from within in compliance with the term (Figure IX).

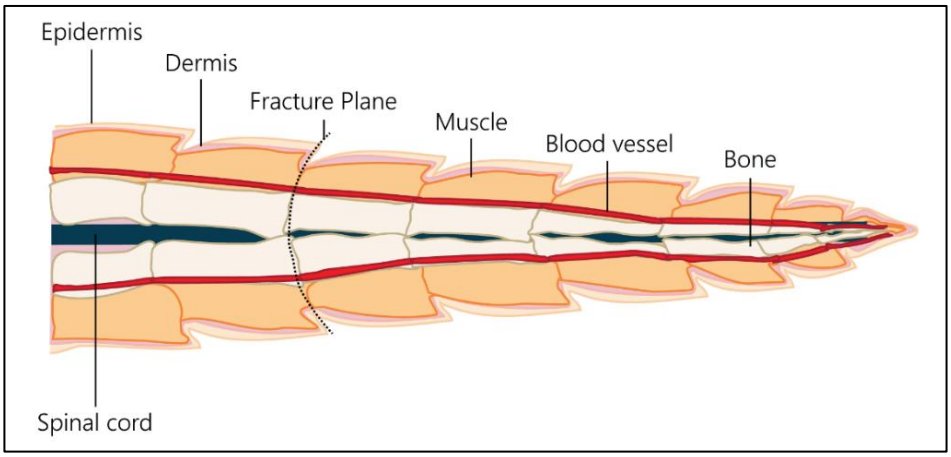

Figure IX: Tissue architecture of lizard tail - original

When any lizard faces a predatory challenge in the wild, it consciously releases its tail from the most distantly placed fracture plane. The released part of the tail wriggles vigorously, thus distracting the predator and gives an escape opportunity to the lizard. This release mechanism is termed 'Autotomy'. We use this tail releasing quality of lizards for our benefit and induce autotomy to observe and examine the following stages of regeneration. Based on the gradual changes and morphological benchmarks achieved, the repair process is subdivided into four main stages: Wound Epithelium, Blastema, and Differentiation. The rationale for choosing these stages has been discussed in detail in the general Materials and Methods section. Post-differentiation, the entire regenerated tail lacks external or internal segmentation including the fracture plane (Figure X). Also, scale pigmentation and pattern vary from the original organ. Structurally, the newly formed tail is cartilaginous to begin with, becoming ossified with passing time. Thus, lizards exhibit 'near perfect' restoration of lost tissue, forming a structural and functional replica of the original organ.

Lizard model stands in the focus of this project which was envisaged to study the impacts of COX-2 derived inflammation on the course and outcome of wound repair, it is crucial to register all the similarities and differences in immune systems of lizards and mammals. Lizards, being reptiles, have different cell types, compared to the mammalian ones, while they perform functions similar to the later ones, their appearance may vary (Zimmerman et al., 2020).

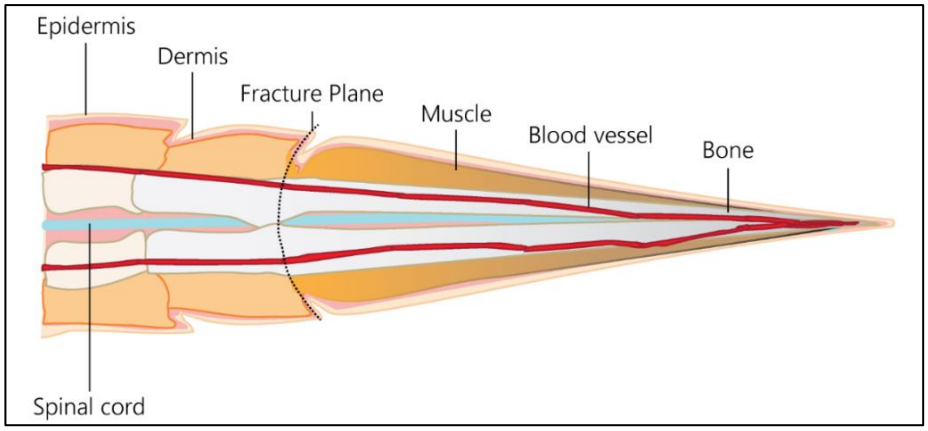

Figure X: Tissue architecture of lizard tail- regenerated

However, the most crucial part for the present study depended on the generation of COX-2 derived immune response post-trauma, which is very relatable in both reptiles and mammals (Zimmerman et al., 2010). The striking similarities in reptiles and mammals' immune system and inflammatory response will be discussed in the following sections.

A previous study from our lab (Ranadive et al., 2018) has shown that the process of wound healing post-amputation is starkly different in the case of tail and limb of *H. flaviviridis*. The autotomised tail heals quickly (by 4-day post autotomy - dpa) without forming a scar between the newly formed wound epithelium and the underlying mesenchyme. The subsequent molecular cross-talk between the stratified wound epithelium (apical ectodermal cap) and the mesenchymal tissues beneath will act as a trigger for epimorphosis. The lizard limb on amputation takes a different route for healing. This wound healing process is much akin to any other higher vertebrates, including mammals. Herein, the wound heals slowly with a remarkable deposition of collagen (that matures to form a scar) between the wound epithelium and the mesenchyme. This scar acts as a deterrent for the molecular interaction between the epithelium and mesenchyme hence the lizard limb fails to regenerate.

As per the results obtained in previous studies conducted in our lab, any attempt to impede the activity of COX-2 resulted in delayed wound healing and loss of regeneration (Sharma and Suresh, 2008). Therefore, we hypothesised that the level of COX-2 induced PGE_2, a master regulator of inflammatory mediator, could be different at the amputation site of tail and limb in lizard which may leads to differential expression of other inflammatory mediators in a context specific manner resulting in scarring of limb and 'superhealing' followed by regeneration of tail post-amputation.

In order to check our hypothesis, a detailed study was devised with one primary objective, which was to study the temporal expression pattern of cyclooxygenase and ascertain its role in the regulation of inflammatory response during wound healing in the appendages of lizard *H. flaviviridis.* Three specific aims were formulated to achieve this objective, results of which are comprehended in the three chapters of this thesis. The timepoints determined for the observation were based on the pivotal hallmarks of healing observed in the tissues - haemostasis, inflammation, granulation followed by proliferation and wound epithelium formation. For tail, 0 (resting), 1, 2, 3 and 4dpa and for limb, 0, 3, 6 and 9dpa (days post-amputation) were considered for this study, owing to the difference in repair time needed for these appendages and based on the previous studies in lab (Buch et al., 2017; Ranadive et al., 2018)

The three chapters entitled below comprehend the specific aims of this study, wherein as a first step, the temporal status of inflammation was probed across the healing frames of tail and limbs, keeping humoral inflammatory mediators in focus. On the second step, expression status of cells of immunity and their recruitment at the site of healing was checked for both superhealing (tail) and scarring (limb) appendages. As a final step, the regenerative tail blastema was used to simulate 'tail-like' conditions in scarring limb. Detailed explanation of rationales considered and experimental protocols are discussed in the respective chapters. Title of the chapters are as follows:

Chapter 1: Inflammation orchestrates differential wound healing in lizard appendages
Chapter 2: Possible role of blood cells in differential wound healing of lizard appendages
Chapter 3: Impact of blastema homogenate application on inflammation and wound healing in lizard limbs