Autonomous self-repair systems

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Regeneration is an important and wonderful phenomenon in nature and plays a key role in living organisms that are capable of recovery from trivial to serious injury to reclaim a fully functional state and pattern/anatomical homeostasis (equilibrium). Studying regeneration can help develop hypotheses for understanding regenerative mechanisms along with advancing synthetic biology for regenerative medicine and development of cancer and anti-ageing drugs. Further, it can contribute to nature-inspired computing for self-repair in other fields. However, despite decades of study, what possible mechanisms and algorithms are used in the regeneration process remain an open question. Therefore, the main goal of this thesis is to propose a comprehensive *hypothetical conceptual framework with possible mechanisms and algorithms* of biological regeneration that mimics the observed features of regeneration in living organisms and achieves body-wide immortality, similar to the planarian flatworm, about 20mm long and 3mm wide, living in both saltwater and freshwater. This is a problem of collective decision making by the *cells in an organism* to achieve the high-level goal of returning to normality of both anatomical and functional homeostasis. To fulfil this goal, the proposed framework contains three sub-frameworks corresponding to three main objectives of the thesis: self-regeneration or self-repair (anatomical homeostasis) of a simple *in silico* tissue and a whole organism consisting of these tissues based on simplified formats of cellular communication, and an extension to more realistic bioelectric communication for restoring both anatomical and bioelectric homeostasis.

The first objective is to develop a simple tissue model that regenerates autonomously after damage. Accordingly, we present a computational framework for an autonomous self-repair system that allows for sensing, detecting and regenerating an artificial (*in silico*) circular tissue containing thousands of cells. This system consists of two sub-models: Global Sensing and Local Sensing that collaborate to sense and repair diverse damages. It is largely a neural system with a perceptron (binary) network performing tissue computations. The results showed that the system is robust and efficient in damage detection and accurate regeneration.

The second objective is to extend the simple circular tissue model to other geometric shapes and assemble them into a small virtual organism that regenerates similar to the body-wide immortality of the planarian flatworm. Accordingly, we proposed a computational framework extending the tissue repair framework developed in Objective 1 to model whole organism regeneration that implemented algorithms and mechanisms to achieve accurate and complete regeneration in an (in silico) worm-like organism. The system consists of two levels: tissue and organism levels that integrate to recognise and recover from any damage, even extreme damage cases. The tissue level consists of three tissue repair models for head, body and tail. The organism level connects the tissues together to form the worm. The two levels form an integrated neural feedback control system with perceptron (binary) for tissue computing and linear neural networks for organism-level computing. Our simulation results showed that the framework is very robust in returning the system to the normal state after any small or large scale damage.

The last objective is to extend the whole organism regeneration framework developed in Objective 2 by incorporating bioelectricity as the format of communication between cells to make the model better resemble living organisms and to restore not only anatomy but also basic functionality such as restoring body-wide bioelectric pattern needed for physiological functioning in living systems. We greatly extended the second framework by conceptualising and modelling mechanisms and algorithms that mimicked both the pattern and function restoration observed in living organisms and implemented it on the same artificial (in silico) organism developed in Objective 2 but with greater realism of the anatomical structure. This proposed framework consists of three levels that collaborate to fully regenerate the anatomical pattern and maintain bioelectric homeostasis in the *in silico* worm-like organism. These three levels represent tissue and organism models for regeneration and body-wide bioelectric model for restoring bioelectric homeostasis, respectively. They extend the previous neural feedback control system to integrate another (3^{rd}) level, bioelectric homeostasis. Our simulations showed that the system maintains and restores bioelectric homeostasis accurately under random perturbations of bioelectric status under no damage conditions. It is also very robust and plastic in restoring the system to the normal anatomical pattern and bioelectric homeostasis after any type of damage.

Our framework robustly achieves some observations of extreme regeneration of planaria like body-wide immortality. It could also be helpful in engineering for building self-repair robots, biobots and artificial self-repair systems.

Keywords: Self-repair, Regeneration, Anatomical and Bioelectric Homeostasis, Multi-Cellular Structures, Planaria, Collective Intelligence, Computational Tissues, Somatic Cell Network, Perceptron Networks, Neural Networks, Signalling Entropy, Information Fields, Auto-Associative Neural Networks, Robotics.

Publications

The thesis has published and prepared the following articles/manuscripts:

- Minh-Thai T.N., Aryal J., Samarasinghe S., Levin M. (2018) A Computational Framework for Autonomous Self-repair Systems. In: Mitrovic T., Xue B., Li X. (eds) AI 2018: Advances in Artificial Intelligence. AI 2018. Lecture Notes in Computer Science, vol 11320. Springer, Cham. https://doi.org/10.1007/978-3-030-03991-2_16

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This thesis is dedicated to my parents for

their dream and expectations!

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Chapter 1

Introduction

Regeneration is an essential phenomenon in nature. While it plays a crucial role in living organisms that are capable of recovering a fully functional state from diverse forms of injury, it is not entirely understood (Elliott & Sánchez Alvarado, 2013; Maden, 1992). Although molecular mechanisms required for regeneration are being discovered, the algorithms sufficient for the simulation of regeneration of complex anatomical structures represent a significant knowledge gap that holds back progress in evolutionary developmental biology and regenerative medicine. Currently, there is insufficient knowledge of regeneration in living organisms, making it essential to develop conceptual frameworks to promote understanding of regeneration and translate them into computational models of cell activity and communication dynamics that can implement complex repair of anatomical structures and restore functionality. It is especially important to explore connectionist (neural-like) models in the control of regeneration to begin to formulate rigorous formalisms for pattern memory and decision-making during anatomical remodelling. *The overall goal of this research is to develop a conceptual framework for biological regeneration that enables recovery of the complete body pattern from any small or extreme damage anywhere in the system (aka body-wide immortality) and restores basic functionality. The purpose of the framework is to promote the generation of hypotheses about the potential mechanisms and algorithms of regeneration that allow such extreme form of recovery*. The developed framework is a multi-level modular neural feedback control system representing collective intelligent decision making in an organism consisting of multiple tissues containing a large number of cells. A main consideration for the framework is to accomplish complete recovery with minimum information and computational burden on cells that interact and implement algorithms of regeneration. The framework also contributes to nature-inspired computing for self-repair in other fields. In order to formulate the research problem, this chapter provides an overview of regeneration, the importance of regeneration, planarian flatworm as an excellent model organism for studying regeneration and current knowledge gaps. Then, the chapter presents the terminology used in the thesis. It then presents the research objectives and concludes with an overview of the thesis chapters and main contributions.

1.1 What is regeneration?

Regeneration in biology is a set of biological processes through which an organism replaces and regrows lost or amputated parts to the original state. In nature, all living organisms need to be resilient in order to survive. Animals can be injured at some point in their life; and their ability and rate of

regeneration will determine whether they survive or die. Regeneration process usually occurs through specialised cells (called adult stem cells) that are randomly distributed in an organism in numbers that vary by species. Stem cells are cells that can renew themselves and produce other types of cells in the body. Regeneration is one of the oldest fields of study and a fascinating phenomenon in biology, but this process is not completely known (Maden, 1992; Needham, 1961). Many biological organisms are able to regenerate some of their tissues and complex body organs after amputation (Birnbaum & Alvarado, 2008). Vertebrates such as amphibians and fish have high regenerative capacity while mammals have a limited ability to regenerate their body parts, such as cells, tissues, and organs. Each organism differs in its ability to regenerate body parts. Self-repairing or regeneration in different species can occur to varying extents: whole body, parts of the body, organs, tissues, and cells. For example, hydra (tiny aquatic animal) and planarian (free-living flatworms) can regenerate the whole body from a small part (Ivankovic et al., 2019; Lengfeld et al., 2009; Levin, Pietak, & Bischof, 2018), and mammals also can restore complex structures such as children's fingertips (Illingworth, 1974), liver (Michalopoulos, 2007) and deer antlers (Li, 2012; Nieto-Diaz et al., 2012). Likewise, many plants have a great capacity for regeneration when they are damaged (Ikeuchi, Ogawa, Iwase, & Sugimoto, 2016). In fact, most natural systems and organisms are able to self-repair, which significantly contributes to their robustness and resilience. Understanding how to decipher the capability and mechanisms of regeneration of organs and body parts is a crucial need in regenerative medicine. Regeneration research has existed for close to a century, but the study of the mechanisms and algorithms of regeneration is still in its infancy. *Therefore, this research proposes a conceptual and computational framework for the regeneration of multicellular artificial (in silico) tissues and organisms with the focus on computationally mimicking some observed regeneration patterns in living organisms.*

Nature has also inspired self-repair in other fields such as robotics, software, electronic, etc. Multi-robot systems have been designed with the ability to form patterns or configurations to achieve the desired goals and functions and detect and recover from faults or attacks (Arbuckle & Requicha, 2010; Edwards, 2016; Rubenstein, Sai, Chuong, & Shen, 2009). In software engineering, self-repair systems have been inspired by the ability to self-heal in natural materials and structures. It could be less challenging to build a self-healing software system than a self-repair robot or a mechanical system and the self-repair software systems often apply when working with agent-based systems or service-oriented architectures, that is still the subject of intense research (Frei et al., 2013). Due to the extreme complexity of self-repair in natural systems, its technical adaptation is not an easy task, and building an engineering system similar to natural systems needs careful study of the biological system and an understanding its material structures and processes of regeneration. In the literature, the term self-repair in software tends to refer to automatic software repair that consists of automatically finding a solution to a software fault without human intervention. Automatically repairing software is both

challenging and important because it is a difficult task and could bring value to society and humanity (Monperrus, 2018). The main task of automatic software repair is to develop systems with the capacity to maintain its intended function even when facing faults or errors. Self-repair is also one of the important aspects of self-management systems that are able to diagnose malfunction of the system and take repair actions to recover system function from any attack or damage. This is analogous to wound healing in living organisms. From the perspective of these artificial self-repair systems, the regeneration framework proposed in this research is analogous to self-repair in self-management systems (living organisms). It mimics some currently known fundamentally important biological regeneration processes at the cell level in order to produce an improved regeneration system that mimics *in silico* some extreme regeneration patterns observed in biology. *Therefore, as a software framework, it can be applied to software engineering systems or self-repairing robots.*

1.2 Why study regeneration?

Regeneration of missing parts is an intriguing mystery in biology and happens in all species. Regeneration has been studied for centuries, and some aspects of regeneration have been uncovered as described in this thesis. For example, snails can regrow their heads, axolotls (amphibian known as walking fish) can regenerate their limbs and zebrafish can reproduce new hearts (Rabinowitz et al., 2017). However, a full description of the regeneration processes is far from clear. When we understand the natural regeneration processes, this information will be useful for regenerative medicine fields with applications to birth defects, traumatic injury, cancer, and degenerative diseases. The regenerative medicine aims to control pattern formation of tissues, organs and body parts that allow living organisms to detect and repair damage to complex forms. Computational frameworks and algorithms that mimic regeneration can aid a greater understanding of complete regeneration. Engineers are also seeking methods to create fault-tolerant and robust self-repair systems by mimicking biological regeneration (Bradley & Tyrrell, 2000; Bremner et al., 2013; Frei et al., 2013).

In most animals and humans, many tissues and organs do not have a great capacity to regenerate, but scientists believe that they could unlock or induce regeneration processes to enable recovery from any damage. In some animals, stem cells can repair or replace damaged parts and prevent damage to other organs after an amputation. For example, zebrafish can repair its heart and axolotls can regenerate their limbs, even as adults (Major & Poss, 2007; Rabinowitz et al., 2017; Roy & Lévesque, 2006). In planaria, stem cells have special capabilities of regeneration and work as a repair mechanism for injured or damaged organs or the whole body (Rink, 2013). However, what mechanisms and algorithms are used in regeneration and how stem cells can accurately regenerate amputated parts are still not clearly understood. Studying regeneration processes in other species may help us understand the fundamentals of regeneration and regeneration in humans and shed light on the

essential steps in the development of regenerative medicine and self-repair systems. *This study, therefore, seeks to develop a conceptual basis for a full description of regeneration based on known regeneration principles and various new hypotheses, and transform it into a computational platform of regeneration and demonstrate its regenerative capacities on an artificial organism.*

1.3 Why planaria?

The main reason for choosing planarian flatworms as the biological inspiration for our frameworks is that planarian is one of the simplest metazoans with powerful regenerative capabilities. Planaria are small flatworms about 3 to 20mm long and 2 to 3mm wide, living in both saltwater and freshwater (Lobo, Beane, & Levin, 2012; Reddien & Alvarado, 2004). T.H. Morgan was one of the first researchers to study planarian regeneration in the early 20th century (Morgan, 1900). Morgan found that planaria had an amazing ability to restore lost parts or organs to the original form when amputated along any plane. As shown in Figure 1.1, after an injury, three different head, body and tail parts regrow quickly into the full worm. Even more amazing is that a tiny piece as small as 1/279th of a planarian (Morgan, 1898) or a piece with at least 10,000 cells could successfully regenerate a new planarian within several weeks (Montgomery & Coward, 1974). A 6 mm long planarian has approximately 6×10^5 million cells (Figure 1.1B) (Takeda, Nishimura, & Agata, 2009).

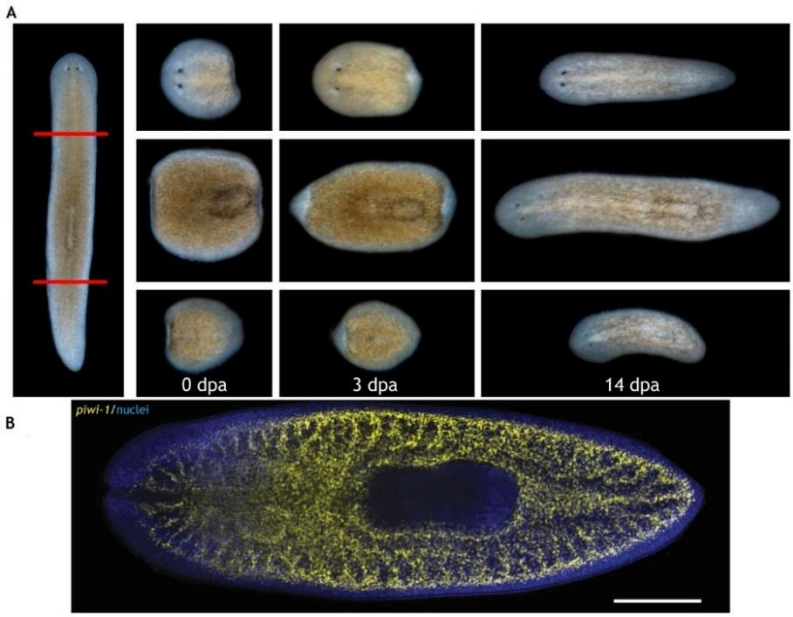


Figure 1.1. Planarian regeneration and stem cells distribution. (A) Regeneration of head (top), body (middle) and tail (bottom) parts. Red lines indicate the cutting planes. Days post-amputation (dpa) are observed time points. (B) The distribution of stem cells (yellow) in a cross-section of a planarian. Scale bar: 500 µm. (Sourced from (Ivankovic et al., 2019) with permission)

Regeneration in planaria is a complex mechanism that involves interactions and communication between cells at the organismal level to maintain complex morphology (body pattern) after amputation (Reddien, 2018; Sheiman & Kreshchenko, 2015). Planaria produce new tissues from the unique stem cells known as neoblasts that can migrate through the whole organism to the damage site to regenerate missing parts by stem cell division (Wagner, Wang, & Reddien, 2011a). Here, a stem cell makes an identical copy of itself and a new tissue cell (also called a differentiated or somatic cell) depending on the type of damaged tissue, and the process repeats over and over until the damage region has been restored to the original morphology.

Apart from a tiny piece regenerating into the full form, another interesting aspect of planarian regeneration is that the tiny piece first grows into a small worm that grows into the full form by concurrent regeneration of body parts (Figure 1.1A) – planaria constantly remodel themselves to maintain proportion among body structures (Beane, Morokuma, Lemire, & Levin, 2013; Oviedo, Newmark, & Sanchez Alvarado, 2003). Another exciting fact is that a small trunk fragment of a planarian left after cutting away the head and the tail always regenerates the head and tail in the correct orientation as in the original planarian (Fig.1.1A). It indicates that the remaining tissue identifies the anterior-posterior (abbreviated as A/P) polarity that helps new parts to grow in the correct positions (Pietak, Bischof, LaPalme, Morokuma, & Levin, 2019; Yazawa, Umesono, Hayashi, Tarui, & Agata, 2009). A/P polarity in planarian is operationally similar to a bar magnet in that a new bar magnet after being cut transversely also restores the original North-South polarity (Lobo et al., 2012).

Despite the advances in the knowledge of stem cell division (Hill & Petersen, 2015; Kobayashi et al., 2008; Lei et al., 2016; Nodono & Matsumoto, 2012; Salvetti et al., 2009; Sasidharan et al., 2013; Wagner, Wang, & Reddien, 2011b), many questions remain about how planaria control the anatomy (Levin et al., 2018). Planaria are thus an ideal test case for developing constructivist models that show the dynamics sufficient for complex pattern homeostasis (Cervera, Meseguer, Levin, & Mafe, 2019; Lobo & Levin, 2015; Stuckemann et al., 2017; Werner et al., 2015). The amazing regenerative abilities of planaria largely inspired this research. The expectation is that knowledge of regeneration in these versatile organisms could one day help unlock the potential of regeneration in the human body. Conceptual frameworks can be an important bridge between what is known and what is possible in future.

Recent studies show that communication mechanisms play a pivotal role in planarian regeneration (Lobo et al., 2012). The question of how old and new tissues communicate with each other or how old and new cells communicate and work with each other to perform complete regeneration is still unclear. After amputation, stem cells migrate to the wound site and divide to produce new cells and

form a blastema (an unpigmented structure) and then regenerate lost tissues until the exact pattern of the original anatomy is recovered (Elliott & Sánchez Alvarado, 2013; Montgomery & Coward, 1974). This requires stem cells to know where the damage is, what is missing, what structures to regenerate and to stop when regeneration is complete. This seems to be achieved through communication between stem cells and other tissue cells (aka somatic cells or differentiated cells) involved in the regeneration process (Oviedo & Levin, 2007).

Gap junctions (GJ) between somatic cells play a vital role in cell communication within a tissue. GJs are direct physical channels that allow cells to transfer ions (bioelectric currents) and small molecules directly into adjacent cells. It has been shown that bioelectric signalling can alter cell division patterns and migration of adult stem cells (Levin, 2007a, 2009; Sundelacruz, Levin, & Kaplan, 2009) indicating the role of bioelectricity in communication between stem cells and somatic cells. All cells in the body produce and receive bioelectrical signals with changes in their cell membrane voltage (V_{mem}) due to ion fluxes moving through ion channels located on the cell membrane (Levin, 2007b; Levin & Stevenson, 2012). Ion fluxes allow communication between cells by exchanging ions between intracellular and extracellular environments that create a voltage gradient that affects the activity of both neighbour cells and distant cells in the body (Barghouth, Thiruvalluvan, & Oviedo, 2015). These bioelectric signals can be transmitted from a cell to long-distances directly through GJ between neighbouring cells as well as indirectly through the extracellular environment and sensed by itself, adjacent cells or distant cells (Adams, 2008; Esser, Smith, Weaver, & Levin, 2006; Levin, 2009). The bioelectric signalling can carry A/P polarity and patterning information in the planarian regeneration (Beane, Morokuma, Adams, & Levin, 2011; Nogi, Yuan, Sorocco, Perez-Tomas, & Levin, 2005). However, how the remaining cells re-establish the polarity in the process of regeneration is still unclear. These complex signals through GJs and ion fluxes are controlled by as yet unknown mechanisms that promote collaboration within the cell collective to maintain the correct geometry of planaria.

The idea that bioelectricity can sense injuries and trigger some of the key factors in regeneration has driven regeneration research for decades to this day. Recently, Durant et al. (Durant et al., 2019) found that the membrane voltage changes very early in the regeneration stage before there is a difference in gene expression (that produces proteins required for cell signalling to initiate repair) and the early disruption of this membrane voltage affects polarisation information during regeneration. Based on endogenous (internally generated) electrical signals, cells communicate with each other and make decisions about what shape and size of what to recover and which genes need to be turned on (Durant et al., 2019) to do the repair. This indicates that bioelectricity plays an important role not only in confirming polarisation after injury in planaria but also in providing the information needed for

regeneration of damaged or missing tissues and organs. However, the exact mechanisms of bioelectric mediated communication between cells to perform the coordinated activity at both the cell and organism level is not fully understood. Also unknown is how the perturbed bioelectric homeostasis (equilibrium) due to injury is restored after recovery to resume the functionality of the organism. Therefore, hypothetical frameworks are useful tools to explore the potential role of body-wide voltage gradients in the maintenance and repair of mature tissues and restoration of voltage homeostasis after recovery.

As fundamental regeneration mechanisms are generally similar among organisms, these model concepts can apply more widely to all organisms, including humans, either to invoke biological regeneration or to develop artificial organs or limbs. Humans and planarians share some of the same organ systems such as a brain, eyes, musculature, intestine, epidermis, reproductive structures. However, unlike planaria, the regenerative capacity of humans is very limited. Researchers attempt to enhance the understanding of regeneration processes by studying simple life forms, like planaria. These findings could potentially reveal clues to affect human cells to behave similarly and improve the ability of regeneration in humans.

1.4 Terminology

In recent years, there has been an increasing interest in self-repair research, and various definitions of the term self-repair have come to existence. Literature has used the same term with different meanings, and conversely, many terms with the same meanings.

- The term *self-repair* applies to a system that is capable of maintaining and repairing themselves (Frei et al., 2013). As an example, a broken fingernail in a hand can be filed to a suitable form by using the other hand.

- The term *self-healing* applies to a system where parts of the system heal damage from the inside (Frei et al., 2013). For example, a broken fingernail can grow by itself and remove a damaged area.

- The term *regeneration* applies to a biological system with genomes, cells, and organs that can renew, restore, and grow after a natural disturbance or damage (Carlson, 2007). A simple example is a tissue that grows by itself after fibrosis.

In this research, these terms self-repair, self-healing and regeneration are used synonymously to refer to a system or an organism that has the capacity to maintain and regenerate itself after a part is amputated. Similarly, other terms *tissue cells, somatic cells, and differentiated cells* are used

synonymously to refer to a cell, which is not a stem cell. Figure 1.2 shows an example of the tissue where a stem cell (red dot) is at the centre and surrounded by tissue cells (blue dots).

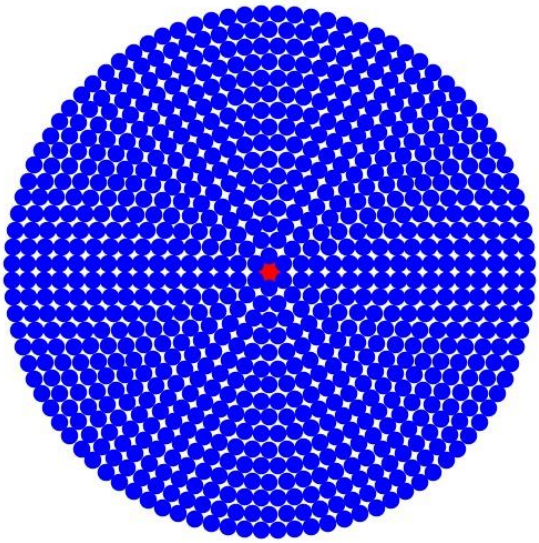


Figure 1.2. An example of the circular tissue with a stem cell (red dot) at the centre and surrounding tissue cells (blue dots)

1.5 Objectives

The primary focus of this thesis is on proposing a *hypothetical* conceptual framework for regeneration with an extensive repertoire of regeneration mechanisms and algorithms using planarian as an example. It contributes to generating new hypotheses to advance the understanding of regeneration by exploiting what is known to explore what is possible in future. Most of the previous regeneration studies have focused on questions related to genes, stem cells, molecular mechanisms, cell signalling mechanisms, and generating models to understand regeneration experiments. While there are not that many computational models of regeneration, most of these require remembering too much information or processing too many computations during pattern discovery and regeneration. There is still a need, and a great scope, for fundamental knowledge from computational fields such as computer science and AI for sufficient and efficient methods to mimic the regeneration processes of living organisms. **Therefore, the overall goal of this research is to propose a conceptual framework based on intelligent systems for regeneration in organisms that have the capacity to self-detect and self-repair any damage or fault by integrating observed features of regenerative biology, especially that of planarian regeneration.** The study intends to develop a comprehensive framework for computational (*in silico*) modelling of regenerative processes that approximates even some of the most extreme regenerative processes observed in biology. The proposed framework is developed in stages starting with a simple tissue (computational tissue) as shown in Figure 1.2 and extending to multiple

tissues and then to a tissue system (organism) which in the final stage incorporates physiological attributes such as bioelectricity and related communication. Accordingly, to fulfil the research goal, this study aims to achieve the following specific objectives.

Objective 1: A Self Repair Model for Simple Computational Tissue. The first model framework represents an autonomous self-repair system that has the capacity to sense, detect and regenerate missing cells in an artificial (circular) tissue (Figure 1.2) under any damage condition, resembling some related processes in biological systems.

Objective 2: A Comprehensive Conceptual Framework for Whole Organism Regeneration. This develops the second model framework that extends the circular tissue model, with further enhancements, to more tissue shapes, such as rectangular and triangular shapes, and assemble these tissue models into a conceptual regeneration framework for an artificial organism that recovers from any damage in the system.

Objective 3: Extended regeneration framework for a Model Organism (Computational Tissue System) with Physiological Attributes of Bioelectric Communication to Maintain both Pattern and Bioelectric Homeostasis. This model greatly extends the organism model proposed in objective 2 to incorporate more features of planarian biology and physiology with a large number of stem cells and bioelectric homeostasis. Bioelectricity is the predominant mode of communication between cells in an organism. This model not only maintains the bioelectric homeostasis under various random physiological perturbations under no damage conditions and efficiently and correctly recovers the full body pattern and bioelectric homeostasis after any damage anywhere in the system.

1.6 Summary of the framework

The proposed framework consists of three sub-frameworks corresponding to the three objectives.

In the first sub-framework, we present a computational framework for modelling a system that allows for sensing itself, damage detection and regeneration in *an artificial circular tissue of cells*. We represent the tissue, which consists of a stem cell surrounded by more than two thousand tissue cells, as an Auto-Associative Neural Network (AANN) consisting of a single layer of perceptron neurons (representing tissue/somatic cells) with local feedback loops (local communications) that allows the system to recognise its state and geometry. Here the communication is local and binary indicating presence or absence of a cell. The stem cell is free to move within the system. Signalling entropy is used as a collective or global property of communication between the stem cell and tissue cells and managed by the stem cell to sense any change in the whole tissue and detect the general damage region in the tissue. Then the exact damage location in the detected region is quickly identified by the perceptron network through local communication. This system consists of two submodels - global

sensing and local sensing that collaborate to sense the change in the system state, detect general damage region, find the exact damage location, and then repair the damage. It can detect and repair multiple concurrent damages and any damage anywhere in the system as long as a single array of cells from the centre to perimeter is left in the damaged tissue to provide minimum pattern information.

In the second sub-framework, we present a conceptual and computational framework for modelling a system that allows for sensing itself, damage detection and regeneration in *a simple artificial organism* consisting of multiple tissues. Here, we first extend the circular tissue model to other tissue shapes of rectangular and triangular tissues, each with a stem cell. We also introduce a new concept of a virtual '*information field*' to the stem cell to store some minimal tissue pattern information to assist complete self-repair of its tissue thereby freeing the damaged tissue of the burden to carry minimum pattern information. Then, we place one rectangular tissue between two triangular tissues to form a simplified worm-like organism with head, body and tail tissues, similar to the planarian. We present two new concepts: at the tissue level, we enhance the stem cell capability with the virtual information field. At the organism level, we introduce a stem cell network consisting of the stem cells in each tissue, which can identify and regenerate missing stem cells and large body parts. This two-level organisation of the model organism, with the stem cell network regulating organism wide large scale repair and local individual tissue repair models regulating tissue repair, allows complete regeneration from any damage anywhere in the organism.

The third sub-framework not only more resemble planarian type regeneration where the artificial organism contains a large number of stem cells but also maintains and restores bioelectric homeostasis. This framework integrates three networks: individual tissue models, stem cell network, and a higher level nodal (global aggregate) network. The first two networks are tasked with pattern regeneration as conceptualised in models 1 and 2 above. The latter high-level network divides the whole organism into 13 segments (i.e., global nodes each consisting of a group of stem cells and surrounding tissue) and assemble these global nodes into network represented by an Associative Memory neural Network (AMN). This AMN remembers and recognises the body-wide bioelectric state of the nodes and restores it after any bioelectric perturbation under no damage conditions. The three levels operate seamlessly in damage to restore anatomical pattern and bioelectric homeostasis.

1.7 Chapter overview and main contributions

This thesis comprises six chapters as described below.

Chapter 1: Introduction

This chapter introduces biological regeneration and the planarian, which is one of the most popular model organisms used in the study of regeneration, and aspects of its incredible regeneration capacity. Then, we present the research problem with a brief overview of background research followed by the objectives of the study and chapter overview of the thesis.

Chapter 2: Literature review

In this chapter, we present a review of the current state of the art of self-repair/self-healing/regeneration in biological and non-biological systems. We primarily focus on computational and algorithmic systems, which explain the processes used by cells or tissues in a biological system to regenerate a given anatomical pattern. We also review nature inspired self-repair systems in other fields that satisfy at least few requirements of our framework that attempts to mimic biological regeneration on an *in silico* organism.

Chapter 3: A Computational Framework for Autonomous Self-Repair Systems – A simple computational tissue of cells

The third chapter addresses the first specific objective of the thesis. It presents a conceptual framework for a self-repair system that allows for sensing itself, damage detection and regeneration in an artificial circular tissue of cells (representing somatic cells in a living tissue). The tissue structure consists of a stem cell at the centre of tissue of differentiated (somatic) cells. There are two types of communication in the tissue. Local direct communication between somatic cells that is implemented by representing the tissue as a single-layer perceptron network that is locally recurrent meaning only the nearest neighbours communicate with each other. This is a type of an Auto Associative Neural Network (AANN). Distant communication between the stem cell and differentiated cells describes global diffusion of information through the Extra Cellular Matrix (ECM). The stem cell uses signalling entropy as a collective or global property to sense the change in the whole tissue and detect the region in the tissue where the damage is, upon which the AANN in this region gets activated to find the exact location, size and shape/border of the damage. The stem cell then migrates to the damage border and repairs the damage. The model recovers from many types of damage autonomously. The main contributions of our tissue model are that it introduces a computational tissue that: (1) recognise itself (both state and geometry), (2) accomplishes regeneration with limited communication and information, (3) involves limited computation and (4) restores the original pattern correctly and

completely. This significantly extends past tissue models that involve a large amount of computation to achieve limited recovery from damage.

Chapter 4: A comprehensive conceptual framework for an Autonomous Regeneration System for a simple virtual organism consisting of multiple tissues

This chapter addresses the second objective of the thesis to extend the tissue model in chapter 3 with a number of new concepts to mimic some of the observed features of planarian regeneration, including body-wide immortality. The new system is able to regenerate itself from any tiny body fragment after damage which is achieved by using a high-level and flexible algorithm. Here we first extend the circular tissue model concept to other tissue shapes of rectangular and triangular tissues. We also introduce a new concept of a virtual '*information field*' to the stem cell of tissue models to store some minimal pattern information extracted by the stem cell from its communication with tissue cells. We then combine two triangular tissues and one rectangular tissue to form a simplified worm-like organism with the head, body and tail tissues. Another new concept is a stem cell network corresponding to the whole organism that can identify and regenerate missing stem cells after damage to any stem cells. Stem cell network is represented by a fully connected neural network consisting of simple linear neurons that represent stem cells. In this model organism, individual stem cells monitor the state of their corresponding tissue and regenerate the tissue as needed after damage as in the circular tissue model. Additionally, the stem cells network monitors the state of the whole organism and regenerates missing stem cells due to damage in order to fully recover from large scale damages involving missing body parts - head, body or tail amputation. In this case, the individual tissue models (AANN) and stem cell network model collaborate to maintain the normal state of the system under any damage condition, from small to large. The contributions of this chapter are that (1) it extended the circular tissue model to other tissue shapes, (2) enhanced stem cell capability with a new concept "virtual Information fields" to store minimal pattern information of the tissues, (3) a stem cell network that connects tissue stem cells to represent the whole organism, (4) stem cell network and individual tissue models that together regenerate the whole organism accurately, and (5) a simple algorithm with minimum computational burden, (6) a decision-making system that combines the stem cell network (linear neural network) and somatic cell networks (AANN) as a collective intelligence system, (7) stem cell network monitors and repairs missing stem cells and large scale damages and the tissue models recover from tissue damages with the help of their stem cell.

Chapter 5: A Comprehensive Conceptual Framework for Autonomous Regeneration and maintenance of bioelectric homeostasis in a simple virtual organism consisting of multiple tissues

The third objective is addressed in this chapter. The objective of this model is to capture more of planarian type regeneration where the organism contains a large number (about 4% of the total cells)

of stem cells. Further, cells communicate through bioelectric signalling to replace communications of presence or absence in binary format in the previous models in chapters 2 and 3. Moreover, the model also maintains and restores bioelectric homeostasis. The model in this chapter is a combination of three networks: individual tissues represented by tissue models (somatic cell networks) as described in the previous two chapters, Stem cell network represented by a locally recurrent perceptron network and an Associative Memory Network (AMN) of global nodes for maintaining and restoring bioelectric homeostasis. Both somatic cell networks and stem cell network use few perceptron motifs (patterns) that are trained to efficiently identify missing neighbours. The AMN is a modified form of Hopfield network that enables learning and remembering the pattern of the body-wide bioelectric gradient. This AMN maintains the bioelectric equilibrium (homeostasis) under any perturbation to the body-wide voltage pattern due to normal physiological activity and restores the bioelectric pattern after any damage. These three networks collaborate seamlessly as and when needed to robustly restore both the original body pattern and bioelectric pattern after any damage. The contributions of this chapter are that it incorporates: (1) a large number stem cells and tissue cells collaborating to sense, identify and regenerate damage efficiently and effectively, and (2) bioelectric signalling that enables cells to communicate with each other and trigger the regeneration process more realistically, (3) AMN that robustly maintains and restores the bioelectric pattern, and (4) The Perceptron motifs with learning ability in stem cell and somatic cell networks to identify missing neighbours.

Chapter 6: Summary, Conclusions and Future Directions

This chapter presents an overview and the most important findings from achieving the objectives of the thesis. The chapter also provides the conclusions and future directions to extend the framework developed in this study to improve its bio realism and generate more hypotheses to understand even more the mechanisms of biological regeneration.

Chapter 2

Literature Review

Biological regeneration is a complex process involving biochemical, bioelectric and physical signals that can be respectively represented by gene and protein activity networks, bioelectrical circuit dynamics and mechanical models or and neural-like networks. Most research on planarian regeneration is associated with gene regulatory networks and protein interaction pathways. However, these networks do not explain mechanisms of form regulation to regenerate successfully. A few researchers have proposed algorithmic and computational models that determine what needs to be done in each step and explain what is required in order to restore correct sample geometries (*in silico*) from different initial conditions. What information is needed to assist decision-making, how is this information then shared and what computation is done on this information to realise collective intelligence mechanisms in the regeneration process? These are still open questions. The previous chapter described the objectives of this research. From these objectives, it specified the number of unique features of biological regeneration that the framework needs to embody. Thus, it is useful to elaborate on these features as our system requirements and constraints before presenting related previous work. We intend to propose regeneration frameworks to design systems that achieve complete and accurate regeneration from any damage, similar to the body-wide immortality of planaria. This means that even a small body fragment can repair the whole pattern or organism. The systems can regenerate new cells on-demand in repair instead of carrying redundant tissue cells. Stem cells produce these new tissue cells and renew itself. Cells can communicate with neighbour cells and distant cells (short- and long-distance communication). Both geometric (pattern) and bioelectric homeostasis are important goals in our final regeneration framework. Accordingly, this chapter provides an overview of the relevant background information that fulfils some of these requirements. Section 2.1 starts with an introduction to the planarian regeneration process, as has been observed in planarian experiments. Next section provides an extensive review of the current state of the art in understanding biological regeneration, largely inspired by planarian regeneration. We briefly review the history of planarian regeneration models, and then we focus on computational and algorithmic models proposed to explain and execute the processes carried out by cells or tissues to regenerate a given pattern. Section 2.3 provides a review of the current state of the art of self-repair in non-biological systems. We paid attention to self-repair models that satisfy at least some of the constraints of our proposed framework. Section 2.4 presents an overview summary of the existing models and presents research gaps that provided the scope for this research.

2.1 The planarian regeneration process

We present the regeneration process observed in planarian experiments that can be considered as the basic or general process of planarian regeneration. Our frameworks follow this mechanism as a guide in proposing potential mechanisms underlying this regeneration process to develop computational regeneration systems. If we cut a planarian flatworm in two, cells proliferate at the wound site to form a blastemal that will recognise the missing parts and regenerate them to produce two new worms with the same geometry of the original worm. The cells of the blastemal are stem cells (called neoblasts). The stem cells can move into the wound site from distant regions if there are no local stem cells. This way, a very small tissue fragment in planaria can regenerate back into normal body proportions over a few weeks. This type of regeneration is called epimorphosis; that means it regenerates amputated parts from stem cells (Agata, Saito, & Nakajima, 2007).

2.2 The current state of the art in understanding planarian regeneration

Here, we give a brief summary account of long research on planarian regeneration and then we provide a summary of the recent efforts in computationally modelling of regeneration that provided the platform for generating our frameworks that greatly extended the previous models in scope and content with respect to mechanisms and algorithms of regeneration and the type and intensity of damages that can be repaired.

2.2.1 Overview of models of planarian regeneration

In early stages of study of planarian regeneration, Morgan (Morgan, 1901; Morgan, 1904) proposed a descriptive model to explain planarian regeneration using the concept of morphogenetic gradients and was concerned about how polarity is re-established in the regeneration process. A trunk of a planarian after cutting head and tail can regenerate a head at the anterior and the tail at the posterior (Fig. 1.1A). However, the head regeneration rate reduces posteriorly to the tail (Sivickis, 1931). From this observation, Sivickis (Sivickis, 1931) proposed a concept of “head-frequency curve” and presented a graph of the regeneration rate relative to the location on the anterior/posterior (A/P) axis (head to tail) (BRØNDSTED, 1955). Child (Child, 1911) recognized the existence of a dynamic gradient along the A/P axis but Morgan showed that the polarity is identified by the structure or different substances along the A/P axis (Morgan, 1905). The head-to-tail gradient is considered due to a factor that is of high concentration in the head but low in the tail (Adell, Cebrià, & Saló, 2010). Recently, gradient models have also explained planarian regeneration with morphogen concentration gradients (Adell et al., 2010) and mitotic activity gradients (Oviedo & Levin, 2007). However, these descriptive models do not explain a mechanism for a tissue to identify where to regenerate a head or a tail. When the worm is cut in the trunk, the cells at the cut have the same information about the gradient and position, but one side regenerates a head, and a tail is reproduced on the other.

The second type- mechanistic models- was first proposed in (Slack, 1980b). This model proposes an ordered set of areas along the A/P axis of the planarian where each area is represented by an ordered number. After damage, stem cells determine the missing area based on the difference between the sequence code for each region and the code at the wound site and then restore the pattern. By this way, the model is sufficient to regenerate the correct pattern of the worm by following this step-by-step mechanism. Another similar model, an intercalary model (Agata, Tanaka, Kobayashi, Kato, & Saitoh, 2003), assumed that each region has a positional value which is used to identify the missing region. This model describes a mechanism for generating missing intermediate fragment by joining the remaining two fragments and then regenerating the missing fragment (Slack, 1980a). However, the location-based models are not consistent with specific experiments, as shown in (Morgan, 1898).

In 1952, Turing (Turing, 1952) presented a system in which chemical substances (called morphogens) react with each other to form self-generated and self-regulated spatial patterns. Turing showed that two or more substances with different diffusion degrees that react with each other can explain the formation of spatial patterns. The other type of regeneration models is dynamical chemical systems inspired by Turing's reaction and diffusion model. Meinhardt and Gierer (Gierer & Meinhardt, 1972; Meinhardt, 1978; Meinhardt & Gierer, 2000) have proposed some dynamical models which use Turing model to generate patterns based on local activation and long-range inhibition. These reaction-diffusion dynamical models can reproduce most biological patterns (Meinhardt, 1982). They can explain the polarity maintenance, the correct regeneration, and the scaling ability of the planaria (Meinhardt, 2009b; Werner et al., 2015). Recently, a model of small molecule components such as cAMP and ATP diffusion through gap junctions based on the reaction-diffusion mechanism was proposed for planarian polarity (Schiffmann, 2011). In this model, a single reaction-diffusion gradient is used in which the head has a high concentration and tail has a low concentration. This model explains the transition of a single headed worm to double-headed worm by using GJ inhibition in which the gradient changes from a high-low to a high-low-high that occurred by the reduction of diffusion coefficient. However, these reaction-diffusion models do not explain other observed regeneration aspects such as the mechanism of migration and differentiation of stem cells (Meinhardt, 2009a), regeneration of dorsal-ventral (D/V) tissues by joining existing tissues (Kato, Orii, Watanabe, & Agata, 1999) (D/V refers to the upper and the lower direction of the anatomy, which is orthogonal to A/P direction), and regeneration of independent A/P axes in the four-headed worm (Oviedo et al., 2010).

Regeneration models based on bioelectrical signals, bioelectric-electrophoretic models, have also been proposed that have given explanations for long-range signal transmission which enables polarity controlling and patterning in the regeneration process (Lange & Steele, 1978). This model was inspired by the effect of external electric fields causing polarity reversals of the worm (Dimmitt & Marsh, 1952; Marsh & Beams, 1952). This model is based on a finding that the brain tissue in the planarian produced

continually negatively charged substances, which diffuse throughout the worm along bioelectrical A/P axis (negative in the head and positive in the tail) and inhibit or abolish brain regeneration of additional brains. The model describes the process of regeneration as follows: After cutting the head (as an example in Fig. 1A), the remaining region (body and tail) does not receive the charged inhibitory substance produced by the missing brain (from head tissue) while the bioelectrical field drives the substance towards the tail; therefore, the substance at the wound will be depleted and the concentration of substance falls below a threshold for inhibition of head regeneration. This triggers new brain tissue regeneration at the wound site. On the other hand, in the case of a tail missing while head and body remaining, the production of the charged substance is continual because the brain is retained as normal. Then, its high concentration at the wound side will inhibit head regeneration at the damage site, and then it regenerates a tail. This model can describe the regeneration of double-headed worm or polarity reversal (reversal of the direction of the charged substance) by external electric fields (Marsh & Beams, 1947; Marsh & Beams, 1952). Other diffusion models have used bioelectric gradients for controlling serotonergic signals during regeneration (Esser et al., 2006; Zhang & Levin, 2009). However, this model cannot explain the patterning along the other axes because it only presents a single direction for one bioelectrical field. Further, there are no comprehensive physiological models that incorporate bioelectric gradients and long-range gap junctional communication.

2.2.2 Recent computational regeneration models

Some researchers have introduced *in silico* models of biological form (geometric pattern) regeneration inspired by planarian regeneration. They have proposed algorithmic models focussing on signal communication between stem cells and tissue cells or between tissue cells only (Bessonov et al., 2015; De, Chakravarthy, & Levin, 2017; Ferreira, Smiley, Scheutz, & Levin, 2016; Ferreira, Scheutz, & Levin, 2017b; Ferreira, Scheutz, & Levin, 2017c; Ferreira, Scheutz, & Levin, 2018; Gerlee, Basanta, & Anderson, 2011; Tosenberger et al., 2015). Specifically, Bessonov et al. (Bessonov et al., 2015) presented regeneration as an error minimisation problem. In this work, tissues of simple (rectangular) and some more complex shapes were modelled to regenerate damaged parts. In this model, there are cells, which can divide to produce new cells, create and send signals to other cells and receive signals from other cells. Each cell communicates with other tissue cells and keeps a record of the total distance to other cells. After damage, the tissue attempts to restore the total signal of each cell through error minimisation by adding new cells. The accuracy and completeness of regeneration depend on the distance between cells, the shape of the cell structure, and a communication function thus limiting the success to smaller and simpler shapes while still involving a significant amount of communication between cells and computation.

Tosenberger et al. (Tosenberger et al., 2015) developed a cellular dynamics model for describing morphogenesis and regeneration of complex patterns. This model contains two sub-models – a global model for stem cell communication as in the model in (Bessonov et al., 2015) and a local model for tissue growth around a stem cells. In the global model, they alter the position of stem cells and make them return to the initial positions, but the assumption in the global model is that stem cells do not get damaged and cannot produce new stem cells. They proposed different types of signal to restore the exact initial stem cell configuration which is successful in some cases and not in others. The local model described the development of a circular tissue from a stem cell. The stem cell continuously produces tissue cells, and those that are beyond the radius of the tissue are killed. Thus, a significant effort is expended in maintaining the tissue shape.

An agent-based model is proposed in (Ferreira et al., 2016) to discover a 3D structure of only stem cells and self-repair from damage or cell death. Each cell creates, sends and receives messages (or information packets) from other cells. The information in the packets is used to recognise the pattern of the structure as well as to detect and repair any dead/missing cells. In a later version, they improved the performance of the model by applying noise to the direction and distance travelled by packets (Ferreira, Scheutz, et al., 2017c). Another of their improvements is presented in (Ferreira, Scheutz, et al., 2017b) with the addition of tissue cells to the structure to reduce the number of stem cells and the number of packets. Packets are only produced by the stem cells while the tissue cells only relay such packets. However, a large number of packets need to be kept in the stem cells as well as a significant amount of communication between cells takes place when they maintain and regenerate the system.

Another computational approach using interactions between a neural network (nervous system) and non-neural (tissue) cells to help an organism grow and recover its initial form from damage is presented in (De et al., 2017). In this model, tissue cells communicate with the neural cells as well as each other in the neighbourhood to modulate four physiological variables while neural cells provide patterning cues to tissue cells in terms of A/P polarity based on bioelectric signals. This is by far the most bio-realistic model with a reasonable regeneration success (55-80%). Limitations of the model are that excessive pruning is required during regeneration and difficulty in regenerating the exact final form.

A genetic algorithm model proposed in (Gerlee et al., 2011) evolves and maintains a 3D spherical cellular tissue structure. Cells communicate with neighbours based on the concentration of oxygen and a generic factor. The algorithm shows that one cell evolves into a network that is similar to cells organised in some of the tissues of the body. They perturbed the original shape to elliptical shape to verify that the system returns to the original pattern. This is only a growth model where the network needs a predefined area to grow into, and it does not include recovery from damage.

Regeneration mechanisms are extremely complex. For this reason, despite the experimental efforts to understand the mechanisms of regeneration and recent computational and modelling studies to explain or mimic regeneration, essential questions about regeneration processes are still unclear. Some of the important questions awaiting answers include: How does the remaining tissue after damage sense and detect damage? What triggers stem cells to react to the damage and start regenerating? How do stem cells carry out the processes of regeneration? How does the system know what exactly is missing and what it needs to build or regenerate next? Where is the pattern memory stored? How does the system know when to stop regenerating? Thus, the biggest knowledge gap concerns the dynamics of large-scale pattern homeostasis, which allows an organism to maintain or regenerate toward a normal or original geometric pattern. Additionally, how the recovered organism restores bioelectric homeostasis is not known and not modelled; how the intact organism maintains bioelectric homeostasis under random physiological perturbations during regular functioning is not known either.

The goal of this research is to produce a conceptual framework for large scale pattern regeneration that captures many of the observed processes and versatility of regeneration in planaria and conduct computational dynamics simulations on an artificial tissue system to demonstrate the efficacy and versatility of the framework. The framework attempts to closely follow the natural regeneration processes in planaria by exploring potential mechanisms and algorithms that mimic those used by cells and tissues to regenerate the morphology and stop recovery after attaining the correct form. Further, it proposes mechanisms and algorithms for restoring bioelectric homeostasis under normal physiological conditions and after recovery from damage.

2.3 The current state of the art of self-repair in non-biological systems

Over the last few decades, the number of studies on nature-inspired self-repair has increased significantly in other fields. Here, we give an overview of self-repair/self-healing systems in these fields in relation to our constraints and requirements described earlier. Some of these self-repair systems include nature-inspired self-repair and self-healing systems in robotics, software and electronics. It is worth noting that there are two aspects to self-repair/regeneration: repair of the form (geometric or anatomical structure) and repair of function. In biological regeneration models, the focus has exclusively been on the repair of anatomical form. In contrast, the focus of self-repair models in other fields has almost exclusively been on the repair of function. Most have been inspired by biology.

2.3.1 Self-healing software

In the software engineering field, software systems may consist of many entities and components such as agents and services that can communicate with each other to perform all functions of the systems.

There is usually redundancy in a multi-agent system so that one agent can replace another to complete a task. These agents can collaborate or compete, depending on the desired goals. A software system cannot repair itself by rewriting codes or correcting codes itself when facing faults. Instead, the system can recover from failures and damages by restoring the codes from backup places.

A series of approaches has been introduced to self-repair computer networks for the mutual repair of anti-virus software (Ishida, 2005, 2008; Ishida & Tanabe, 2010). These networks consist of nodes where each node can be a computer that communicates with others. Individual computers have a unique state at a given time where the normal state is a clean computer without viruses, while abnormal state describes a viral infection. Self-repair in this network is detection, deletion of viruses and restoring anti-virus software that occurs in two cases: (1) a node repairs itself when it can detect and delete viruses; and (2) each node repairs its neighbour nodes with a certain probability when the neighbour nodes cannot self-repair themselves. Normal nodes and damaged nodes can repair other nodes with different probability of success. Multiple nodes can repair a node, and success is defined in two ways- either all repairs are successful (AND-repair), or at least one repair succeeds (OR-repair). Normal computers can successfully detect and delete viruses, while abnormal computers infected by viruses can repair successfully if the anti-virus program is keeping itself up-to-date and its functions are not infected by viruses. The repairs by abnormal nodes could contaminate other nodes because the repair process copies the content (anti-virus software) from repairing nodes to repaired nodes. In this system, each computer carries the antivirus software. This repair system fulfils some of our requirements like communication between nodes and collaboration to sense and repair damage. However, it only restores function, and if many nodes are abnormal, the remaining normal nodes may not repair successfully.

Part el al. (Jeongmin, Giljong, & Eunseok, 2005; Park, Youn, & Lee, 2005) proposed multi-agent-based self-healing systems that can monitor and restore function (malfunction). The repair systems consist of six steps executed through six agents: Monitor agent, Component agent, System agent, Diagnosis agent, Decision agent, and Searching agent. Six agents cooperate to detect and repair problems in the system. In the case that systems cannot repair using local information within the system, the Searching agent can search for the required information on the Internet to assist repair. This system also utilises communication between agents that helps them detect malfunction and fix the issue. However, like the other systems above, these systems cannot create new agents or entities and only restores system function.

Other self-healing systems can be found in fault-tolerant systems (Nelson, 1990), self-stabilising systems (Chernoy, Shalom, & Zaks, 2010), survivable systems (Linger, Mead, & Lipson, 1998), and autonomic computing (Kephart & Chess, 2003) where the goal is to maintain their function when the

system experiences faults. These systems operate based on the strategies for damage detection, identification, and recovery.

2.3.2 Self-healing electronics

In electronics, researchers have developed techniques to make electronics more reliable. One way is to make the electronics more fault-tolerant. For example, if a part breaks, the system will still work. Another is to make the systems self-repairing if something breaks.

In (Bremner et al., 2013; Samie, Dragffy, Tyrrell, Pipe, & Bremner, 2013), inspired by bacteria, the authors in the two studies proposed similar artificial electronic systems that are fault-tolerant VLSI systems on silicon integrated circuits consisting of electronic cells for performing logical computations (with XOR gates). The systems consist of multilayer structures: cells, clusters, colonies, system, and Bus-Routing layers that collaborate to recover the *functionality* of cells. They implemented a Boolean difference approach to perform the computations with a total of 15 cells. Electronic cells are the bottom-level elements of the system where each holds genetic information and additional information to describe algebraic or logical functionality of cells and repair the system function. Cells with similar genetic information are grouped into a cluster to assist self-repair. These systems have four clusters and a fixed number of redundant cells attached to the circuit to replace faulty cells. The systems can repair soft errors (malfunction) by restoring the information borrowed from other cells and hard errors (damaged physical cells) by assigning the function of damaged cells to the spare cells. There is no movement of cells as all are fixed to the circuit. The results show that these systems are fault-tolerant and continue operation when facing faults until they run out of spare cells. These systems satisfy some of our constraints such as short-range and long-range communication. However, the extent of their self-repair of physical damages is very limited and depends on the number of spare cells as they do not produce new cells. Second, information is stored in every cell that leads to a high level of redundancy as most of this data is never used. Third, the systems cannot restore the information in cells when a cluster of these cells fail.

Some other fault-tolerant systems have been proposed based on cellular automata models (Jones, McWilliam, & Purvis, 2010; Kawanaka, Tsunoyama, & Naito, 1994); when a fault is sensed, the system reconfigures to normal state by using redundant elements. Another system inspired by molecular biology, the BioWatch (Stauffer, Mange, Tempesti, & Teuscher, 2001), was developed with self-repair ability. The BioWatch is a bio-inspired electronic watch that consists of logic circuits, input entities (touch-sensitive buttons) and output entities (LED displays), namely reconfigurable computing tissue. The BioWatch displays the time as a normal digital watch and can receive input from human through touch-sensitive buttons. The tissue consists of a finite number of cells with identical physical structure and programme (called genome), a small processor and memory. Individual cells have the compete

blueprint of this system (genome) and realise a unique function (gene) depending on their locations. These cells communicate with others through outputs/inputs of individual cells. They collaborate to display the time. A fault or an error to a cell is created by pushing a button that makes the cell stop working and other cells receive wrong input values. The tissue needs to have spare cells in the system so that the watch can repair by replacing dead cells. To repair, a spare cell will be assigned the same function of the faulty cell before the damage. The watch stops working when the number of faults is higher than a predefined number.

Another study on self-healing based on Field-Programmable Gate Array (FPGA-based) embryonic network used spare cells and information redundancy to recover the system if cells were faulty (Szász & Chindriş, 2010). Some other self-repair mechanisms used spare elements for recovering, for example, the motherboard of IBM POWER6 Microprocessor and Intel Itanium processors (Quach, 2000; Reick et al., 2007), digital systems based on PrT (predicate/transition) net system (Shen & Shen, 2002), and an automatic navigation system using Markov models (Kabuka, Harjadi, & Younis, 1990). However, the use of redundancy strategies leads to significantly increased costs in resources.

2.3.3 Self-repair robotics

In the field of robotics, a few to a few hundred robots (modules) can connect to each other to form a large geometric configuration or shape with a specific designed function. A self-repair system allows a robot to repair itself (Dai, Hinchey, Madhusoodan, Rash, & Zou, 2006; Timmis, Ismail, Bjerknes, & Winfield, 2016) and keep working which is a type of self-reconfiguration (White, Zykov, Bongard, & Lipson, 2005). The system usually includes the ability to self-modify and replace new parts to fix the damaged ones. Self-repair process usually consists of two steps: failure detection and then replacing the deficient module with extra modules which are attached to the system (spare modules) or received from outside.

In (Arbuckle & Requicha, 2010; Murata, Yoshida, Kurokawa, Tomita, & Kokaji, 2001; Rubenstein et al., 2009), a swarm of robots has been programmed to construct and self-repair a number of simple two-dimensional pattern structures (triangular, star, key-like shapes). Robots are identical and programmed to remember a set of rules – information pertaining to each pattern. These robots are physically attached to and communicate with a maximum of four neighbour robots to build a structure. When a robot is faulty, it detaches from neighbours leaving a gap in the structure. This gap is then filled by a new robot from the outside to restore the pattern. Physical connections between robots and their neighbourhood communications resemble biology. However, every robot carrying all the pattern information can create information redundancy in the system, and these robots also cannot communicate long-distance with other robots. This system can recover the pattern structure but assumes an inexhaustible supply of robots to replace the faulty ones, which can be a burden in terms

of cost and space. In biological regeneration, there is no inventory of spare cells and they are generated on demand to optimise the mass and energy efficiency of the biological system.

Inspired by biological stem cells, robots in (Rubenstein et al., 2009) are robotic stem cells that have the capacity to self-organise and self-heal a damaged structure. Robotic stem cells are each programmed to remember a copy of the whole structure. They reconstruct when the original structure is damaged, or there is a difference between the desired and the current shape. When robots suffer damage, the remaining robots reconfigure and reorganise the same pattern but on a smaller scale (smaller size) and continue to function. It cannot restore the original structure due to the lack of new robots. Similar robotic systems are the Swarmanoids (Dorigo et al., 2013; Ducatelle, Caro, & Gambardella, 2010) that consists of three basic types of robots: eye-bots, hand-bots and foot-bots. A main feature of this system is that a swarm of these heterogeneous smaller mobile robots self-organise into a larger 3D robotic structure with eyes, hands and feet to achieve a particular task. Inspired by ants, the system uses local and long-distance interactions between robots in this self-organisation. However, these systems have no capacity to self-repair when a robot is faulty.

2.3.4 Self-repair in mechanical systems, materials, and others

Self-repairing mechanical systems can self-assemble and self-repair using spare elements. The idea is that the system requires component redundancy existing in the system, which can then be used to replace faulty components and re-establish the function with existing elements. Self-repair in mechanical systems is a basic ability of self-assembly systems; when a module or part of the system breaks, it can easily be replaced by another module or part of the system that already exists or can be received from the environment (Gross & Dorigo, 2008). Self-repair systems in these other the fields (e.g., mechanical systems, materials, etc.) have also received the attention of many researchers. However, these systems are less relevant to our requirements and constrains that we have mentioned above.

2.4 An overview summary of biological and non-biological self-repair models

In biological systems, past models of biological regeneration have covered some of our requirements for repair of different forms, including stem cell and tissue cell communications and sharing patterning information. They repair damages to varying degrees of success and most require cells to store a significant amount of pattern information and/or carry out a large amount of communication between cells for regeneration. None of the previous models regenerates completely and accurately, and none can explain how a small tissue fragment can support all of the computations needed to regenerate a whole pattern while the tissue itself is being remodelled. These existing computational models have various limitations and above all they do not explain most of the important observations from

planarian regeneration experiments. There is still a need for conceptual and computational frameworks that can shed light on the mechanisms and algorithms by which planaria restore the correct morphology (size and shape) despite the severity of the damage. In this research, we closely follow the experimentally observed features of planarian regeneration as our system requirements and constraints for building conceptual and computational frameworks at tissue and organism levels. Thus, regeneration research can benefit from our proposed framework that conceptualises regeneration of a whole pattern from diverse damages and even from a small tissue fragment with the minimum computational burden.

In non-biological self-repair systems, the commonality between the approaches is that they require spare parts or information, from inside and/or outside the system, to recover the system function and/or structure. The spare parts should exist in the system environment as neutral elements, which can be used to replace faulty parts by assigning to them the function of the faulty parts. Every part stores all the information required for function and repair (Mange, Sipper, Stauffer, & Tempesti, 2000) or every part stores specific information for restoring the function (Samie et al., 2013) by which they can maintain all necessary information for self-repair. However, most parts never use this information for repair. The use of spare parts is also a weakness in self-repair systems that recover the form. With limited spare parts, the system cannot repair large damages, or any damage after all spare parts are used up, leading to system malfunction or shutdown. Additionally, too many spare parts make the systems bulky and increase the price of products. Moreover, only a few systems restore the structure, but they need to get new entities from outside that is a stark departure from biological systems that regenerate everything from within. These self-repair models share some similarities with the few existing biological regeneration models, the most prominent being short- and long-range communication between entities. In particular, long-range communication between robots via wireless signals can specifically represent long-distance communication between cells via bioelectric signals in biological regeneration models. Further, accessing information from an outside field (internet) is an efficient way to supplement information stored within a system; our framework uses a similar concept, virtual information fields, for enhancing regeneration capability of our framework. Our overall framework could be useful in engineering for building self-repairing biobots and artificial robots.

In this research, we used the information presented in this chapter as the platform to ask some important fundamental questions of regeneration and propose a comprehensive conceptual and computational framework for explaining and simulating the mechanisms and algorithms of regeneration capable of mimicking extreme and versatile regeneration ability of planaria by regulating the interaction of cells, tissues, and the organisms. The proposed framework has the capacity to self-detect and self-repair any damage anywhere, thus capturing the most extreme regeneration processes

observed in biology. Further, it restores bioelectric homeostasis after recovery as well as under any normal physiological fluctuations. This proposed framework that exceeds the capacity of existing regeneration models provides better hypothetical explanations of the regeneration process in biological organisms, especially in planaria, as discussed in the rest of this thesis. This framework detects damage and regenerates the complete form and function of tissues and organisms using simpler and more efficient computations than the other models.

Chapter 3

A Computational Framework for Autonomous Self-Repair Systems (A single tissue system)

Abstract

This chapter describes a new computational framework for damage detection and regeneration in multicellular structures resembling living systems. The artificial model (*in silico*) organism consists of a stem cell and surrounding tissue of cells that is capable of regenerating after diverse forms of damage. We represent the tissue as an Auto-Associative Neural Network (AANN) consisting of a single layer of perceptron neurons (cells) with local feedback loops. This allows the system to recognise its state and geometry in the form of collective intelligence. Signalling entropy is used as a global (emergent) property of the system maintained by the stem cell. The model represents the case where the stem cell and the cell collective collaboratively detect damage of any size in any location and regenerate the structure. It has two submodels – global sensing and local sensing. In global sensing, the stem cell senses the change in the whole system state and detects the general damage region based on system entropy change. Then, local sensing is applied to find the exact damage locations using neighbourhood communications among perceptrons in the identified damage region of the AANN. Results show that the method allows robust and efficient damage detection and accurate regeneration from very small (even single cell) to very large damages. This kind of approach to regeneration would be helpful in biology for regenerative medicine and in engineering for building self-repairing robots.

3.1 Introduction

Regeneration of tissues and organs happens in many living organisms. The capacity for regeneration varies greatly among animals. For example, Hydra and planaria can regenerate the whole animal from small tissue fragments (Birnbaum & Alvarado, 2008; Lengfeld et al., 2009; Sánchez Alvarado, 2012) while others, including humans, have limited regeneration ability (Baddour, Sousounis, & Tsonis, 2012; Sousounis, Baddour, & Tsonis, 2014). Although regeneration studies on the genetic networks and molecular mechanisms have increased significantly, the algorithm sufficient for the simulation of regeneration of complex shapes is lacking. There is a need for testable models explaining what methods maybe used in an organism to bring the morphology (anatomical size and shape) back to the correct state and stop when the correct morphology has been reached.

Mechanisms of regeneration in biology are complex and not totally understood. The dynamics of large-scale remodelling of anatomy that allow organisms to maintain and regenerate toward a specific shape are still the biggest knowledge gap. Recent evidence suggests that these mechanisms classify into two groups: pattern formation (Badugu, Kraemer, Germann, Menshykau, & Iber, 2012; Meinhardt, 2008) and tissue memory (Kragl et al., 2009; Kragl et al., 2008). The pattern formation includes cell-cell communication and other processes (e.g., cell division) that use the currently available information (attained through evolution) while tissue memory means storing the information on the former state of the tissue for future use. Recent studies show that communication mechanisms play a pivotal role in planarian regeneration (Lobo et al., 2012). The question of how old and new tissues communicate with each other or how old and new cells communicate and work with each other to perform complete regeneration is still unclear. After amputation, stem cells migrate to the wound site and divide to produce new cells and form a blastema (an unpigmented structure) and then regenerate lost tissues until the exact pattern of the original anatomy is recovered (Elliott & Sánchez Alvarado, 2013; Montgomery & Coward, 1974). This requires stem cells to know what is missing, where the damage is, what structures to regenerate and to stop when regeneration is complete. This seems to be achieved through communication between stem cells and other tissue cells (aka somatic cells, differentiated cells) through ion fluxes during the regeneration process (Oviedo & Levin, 2007). Ion fluxes allow communication between cells by exchanging ions between intracellular and extracellular environments that affect the activity of both neighbour cells and distant cells in the body (Barghouth et al., 2015). Moreover, gap junctions (GJs) between somatic cells also play a vital role in cell communication within a tissue. GJs are direct physical channels which allow tissue cells to transfer ions and small molecules directly into adjacent cells. However, the exact nature of these signalling mechanisms that collaborate to maintain and regenerate the correct anatomical state of the organism are still unclear. Here, we present a conceptual framework and a computational model based on the assumption that regeneration uses both pattern formation (to recognise damage and repair the tissue) and tissue memory (to keep original pattern information). We develop an artificial computational self-repair system that mimics some basic processes in biological self-repair systems in order to improve the capacity to recover the exact original form such as the one shown in Figure 3.1A&C from any damage to parts of it.

3.2 Objectives

In this chapter, we propose a concept for an autonomous self-repair system that has the improved capacity to sense, detect and regenerate a tissue of cells under any damage condition induced by an injury that in a simple way resembles some related regeneration processes in biological systems. When the damage of any size happens in any location in the tissue system, it needs to efficiently sense the change, detect the location and extent (size and shape) of damage and regenerate missing cells to

bring the system to its exact original state. The model is applied to an artificial circular tissue consisting of a regenerative stem cell surrounded by (non-regenerative) tissue (differentiated or somatic) cells Figure 3.1A&C. The main objective is to make the stem cell and cell collective collaborate robustly to maintain the normal state of the system under any damage condition. We conceptualise efficient communication mechanisms that facilitate optimum collective decision making (collective intelligence) with the minimum informational and computational burden on the system to achieve complete regeneration and stop when the exact pattern has been reached.

3.3 Summary of the tissue repair system framework

This chapter presents an efficient computational framework for damage detection and regeneration in an artificial (in silico) circular tissue resembling a living tissue. The circular tissue consists of a stem cell and surrounding tissue cells with two types of communication - local direct interaction between tissue cells and long-range communication between stem cell and tissue cells. The tissue is considered as an Auto-Associative Neural Network (AANN) represented by a single layer of perceptron neurons (tissue cells) with locally recurrent (bidirectional or feedback) interactions. A global system state is represented by signalling entropy that is kept by the stem cell. The system contains two submodels – Global Sensing (GS) and Local Sensing (LS). In GS, the stem cell uses system entropy to sense the change in the whole system state and detect the general damage regions. In LS, the threshold neurons in the AANN identify the exact damage locations in the damaged regions identified by the GS. Then, the stem cell moves to the damaged locations and regenerates missing cells.

3.4 Methodology

In this section, we propose the conceptual framework for Autonomous Self-Repair Systems that are capable of regenerating parts or the whole system after diverse forms of damage. This resembles the ability of a living organism to adjust its internal environment to maintain a defined stable equilibrium (anatomical homeostasis). Broadly speaking, homeostatic systems are control systems that can bring a system back to stable equilibrium after a change, including detecting and repairing damage, to sustain its form and function. Accordingly, we propose a control system for an artificial organism similar to a biological tissue that can sense and detect damages and take actions to rectify them in a self-stabilising manner. This framework addresses the first objective of this thesis and aims to develop a comprehensive self-repair system for a functioning tissue.

We assume that an artificial (in silico) circular tissue pattern consists of a stem cell surrounded by more than 2000 differentiated cells in a 2-dimensional plane D as in Figure 3.1A&C. A cell in a plane is identified by a distance, radius, from a reference cell (stem cell) and an angle θ from a polar axis (r, θ) (Figure 3.1A). The stem cell can renew itself through a process known as asymmetric cell division where

it produces a differentiated tissue cell while keeping a copy of itself. These differentiated cells surround the stem cell in our circular tissue pattern. The tissue structure is described by a network of cells embedded in a medium similar to Extra Cellular Matrix (ECM) in a living tissue where it plays a vital role in nutrient, information and energy transfer into cells. ECM is the space between cells and consists of a network of functional and structural proteins aggregated in a uniquely tissue-specific manner.

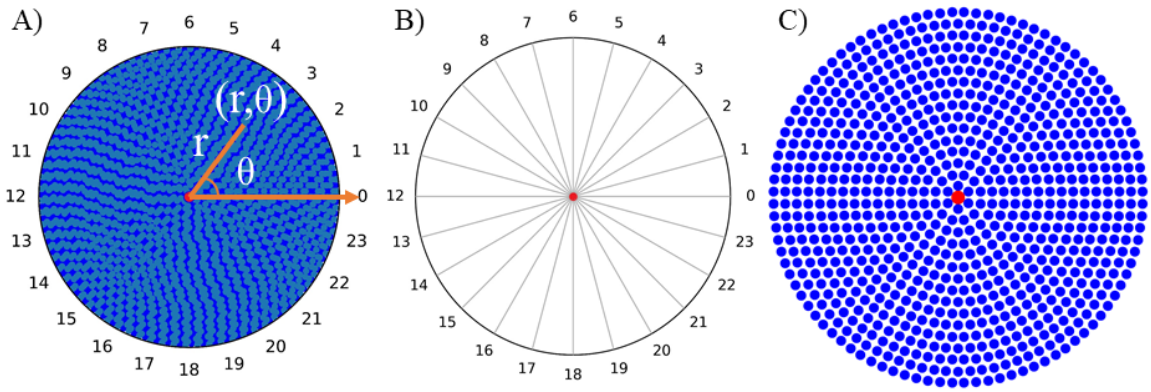


Figure 3.1. Circular tissue pattern. A) A stem cell at the centre surrounded by differentiated cells with the location identified by polar coordinates (r,θ) B) Tissue pattern divided into n segments (e.g., n=24) and C) A close-up look at the tissue structure

There are two types of communication in the tissue -- local direct communications between neighbour cells and global diffusion of information through the ECM -- the form of communication between a stem cell and differentiated cells. This diffusion means that longer the distance the information travels, the greater the uncertainty of its content, so we use the concept of entropy to encode this information (Section 3.4.1). Direct neighbourhood communication is facilitated by representing the tissue as an Auto-Associate Neural Network (AANN) consisting of a single layer of perceptrons (threshold neurons) that represent cells that are connected with local feedback loops (Figure 3.3a). AANN are networks where neurons influence themselves through feedback loops that typically give rise to emergent systemic properties. Thus the tissue is represented as a locally recurrent dynamical system that collectively maintains tissue states. This also minimises the informational and computational burden on the tissue. Further, in living tissues, cells dynamically exchange matter, energy and information with inherent stochasticity. Our model considers stochasticity in cell position due to cell movement and is approximated by white noise $\epsilon(\mu, \sigma)$ that adjusts cell position as $(\text{radius}, \theta) \pm \epsilon(\mu, \sigma)$. The $\epsilon(\mu, \sigma)$ is assumed to be a Gaussian noise distribution with 0 mean (μ) and standard deviation (σ) with a value determined heuristically (e.g., μ =0 and σ =0.03) as explained later. Our framework is designed to regenerate this system after damage.

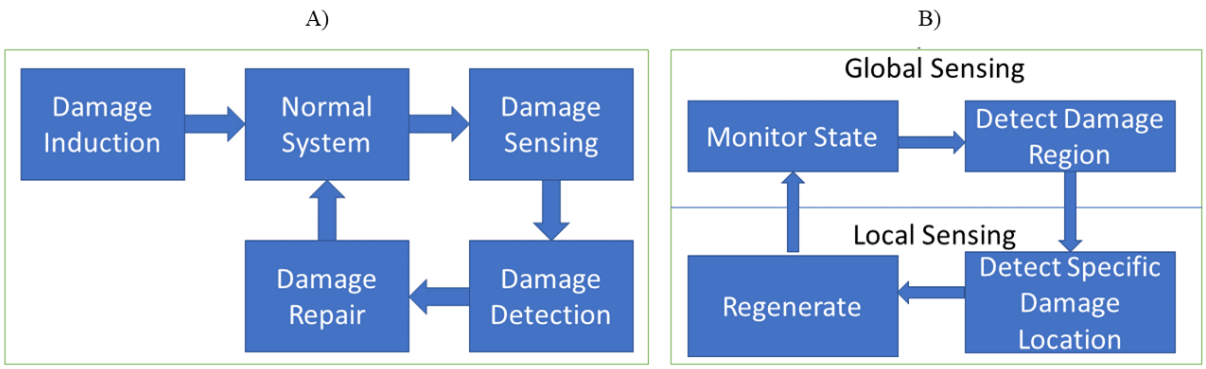


Figure 3.2. The framework of the Self-repair system: (A) Schematic diagram showing the components of the framework, and (B) Functional aspects of the framework

Figure 3.2 presents the framework of the autonomous self-repair system. With the AANN representation with learning capabilities, our organism (normal system in Figure 3.2A) retains the memory of its state and geometry. Additionally, with entropy, we introduce the possibility of maintaining a global (emergent) system property as an indicator of the normal state of the whole system, similar in concept to maintaining a constant body temperature by an organism as an indicator of general whole-system health. In real biological systems, maintaining a relevant emergent property could constitute a higher-level goal of the system.

Our repair system consists of two submodels - Global Sensing and Local Sensing. In global sensing, the stem cell can sense globally when the system changes from its original state. We use entropy and change in entropy to represent changing states. In a real biological system, damage can spill electrolytes and signalling molecules into the ECM that can be sensed by the stem cell. An analogous action in our model triggers the stem cell to sense a change that initiates an autonomous damage identification and recovery process where it first starts looking for the general region of damage from global entropy change; and then, it triggers the second type of communication - local neighbourhood communication - in the identified region to find the exact location, size and shape of the damage. Here, threshold neurons in the AANN communicate via local feedback loops to sense any missing neighbours. Neurons with missing neighbours make up the damage boundary. Finally, the stem cell migrates to the damaged location through ECM to regenerate missing cells by asymmetric cell division until complete and correct recovery of the original tissue pattern.

3.4.1 Normal (undamaged) System

The tissue system remembers its initial state and system boundary through local communications within the AANN where perceptrons repeatedly send and receive signals from neighbours and generate outputs until the whole system reaches equilibrium. This creates a state matrix that defines the memory of its normal or homeostasis state and tissue geometry. This information is distributed within the AANN in the form of collective intelligence enabling the tissue to know/recognise itself. In

this preliminary model, only the presence or absence of a cell is communicated. (But in the final framework under objective 3, bioelectric communication will be added to the model to increase biorealism and incorporate bioelectric homeostasis that would reveal a number of possible dynamical equilibrium states including fixed point or limit cycle attractors and other emergent properties). Figure 3.3 shows an example of perceptron within AANN and its processing. Algorithm 1 (in the next section 3.4.2) captures this processing in the whole system. We assume that each interior cell has four neighbours that communicate with it as in Figure 3.3A, and the cell thus receives four inputs from its neighbours. The perceptron computes the output from the received inputs and connection weights (Figure 3.3 b). Figure 3.3c presents a sample dataset showing that a perception responds with 1 only if all neighbours are present and 0 otherwise. A boundary cell has only 3 neighbours, and the corresponding perceptrons respond with 1 only if all three neighbours are present and 0 otherwise. This way, tissue border is identified.

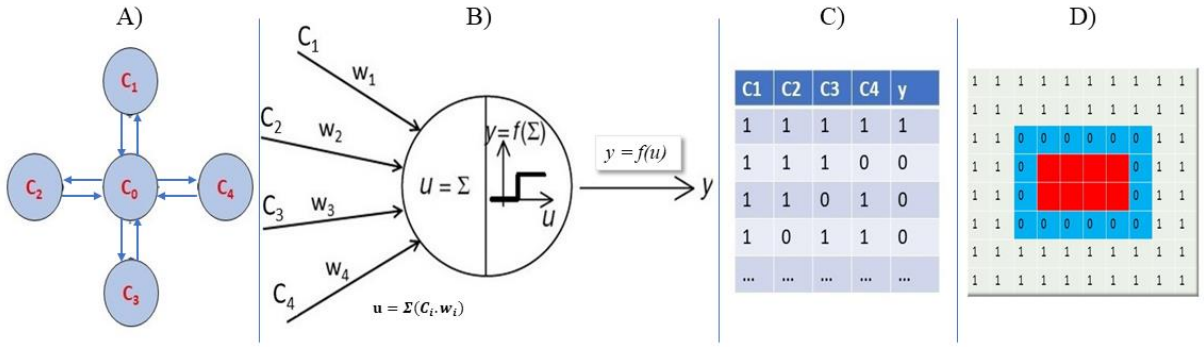


Figure 3.3. Single-layer perceptron in AANN - A) Two-way communication of a perceptron with its neighbours, B) Computation in a Perceptron C) Sample data showing perceptron response to inputs from neighbours and D) Output of a damaged segment of AANN

A state of 1 is assigned to the cells in the original tissue and weights are also assigned 1.0 (fixed weights used in this model to test the overall model concept; learning will be introduced in the final framework under objective 3). A perceptron sums all the weighted inputs, and if the sum is equal to a threshold (e.g., threshold = 4 for interior cells), the neuron produces an output = 1 (Eq. 3.1). It means that all its neighbours are present. This allows the system to know its intact state and geometry. In contrast, if the sum is less than the threshold, the output is zero indicating the absence of the full set of neighbours, i.e., damage, which is used in the local sensing model.

$$\text{Interior cells: } u = \sum_{i=1}^{4} C_i * w_i \; ; \quad y = \begin{cases} 1 & if\ u = 4 \\ 0 & otherwise \end{cases}$$
$$\text{Border cells: } u = \sum_{i=1}^{3} C_i * w_i \; ; \quad y = \begin{cases} 1 & if\ u = 3 \\ 0 & otherwise \end{cases} \tag{3.1}$$

Further, cells in the tissue communicate with the stem cell through information diffusion through the ECM that allows it to sense their location. From these data, original system entropy is calculated (Eq. 3.2). This forms a global measure of the state of the system that it tries to maintain. In our model, we divide the tissue into n equal segments, as depicted in Figure 3.1B. The stem cell scans the segments as it sweeps across the whole system and stores the entropy for each segment as the normal entropy in the homeostatic state. We briefly introduce entropy and our formulation here.

The concept of information theory (entropy) was introduced by Claude Shannon et al. in 1948. The entropy of a system is a measure of uncertainty or the degree of randomness of the system. In systems biology, it has been used to identify signalling pathways, understand drug sensitivity profiles, and determine normal and cancer stem-cell phenotypes (Teschendorff & Enver, 2017; Teschendorff, Sollich, & Kuehn, 2014). In the context of a network of cells, signalling entropy is a measure of overall uncertainty in a desired state, such as strength of signal communication in the tissue. For example, the greater the entropy, the greater the uncertainty of communication between the stem cell and other cells; meaning that the longer the distance of information diffusion, the greater the entropy. In this sense, we assume distance to be a random variable.

Assume that a stem cell SC has k number of differentiated cells with coordinates $(r_1, \theta_1), (r_2, \theta_2), \dots, (r_k, \theta_k)$. (As mentioned previously, we added a small white noise to represent small cell movements around their position using a heuristic measure discussed later in this chapter). Let $\mathrm{d_j}$ be the distance between the stem cell SC and differentiated cell $\mathrm{DC_j}$. We first define a stochastic (probability) matrix P with components p_j and signalling entropy E of the system defined as:

$$p_j = \frac{d_j}{\sum_{j=1}^{k} d_j};\ E = -\gamma \sum_{j=1}^{k} p_j \log p_j\ ;\ \gamma = \frac{1}{\log k} \tag{3.2}$$

where $\sum_{j=1}^{k} d_j$ denotes the total distance from the stem cell SC to all k differentiated cells and γ is a positive constant. The stem cell estimates the entropy of the segments and stores this entropy vector as the original entropy.

3.4.2 Global Sensing – damage region identification

The idea of this submodel is that the stem cell can sense a change in the system due to damage anywhere within it and scans the system for entropy change in order to find the general area of damage. It scans the system by segments, and the difference between the current and original entropy informs the segment(s) that have received damage. A pseudo-code of the damaged segment detection is presented in Algorithm 2. First, it uses the original computed entropy for the normal state as input to the algorithm. Next, the entropy of each segment is calculated after sensing damage and compared

with the normal entropy. A damaged segment D is identified if there is a difference between the old and new entropy values.

Algorithm 1. Normal System Recognition

Data : *A set of cells C*
Result : *A state matrix S*

```
S ← Ø;
For each cell c_i ∈ C do
        S ← S ∧ SLP(c_i);          // SLP(c_i) is a single layer perceptron
End
Return S;
```

Algorithm 2. Detecting damaged segments

Data : *Normal entropy values E and segments S*
Result : *A set of damaged segments D*

```
D ← Ø;
For each segments s_i ∈ S do
        If Entropy(s_i) ≠ E_i then
                D ← D ∧ s_i
        End
End
Return D;
```

Algorithm 3. Detecting specific damage locations

Data : A set of damaged segments D
Result : A set of locations L

```
L ← Ø;
For each cell c_i ∈ D do
        If SLP(isInside(c_i)) ≠ 4 or SLP(isOutside(c_i)) ≠ 3 then
                L ← L ∧ c_i        # isInside() returns True if a cell is in interior.
        End                        # isOutside() returns True if a cell is in border.
End
Return L;
```

Algorithm 4. Regeneration of missing cells

Data : *A set of locations L (output=0)*

```
While L ≠ 0 do
        r_min ← mindist(sc, L);        # find a minimum distance from the stem cell to L
        While r_sc < r_min do
                r_sc += 0.5            # the stem cell moves to the damaged location
        End
        For each cell c_i ∈ P do
                Addneighbours(c_i)     # adds missing neighbours of c_i
                L ← L - c_i            # Removes c_i from L
        End
End
```

3.4.3 Local Sensing – exact damage location identification

The main point of local sensing is for the stem cell to initiate a local search for the exact location of damage in the already identified general area of damage to be able to regenerate missing cells. This is to minimise extensive local search over the whole tissue and reduce the information and

computational burden on the system. Specifically, knowledge of the damaged segment(s) allows it to inform the cells in these segment(s) (corresponding part of the AANN) to process information through local feedback connections to assess any missing neighbours. As illustrated before, in our example, each interior cell has four neighbours that can communicate with it (Figure 3.3a), and the perceptron responds with 1 only if all neighbours are present and 0 otherwise (Figure 3.3c). The output of 1 means that all its neighbours are present. In contrast, if the sum is less than the threshold, the output is zero indicating the absence of the full set of neighbours due to damage. After damage, interior neurons surrounding damage have a maximum of 3 inputs. At the border of the original tissue, perceptrons have 3 inputs. After damage, border neurons near damage have a maximum of 2 inputs. From this, the system can identify the border of damage in the tissue interior or at the boundary that can be used to recognise when to stop growing.

Algorithm 3 shows how to detect specific locations of damage. The output of each cell (perceptron) indicates the state of its neighbours. Figure 3.3d illustrates an example of AANN outcome for each cell in a damaged segment of the single layer of perceptrons. For example, there are eight dead cells in the middle (red colour) that are surrounded by a set of cells with output=0 (Blue colour). The zero values of the output indicate that at least one of their neighbours is dead whereas a cell with its four neighbours intact has an output equal to 1.

3.4.4 Regeneration

The single-layer perceptron outputs help the system identify the boundary of the damage. In order to regenerate, the stem cell migrates to the damage location and fills out the damaged area by asymmetric cell division. Algorithm 4 above shows how the stem cell moves along the shortest path to the damage location and then regenerates missing neighbours of each cell in the damaged area. In the case of damage extending to the original border, it adds new cells until they align with the existing border neurons. When a new cell is added next to a border cell, the new cell becomes the marker for the next border cell. Regeneration continues until the whole set of border cells is added and comes to completion with the renewal of the correct original form.

3.5 Results and Discussion

To determine the level of noise in the cell position the system can handle, we first perturbed the system (original location of all cells) by $\pm$(2%, 5% and 10%) and calculated entropy. Table 3.1 shows mean and standard deviation of change in entropy due to applied noise. The objective of perturbation was to invest our system with some characteristic noise as in a living tissue. Figure 3.4 shows how the average entropy of the 24 segments responds to the three levels of perturbation. The 0% indicates the static position of cells. Our intention was to make the system stochastic but still sensitive to changes

in entropy due to single missing cells. We observe only a relatively small increase in the absolute change in entropy for ±2% and ±5% perturbations but ±10% introduces a significant change. Also, ±10% perturbation introduces disorder into the system producing random cell clusters. Further experimentation with entropy calculation with various cell deletions revealed that ±5% perturbation introduces enough noise into the system but still keeps it sensitive to single-cell deletions; therefore, ±5% perturbation was selected to represent stochasticity in the tissue. The corresponding noise distribution $\varepsilon(0, 0.03)$ had 0 mean and 0.03 standard deviation (Table 3.1).

Table 3.1. Mean and Standard Deviation of entropy change due to noise (perturbation)

Perturbation level (±%)	Mean	Standard Deviation
2	0	0.013
5	0	0.03
10	0	0.06

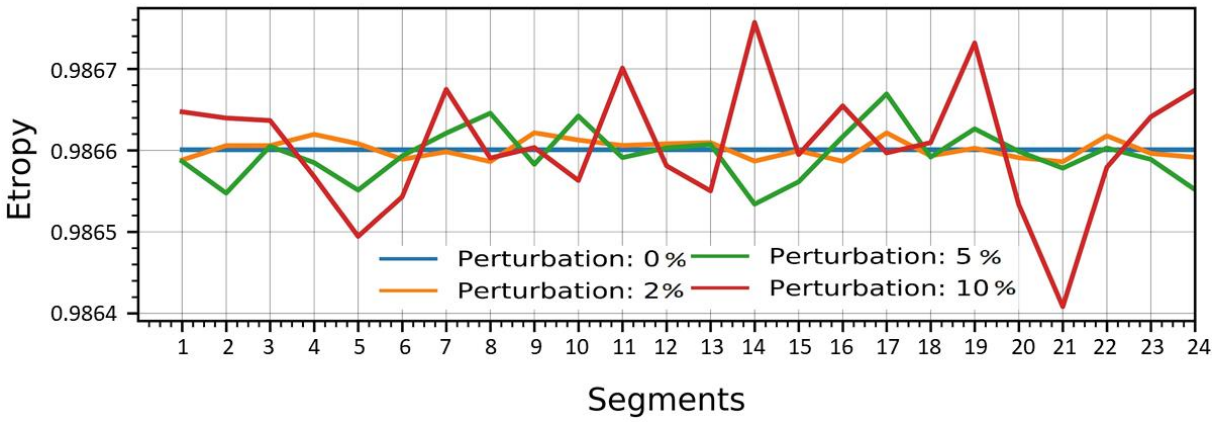


Figure 3.4. System entropy computed over the 24 segments for perturbations of ± [0, 2, 5 and 10]% of the original cell positions. ±10% causes large variations in entropy.

To find the general damage region based on the proposed entropy change, we started with ±5% perturbed cell system and calculated its entropy over all 24 segments as shown in Figure 3.5 (bottom graph - 0 cell deletions). Figure 3.5 (bottom part) presents six cases of randomly introduced damage all over the tissue with increasing damage intensity by randomly deleting one/five/ten cell(s) in each segment or deleting a large area (100 cells) near the stem cell or far from it. After sensing damage, the system recalculates entropy for the whole system, and the graphs in Figure 3.5A show the degree of entropy change in relation to the intensity of the damage. As can be seen, entropy undergoes change due to even single-cell deletions and the larger the number of cell deletions, the larger the change in entropy. The stem cell accurately determined damaged segments from these changes in entropy.

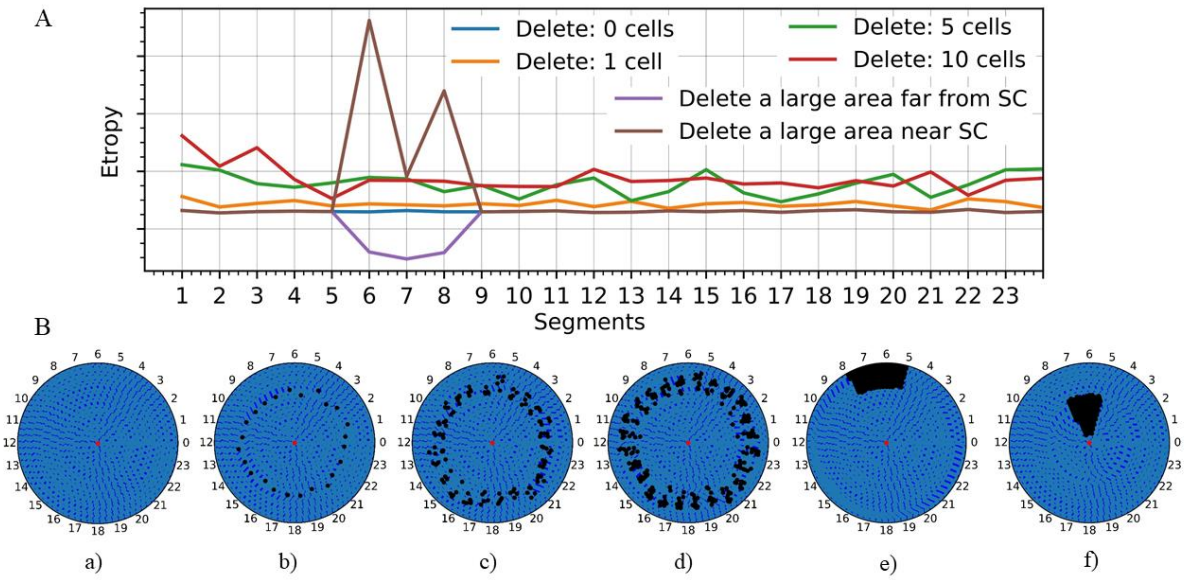


Figure 3.5. Entropy change with damage intensity. (A) System entropy over the 24 segments of tissue for: no damage, and random deletions of 1, 5 and 10 cells in each segment, and 100 cells far from the stem cell (SC) or near the stem cell. (B) Tissues showing the six damage cases (labelled from a to f) indicated in (A).

To identify the exact damage location after identifying the damaged segment(s), local sensing activated the perceptrons in the portion of the AANN corresponding to the affected segments using Algorithm 3 and found the exact location, size and the boundary of the damage. All damages and boundaries were identified correctly. Then using Algorithm 4, stem cell moved to the nearest location of the damage and moved forward filling missing cells. In the case where the damage extended to the boundary as in Figure 3.5B(e), regeneration continued until reaching a boundary cell at which point regeneration continued along the boundary line and stopped when the last missing cell on the boundary was filled as shown in **Figure 3.6**.

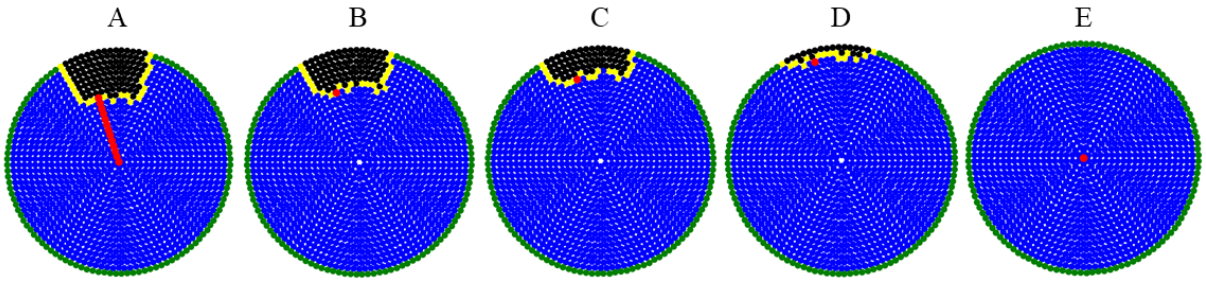


Figure 3.6. Progression of damage repair until complete and correct pattern regeneration (from left to right for damage in Figure 3.5B(e)) showing migration of stem cell to the damage location and continuing repair guided by cells on the border of the damage

For discussion on the model performance, we compare our method with the relevant previous methods. In Tosenberger et al. (Tosenberger et al., 2015) model, stem cells continually divide and send live signals to the surrounding differentiated cells that die if the received signal is less than a threshold. However, this is not only biologically unrealistic but also computationally inefficient due to the need to keep dividing and killing unnecessary cells. In our model, the stem cell is activated only when damage happens and then detects damaged segments (global sensing) with one off calculation and determines specific location of damage (local sensing) focusing only on the region of damage and regenerate missing cells guided by the damage border avoiding overgrowth making the system more robust and efficient computationally.

In Bessonov et al. (Bessonov et al., 2015) tissue regeneration model, all cells send signals to each other, which is not computationally efficient and effective due to a great number of communications between cells. In our model, only the stem cell communicates with all other cells in a limited fashion while differentiated cells only communicate with their (3 or 4) neighbours. Ferreira et al. (Ferreira et al., 2016; Ferreira, Scheutz, & Levin, 2017a; Ferreira, Scheutz, et al., 2017b) models developed to discover the structure and recover dead cells use information packets that travel extensively through the structure. Also, it produces a great number of packets that need to be kept for the purpose of regeneration. Our model not only depends on much less communication between cells but also keep minimal information - just the global entropy (of 24 segments) and state of neurons in the original system. In De et al. model (De et al., 2017), interacting neural skeleton and non-neural cells coordinate tissue growth using biologically realistic variables and concepts. However, this model does not recognise damage and generates and kills many cells before reaching a partially recovered form. In contrast, our model detects damage and recovers the complete form using simpler and efficient computations. However, our model is at a preliminary stage, used simple geometry and relied only on the positional information. Therefore, it can benefit from functional variables as used in (De et al., 2017) to extend its biorealism while retaining its attractive features.

3.6 Conclusions

In this chapter, we describe a new model for damage detection and regeneration in multicellular tissue structures based on concepts and assumptions inspired by the biology of living tissues. It appears to be very robust with greater computational efficiency than previous models as it has much less information processing and correctly regenerates the complete form that none of the previous models has been able to do. This is achieved by enabling a cellular system to - recognise itself (its state and geometry) resembling a form of collective intelligence, maintain a global emergent system property (entropy) as an indicator of normal system state (global sensing) and use it to identify general damage regions, activate local sensing to identify damage location, and finally facilitate stem cell migration to

repair the damage. Thus the model indicates the possibility for a biological system to autonomously sense, detect and repair damages correctly.

The approach allows regeneration of large area damages - for the circular tissue the minimum requirement for repair is that only one single line of cells reaching the border from the stem cell must remain. This encapsulates a huge array of large and small damages anywhere in the tissue. The model can also apply to other shapes such as oval, square, rectangular and triangular shapes. It alludes that noise beyond 5% may reduce the capacity to sense individual or small damages. In the next stage addressing objective 2, the model will be extended by investing the stem cell with greater functionality for improving cognition capabilities to enhance collective intelligence and decision making in terms of information processing, learning, memory and reasoning. Further, the circular tissue repair model concept will be extended to different tissue shapes (triangular and rectangular), while enhancing its current strengths. Finally, a new conceptual framework will be developed for the regeneration of a whole system or organism consisting of multiple tissues. The aim will be to transform and extend the single tissue model into self-recognising, self-organising and self-stabilising multiple cell communities that are intelligent decision-making systems with an increasingly greater resemblance to living cell systems.

Chapter 4

A comprehensive conceptual and computational dynamics framework for Autonomous Biological Regeneration Systems – Whole organism with multiple tissues

Abstract

This chapter offers a novel *generic* conceptual framework that hypothesises mechanisms and algorithms of biological regeneration to mimic observed regeneration phenomena. It extends the single tissue regeneration system presented in the previous chapter to a regeneration framework for a multiple tissue system representing a simple virtual organism like the planarian. Specifically, it addresses how cell collectives in an organism may internally represent and regenerate an *in silico* target anatomical pattern similar to living animals. In biological regeneration, specialised cells called stem cells reach the damage location in a tissue and produce new tissue cells until the full recovery of the original form. Planarian flatworm has been a popular model organism to explore these regeneration processes *in vivo* and *in silico* due to their body-wide immortality - ability to recover the original form from even tiny body fragments. Past computational models proposed to capture some aspects of planarian regeneration *in silico* have achieved limited recovery on simple tissues involving a large amount of computation.

This chapter proposes a hypothetical conceptual and computational dynamics framework for the top-down representation of the mechanisms and information structures by which cellular networks may internally represent and regenerate a target morphology similar to living animals such as planarian flatworms. The framework consists of a two-level neural feedback control system with the minimum computational burden and algorithmic complexity that uniquely achieves complete and accurate regeneration from any damage, much like the body-wide immortality of planaria. The framework that models body-wide immortality of planarian regeneration integrates two levels. Level-1 consists of three 2D-tissues (head, body and tail), each consisting of a stem cell and a large number of tissue (differentiated) cells. Here, differentiated cells identify their tissue pattern through local communication via gap junctions. The stem cells communicate with differentiated cells to- (i) maintain individual tissue integrity, (ii) extract essential tissue pattern information and store it in a newly introduced abstract information field, and (iii) identify and restore damaged tissues. In Level-2, a stem cell network implements a collective information field that shares tissue-level pattern information, enabling recovery from any stem cell or large-scale damage collaborating with Level-1.

Both levels (tissue cells and stem cells) represent networks that perform simple neural computations and form a neural feedback control system. We report results from computer simulations of the framework to demonstrate its robustness in recovering the organism after any injury. This comprehensive hypothetical framework that significantly extends the existing biological regeneration models offers a new way to conceptualise the information-processing aspects of regeneration, which may also help design living and non-living self-repairing agents.

4.1 Introduction

Regeneration of complex biological structures (Birnbaum & Alvarado, 2008) is a remarkable example of the broader phenomenon known as anatomical homeostasis: the ability of cells in an organism to cooperate toward building a specific anatomical shape, which can be reached from a wide variety of starting or damage conditions and which stops when that target morphology (size and shape) has been achieved (Harris, 2018). The question of how cells in an organism know what shapes to build and when to stop can be framed as a problem in collective decision-making, challenging the community to develop conceptual models of how information is processed by cellular networks to reliably achieve anatomical setpoints. Conceptual models are important because very little is understood about the computational aspects sufficient for anatomical homeostasis (Pezzulo & Levin, 2015, 2016) due to the challenging complexity of the regeneration processes.

Our goal is to propose a novel generic conceptual framework with mechanisms and algorithms of regeneration for modelling some functional aspects of regeneration *in silico*, which can be used to motivate hypotheses for advancing the understanding of biological regeneration. Specifically, our framework aims to mimic some essential aspects of complete pattern regeneration observed in the past *in vivo* studies on planarian flatworm, a small organism with extreme regeneration abilities (Cebrià, Adell, & Saló, 2018; Durant, Lobo, Hammelman, & Levin, 2016; Gentile, Cebria, & Bartscherer, 2011; Lobo et al., 2012). As fundamental regeneration processes are generally similar among organisms, these generic model concepts can apply more widely to all organisms, including humans. Our framework significantly extends the mechanisms and capacity of regeneration of past *in silico* models of planarian regeneration that have achieved limited regeneration success on simple tissues requiring a large amount of computation (De et al., 2017; Ferreira et al., 2016; Ferreira et al., 2018). In contrast to these previous works, our framework achieves complete and accurate regeneration of an *in silico* worm-like organism consisting of multiple-tissues, much like the body-wide immortality of planaria, with a minimum computational burden. The problem of how information is processed in regeneration, and therefore conceptual models of regeneration, are also of high importance for regenerative medicine and as a design strategy for synthetic biology. Further, they are important for the efforts to use insights from biology to engineer self-repairing robots and synthetic living machines

(Doursat, 2013; Doursat & Sanchez, 2014; Doursat, Sayama, & Michel, 2012, 2013; Fernandez, Lobo, Martin, Doursat, & Vico, 2012; Kamm et al., 2018).

The extreme regeneration capabilities of planaria raise a number of questions, which remain to addressed. How do (even small) pieces of the body know the whole pattern? Is the process fully emergent, or is some amount of global target morphology or pattern information stored somewhere as a setpoint for pattern homeostasis and error reduction over time? Where could such information be stored, how is it retrieved, and what mechanisms are used to implement it during regeneration? What is the minimum size of the body fragment sufficient for complete regeneration? One benefit of algorithmic models and simulations is that they force one to be explicit in proposing hypotheses on these large-scale issues.

This research offers hypothetical answers to some of these questions in order to make improvements towards efficient regeneration systems that completely and correctly regenerate their form that in a broad sense, resemble biological systems. The novel contribution of our proposed framework is that it conceptualises the mechanisms and algorithms to simulate planarian regeneration (*in silico)* in some important functional aspects observed *in-vivo*: *regeneration of the whole pattern from even a tiny fragment, concurrent regeneration of body parts (head, tail etc.) along with identification of A/P polarity, and complete and accurate regeneration that stops upon reaching the required form.* In particular, our framework captures the observed processes of regeneration and proposes efficient information distribution, storage and access mechanisms for collective decision making by a community of cells with the minimum computational burden.

In our previous chapter, we developed an *in silico* circular tissue regeneration model (chapter 3). We aim to extend this model, with further enhancements, into a conceptual regeneration framework for an *in silico* whole organism that recovers from any damage anywhere in the system. In the circular tissue model, tissue cells were represented by a 2-dimensional perceptron neural network. The stem cell and tissue cells collaborate to identify and recover from damages with two simple communication processes: The tissue cells communicate with their local neighbourhood to establish neighbourhood rules that define the tissue pattern while the stem cell communicates with tissue cells to maintain the pattern and restore damaged tissues in collaboration with them. A novelty of the whole organism regeneration framework proposed in this chapter is that we extend this circular tissue model to other shapes- triangular and rectangular. Further, the circular model recovered from all but total perimeter damages. In the new framework, we make another contribution by enhancing the capability of the stem cell to extract the tissue plan (essential pattern information) from its communication with tissue cells and retain it in its memory in a newly introduced information field to overcome this limitation and extend the recovery to any damage anywhere. Another contribution of the framework is forming

an organism by combining individual tissue models and conceptualising a stem cell network for the whole organism for regenerating missing stem cells and large-scale recovery. Finally, a significant contribution of the work is that the whole framework is embedded in a simple neural computing paradigm that greatly reduces the algorithmic complexity compared to previous models.

When damage happens, our new framework efficiently senses the nature of the damage, whether a tissue or stem cell damage, detects the damage location and regenerates the missing individual cells, stem cells, tissue parts or whole tissues to bring the system to its exact original state. The regeneration framework dynamically maintains pattern homeostasis regardless of the extent of the damage, akin to the body-wide immortality of the planarian. The framework consists of two levels: (1) Tissue level consisting of two-dimensional tissues (head, body and tail), each consisting of a stem cell and a large number of tissue cells. The tissue cells identify their tissue pattern through local communication while the stem cell communicates with tissue cells to- (i) maintain individual tissue integrity, (ii) extract essential tissue pattern information, and (iii) identify and restore a damaged tissue as long as the stem cell is intact; (2) Organism level where the three stem cells form a network, represented by a linear neural network, that enables recovery from any stem cell or large scale (multiple tissues) damage in collaboration with the tissue level. We present the development of the framework and demonstrate its operation through simulation experiments to show that our simple *in silico* organism maintains body-wide anatomical homeostasis under any damage.

Our framework is essentially a conceptual tool, encompassing (limited) available knowledge of regeneration and some proposed new concepts, for motivating hypotheses for advancing the understanding of regeneration. The *in silico* implementation on a simple worm-like structure shows that it mimics some important observations of planarian regeneration, including body-wide immortality. Due to the challenges in understanding the essential processes of regeneration, the current biological regeneration models remain at a conceptual level, as does our framework. Scientists can design experiments to explore these concepts. As more knowledge is thus gained in future, there will be a greater possibility to validate our framework. Concepts proposed could more readily motivate self-repair in artificial systems.

4.2 Objectives

In this chapter, we extend our previous tissue self-repair model (see chapter 3) to other tissue shapes and organise them into a conceptual regeneration system for a whole organism where stem cells detect tissue as well as stem cell damage and regenerate new stem cells and tissues to recover the whole organism after damage. When damage happens anywhere in the organism, it efficiently senses the nature of the damage, whether a tissue or stem cell damage, detects the location and regenerates the missing stem cells, whole tissues, or tissue parts to bring the system to its exact original state.

4.3 Regeneration as a biological computing process

In the previous chapter, we presented an autonomous tissue self-repair system for damage detection and regeneration inspired by biological regeneration. The model considers a circular tissue consisting of a stem cell surrounded by thousands of differentiated cells. The tissue is considered as a system that is aware of its own pattern integrity (self-aware) and reacts to damages to efficiently restore it. To achieve this, the tissue is represented as a self-aware neural network system. Specifically, it is a bi-directional graph in the form of an Auto-Associative Neural Network (AANN) consisting of neurons (differentiated cells) that communicate with their immediate neighbours. Further, the stem cell communicates with the neurons to maintain tissue integrity and direct the repair process after damage and the AANN assists the stem cell to restore the pattern. The system fully recovers from a wide variety of damages as long as at least one string of cells from the stem cell to the border remains to provide pattern information during regeneration. Although this covers a broad spectrum of damages that the system can handle, having to retain pattern information in the tissue, that is not possible for extreme damages where the whole outer region of the tissue goes missing, is still a limitation of this model. Also, it has only been applied to a simple tissue of circular shape.

Many important advantages of our system described above over the existing methods provide the scope for improving it to address its limitations and extending it to other tissue patterns and multiple tissue systems to represent regeneration in simple organisms. This is the aim of the current chapter. This brings out some additional fundamental questions about the mechanism of planarian regeneration and provides the opportunity to offer hypothetical solutions. For example, there are two basic mechanisms of regeneration in planaria: when a local tissue is damaged, stem cells sense and migrate to the damaged area to correctly repair the damage (our previous model has covered this). When any part with stem cells is cut off, the remaining stem cells sense the damage, move to the blastema, produce new stem cells and regenerate the missing tissues concurrently; we aim to incorporate this into our model. The additional questions related to these two mechanisms are: how does a system know the pattern of its various tissues (head, body, tail etc.)?; how are various tissue systems in an organism maintained and coordinated concurrently in regeneration? How do stem cells communicate with each other? How do stem cells and tissue cells in a multiple tissue system collectively implement regeneration? Specifically, what communications and computational algorithms could be involved during regeneration? These questions allow great scope for models that are not only biologically realistic but also computationally efficient. This research attempts to offer hypotheses to answer these questions in order to propose, for the first time, a conceptual framework for efficient whole regeneration systems that completely and correctly regenerate their form that in a broad sense resembles biological systems.

4.4 A new conceptual and computational dynamics framework for whole system regeneration

In this section, we introduce the overall generic conceptual framework with mechanisms and algorithms of regeneration that mimics the body-wide immortality of planarian on an *in silico* worm-like organism consisting of multiple tissues. The questions raised in the previous paragraph in relation to whole system regeneration penetrate into the fundamental issue of regeneration well-articulated by Neuhof et al. (Neuhof, Levin, & Rechavi, 2016): although stem cells are thought to drive regeneration in collaboration with differentiated (tissue) cells, it is unclear precisely which aspects of patterning information are intrinsic to the stem cell and which are computed through this collaboration; and that if tissue cells influence the stem cell during regeneration, tissue cells may play a role in determining the phenotype of the new individual. Our framework aims to address the fundamental issue raised by Neuhof et al. (Neuhof et al., 2016) by providing hypothetical answers to the questions raised above, inspired by planarian regeneration. Some previous regeneration models reported in Section 2.1 have attempted to address aspects of these fundamental issues of regeneration by proposing hypothetical mechanisms exploiting the observations of: stem cell production of tissue cells, stem cell migration to the wound site to initiate repair (as explained in the Introduction and Literature Review) as well as stem cell and tissue cell collaborations. However, conceptual frameworks for computation modelling of whole system regeneration are currently lacking. The above fundamental issues and past work, including our tissue model presented in the previous chapter, formed the basis for the conceptualisation of new mechanisms of our framework to achieve a greater level of capacity for accurately and completely regenerating more complex forms.

We hypothesise that a system that is able to regenerate its original pattern from any tiny fragment anywhere in the body after damage must be extremely versatile and robust, and therefore, it possibly uses a high-level and flexible control algorithm, as opposed to extensive micromanagement, to regenerate itself. Further, such an algorithm may seek efficient processing with the minimum computational burden and algorithmic complexity. Also, since a very tiny fragment can regenerate the whole pattern, we hypothesise that the whole pattern information must either be stored everywhere in the organism or be accessed by any part of the organism from an as yet unknown space. Both are exciting avenues for exploration. The first avenue indicates that all cells carry all necessary information on the body pattern. Although this seems to be the easiest option to implement and has been exploited in some nature-inspired self-repair systems in other fields, it could create information overload or redundancy in the biological system. Further, currently there is no scientific evidence that cells carry any patterning information. However, it has been known for some time of the existence of bioelectric fields and morphogenic fields surrounding biological organisms. It has been shown that

bioelectric fields interact with regeneration processes in important ways to even completely alter the final anatomical pattern achieved (Beisson, 2008; Pezzulo & Levin, 2015). However, the exact mechanism of these interactions have so far been elusive and it is currently an actively topic of research. We, therefore, explore the second avenue and propose the existence of cellular memory space – an information field – from which pattern information can be accessed from anywhere in the organism. We hypothesise that this field belongs to stem cells and exists as part of them. In our proposed framework, stem cells extract minimum geometric pattern information required to rebuild the anatomy from communications with their respective tissue cells. We propose that the information field holds this minimum pattern information of the organism and stem cells share and access this information from anywhere in the organism in the regeneration of extreme damages incurring the loss of stem cells and outer perimeter. As stated earlier, recovering from the total loss of the outer perimeter is a challenge for our circular tissue model.

In our circular tissue model, most damages are recovered by extracting patterning cues left in the damaged tissue. However, the model fails to recover correctly from whole perimeter damage (i.e., small tissue fragment with the stem cell cannot correctly recover the full form). This is not a trivial problem for biology or modelling. The proposed information field enhances the capability of stem cells and allows recovery from all possible damages, thus releasing the damaged tissue of the burden of retaining pattern information. This can open up possibilities for science to investigate the potential of bioelectric or morphogenic fields to hold patterning and other information.

There is value in sharing and accessing high-level information as it can afford greater simplicity in the effective management of large dynamical systems by reducing information overload on system elements. This is especially true for evolving dynamical systems with high-level goals, such as biological systems that need to survive and stay robust. Meadows (Meadows, 2008) states that as complexity increases, evolving dynamical systems tend to self-organise into hierarchies with specialised subsystems as hierarchy offers greater resilience for further experimentation and evolution. This is especially relevant to biological organisms. This hierarchy with some centralised information does not necessary imply central control as in a properly functioning hierarchical system, the goals of the subsystems and the whole system are aligned in a way that subsystems serve the goal of the whole system (global optimisation) while the hierarchy serves the subsystems (local optimisation) and provides overall coordination. This offers an interesting blend of distributed and centralised information arrangements. Our proposed framework is largely a distributed system spread across thousands of tissue cells, with a small central repository of minimum pattern information accessed only by specialised (stem) cells.

In our framework we introduce two new concepts: (i) a number of tissue models (rectangular and triangular) that are similar in operation to the circular tissue model but with stem cells that contain a virtual information field that carry minimum pattern information, extracted by stem cells from the corresponding head, body and tail tissues (tissue memory), so that any damaged tissue can be repaired at the tissue level. (These tissue shapes are idealised patterns for simplicity and proof of concept). (ii) tissue patterns combine to form an organism where the stem cells from a stem cell network, corresponding to the whole organism and creates a shared information field (collective memory) of stem cells. Here, two triangular and one rectangular tissue combine to form an *in silico* worm-like pattern. The stem cells are represented as linear neurons and stem cell network as a fully connected neural network. The stem cell network monitors the state of the whole organism and detects and regenerate any missing stem cells, which in turn can regenerate the whole organism from any small- or large-scale damage. The stem cells thus act as the Global Sensing systems in repair at the tissue level and regenerators of missing stem cells and tissues at the organism level (in severe damage) with access to the information field for tissue pattern.

In this new framework, the stem cell network repair model and individual tissue repair models collaborate seamlessly to maintain the integrity of the system under any damage condition by autonomously regenerating missing stem cells and damaged tissues. In most cases of tissue damage, it would regenerate tissues without tapping into their information fields. However, in extreme cases of tissue damage that create open-ended boundaries, a stem cell accesses minimum pattern information from the field, thereby making it possible to recover from any tissue damage. The combined system mimics some of the main features of planarian regeneration, including regeneration of the whole pattern from any tiny fragment as long as at least one stem cell (regardless of which tissue) remains after damage; and complete and accurate regeneration from minor damages to severe damages incurring the loss of whole-body parts (head, body or tail). Thus, the proposed framework is robust, versatile and flexible with complete regeneration ability achieved with minimum computation. Below we describe the framework starting with the extension of the original circular tissue repair model to triangular and rectangular shapes with stem cells and virtual fields (sections 4.5.1) and finally the assembly of the worm-like organism (section 4.5.2). The framework with all its functional aspects is described in section 4.5.3 and implementation of the framework on the *in-silico* worm in Section 4.6.

4.4.1 Extension of the circular tissue (base) model: A Regeneration system with enhanced stem cell capability and an information field and application to develop new triangular and rectangular tissue self-repair models

In the new framework, we extend the capability of the stem cell by investing it with extra capabilities. Specifically, we hypothesise that a stem cell remembers minimum pattern information of the corresponding tissue, that is stored in an information field surrounding the stem cell and shared as

needed with other stem cells through the collective field. The information field is a mathematical abstraction over traditional components of tissues.

A. Self-repair circular tissue model with enhanced stem cell capability

Figure 4.1 shows the stem cell and information field for the circular tissue. The red and blue dots are stem cells and tissue cells, respectively. Here, the same neighbourhood rules (Eq. 3.1) and perceptron network (AANN) for the circular tissue presented in Chapter 3 prevail. The novelty is that in addition determining entropy from the communication with tissue cells to maintain tissue integrity, the stem cell uses this communication to recognise the specific tissue pattern by extracting minimum pattern information required to regenerate the anatomy. Specifically, from the distance d_j to the tissue cells, the stem cell determines the geometry of the tissue and keeps radius d in the information field that allows it to regenerate the pattern from any damage, thus releasing it from the previous constraint of having to retain minimum information in the damaged tissue. It is important to note that in most cases of damage, the system would regenerate without tapping into the field. The stored information will be used only in extreme cases where the whole outer boundary is lost to damage. This is not unlike the situation where, in most cases of damage to a building, a structural engineer would repair it by taking cues from the undamaged part of the structure; however, when the damage is severe to the extent that it creates open-ended boundaries, they refer to the original plan to repair the damage. We extend this model concept to triangular and rectangular tissue models that are then combined into an artificial organism with a stem cell network and a collective virtual field to form the autonomous whole system regeneration model. Below we present the triangular and rectangular tissue models and example cases of regeneration.

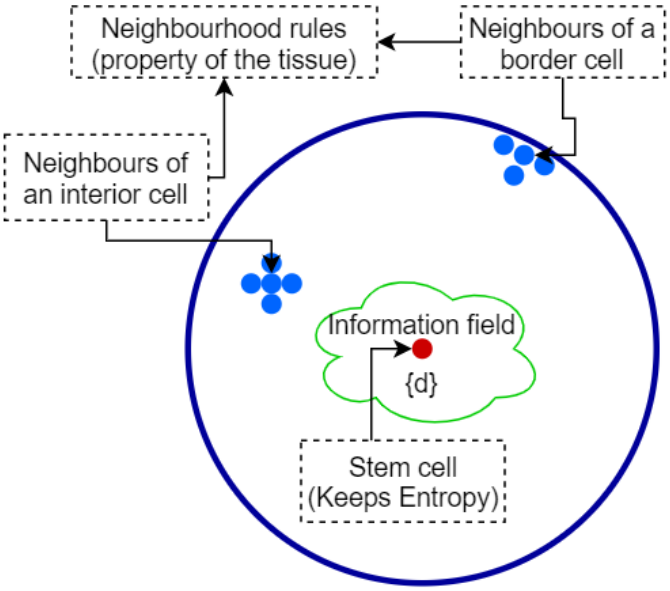


Figure 4.1. Individual tissue repair model – A circular tissue with a computational stem cell and surrounding information field

B. Development of self-repair triangular tissue model with enhanced stem cell capability

The triangular tissue model is similar to the circular tissue model, but different neighbourhood rules apply to the AANN and the stem cell (Figure 4.2). The neighbourhood rules are: the number of neighbours of interior cells, border cells and corner cells are four, three and two, respectively. From these rules, perceptrons in the AANN (Eq. 4.1) can collectively establish its original pattern and border of damage anywhere in the tissue. The stem cell extracts the tissue plan (minimum shape information) into the information field from its communication with the tissue cells (from its distance to the tissue cells, d_j): the length of the sides (d) (e.g., based on the number of cells, d=10 in our examples), Aspect Ratio (AR) which is the height to length ratio (considered equal to 1 in our examples), number of corners (n=3) (Figure 4.2A). The tissue is divided into four segments for monitoring its pattern integrity through entropy by the stem cell (Figure 4.2A). As stated before, the number of segments is arbitrary and is a trade-off between performance (damage detection) and information efficiency. The larger the number of segments, the more accurate the performance but the larger the information burden on the stem cell as it has to carry a larger amount of information on entropy relevant to the segments. Here we use a much smaller number of segments than in the circular tissue model to further improve information efficiency as we found from the circular tissue that the entropy is sensitive to even single missing cells. Further experiments revealed that this sensitivity is retained with even larger segments. As in the circular tissue model, entropy is a property of the stem cell, and neighbour rules are properties of the tissue. As before, entropy is used to detect the general damage region from the affected segment(s) (Global Sensing), and the tissue AANN in the affected region gets activated to detect and locate the border of damage (Local Sensing) upon which the stem cell repairs the damage.

This way, the stem cell achieves complete regeneration with the help of the tissue AANN. The novelty is that in the most extreme damage cases, the stem cell taps into the information field to extract minimum pattern information to regenerate the pattern.

$$\text{Interior cells:} \quad u = \sum_{i=1}^{4} C_i * w_i \; ; \qquad y = \begin{cases} 1 & if\ u = 4 \\ 0 & otherwise \end{cases}$$

$$\text{Border cells:} \quad u = \sum_{i=1}^{3} C_i * w_i \; ; \qquad y = \begin{cases} 1 & if\ u = 3 \\ 0 & otherwise \end{cases} \tag{4.1}$$

$$\text{Corner cells:} \quad u = \sum_{i=1}^{2} C_i * w_i \; ; \qquad y = \begin{cases} 1 & if\ u = 2 \\ 0 & otherwise \end{cases}$$

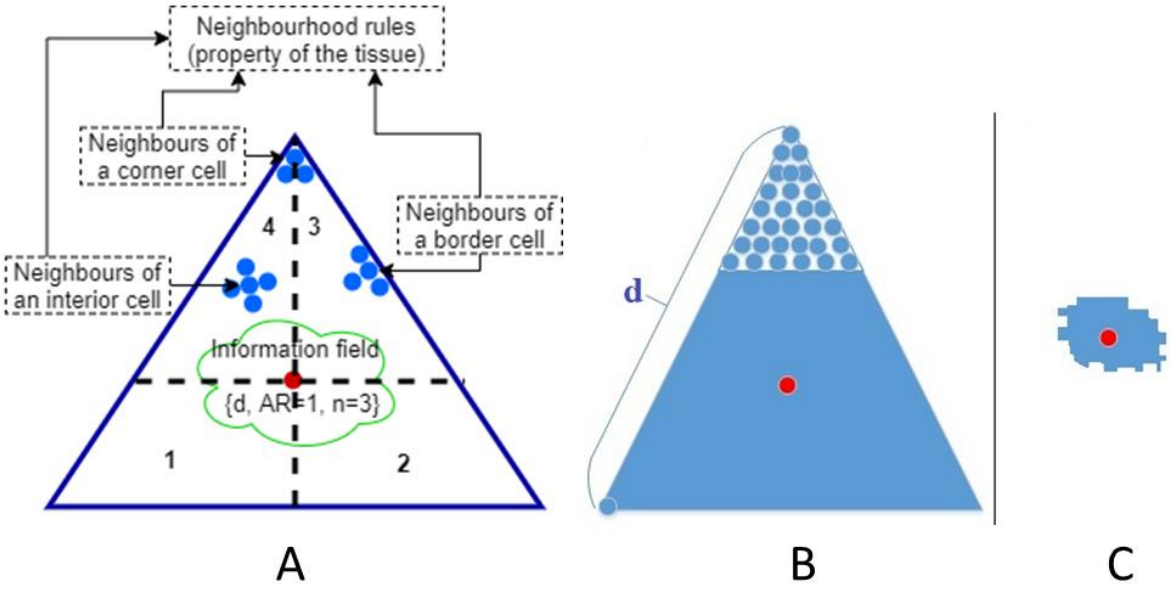


Figure 4.2. Triangular tissue repair model. A) triangular tissue with a computational stem cell and surrounding information field; B and C) Two cases of damage in the triangular tissue. Stem cells and differentiated cells are denoted with red and blue colours respectively.

Two cases of regeneration of triangular tissue

We demonstrate how the system recovers from two types of damage shown in Figure 4.2B, C. In the first case, a part of the tissue is missing, and in the other, it incurs severe damage leaving a small fragment with the stem cell.

a. Recovery from partial tissue damage

For the first damage case, Figure 4.3 shows the repair process. Figure 4.3A, B are the original and damaged tissues, respectively. Global Sensing based on entropy allows the stem cell to identify

the damage regions of 3 and 4 from the entropy of the four segments, activate the corresponding region of the tissue AANN that identifies the exact damage location and damage border (Figure 4.3C) using rules for interior and border cells shown in Figure 4.3A. Then, the stem cell moves to this location and produces new cells row by row along the damaged border to regenerate the pattern without tapping into the field as it can use the existing pattern information in the damaged tissue. Specifically, new border cells are added, guided by current border cells of the structure and neighbourhood rules for the border. When a border cell aligns with two border edges, and it does not satisfy the border rules, then this border cell becomes a corner cell that stops regeneration (Figure 4.3D). The rest of the figures in the bottom row show the restoration of tissue entropy to the original state during regeneration. The x-axis in Figure 4.3 (bottom row) refers to quadrants. The lines connecting the four data points are to emphasise the difference between the entropy for normal and damaged tissue. Lines are simply for visual clarity for the readers to easily see the difference between the two cases.

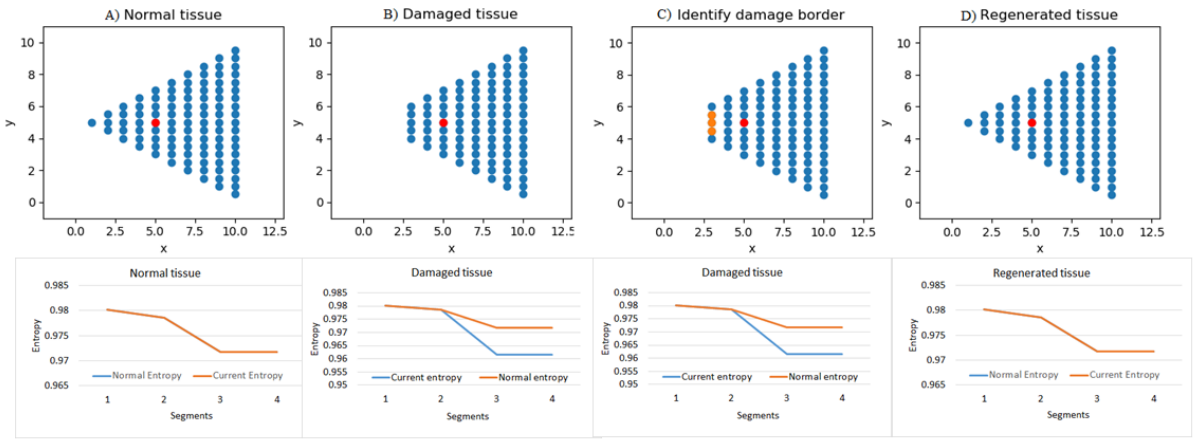


Figure 4.3. Regeneration steps after partial tissue damage. A) Original tissue; B) partly damaged tissue; C) damage border identified by the model (Entropy (GS), (AANN) (LS) and neighbour rules) and D) regenerated tissue. x and y are coordinates

b. Recovery from severe damage leaving a tissue fragment with the stem cell

Figure 4.4 illustrates the progression of regeneration to restore the original form from the severe damage shown in Figure 4.4C. Damage identification is the same as before, with Global Sensing and Local Sensing. The first image in the second row of Figure 4.4 shows entropy before and after damage (Normal and Current Entropy, respectively), which indicates that all 4 segments have received damage. As this is extreme damage to tissue exterior, the system taps into the information field. The stem cell initially produces new cells to form a new small triangular tissue (Figure 4.4B) that then it incrementally grows until completion (Figure 4.4C&D) according to the above neighbour rules and minimum geometry information in the information field (d, AR=1, n=3). The rest of the figures in the second row show the restoration of tissue entropy to the original state during regeneration.

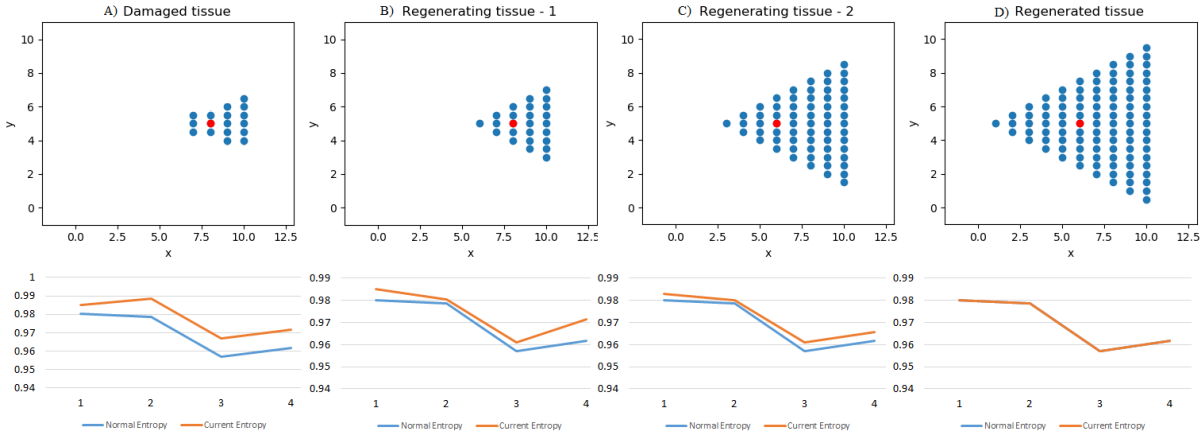


Figure 4.4. Regeneration of a triangular tissue from a small remaining fragment with intact stem cell. The first row shows stages of regeneration of the pattern until complete recovery. From a small damaged tissue, it first recovers a scaled version of the correct triangular form and then incrementally grows it until completion according to neighbour rules and pattern information (d, AR=1, n=3). The second row shows the corresponding tissue entropy for each stage.

C. Development of self-repair rectangular tissue model with enhanced stem cell capability

The rectangular tissue model is similar to the triangular tissue model, but different neighbourhood rules apply to the AANN and different pattern information applies to information field (Figure 4.5). The neighbourhood rules are: the number of neighbours of interior cells, border cells and corner cells are four, three and three, respectively. From these rules, perceptrons in the AANN (Eq. 4.2) can collectively establish its original pattern and border of damage anywhere in the tissue. The stem cell extracts relevant minimum shape information into the information field from its communication with the tissue cells: the length of the sides (d) (e.g., based on number of cells, d=10 in our examples), Aspect Ratio (AR= height to length ratio=3 in this case), number of corners (n=4). Figure 4.5 also shows the four segments used for entropy-based damage region identification. In this tissue, the system can recover from any damage without tapping into the field as long as one whole side or whole exterior does not receive damage. For only such damage to the exterior, it taps into the field for pattern information.

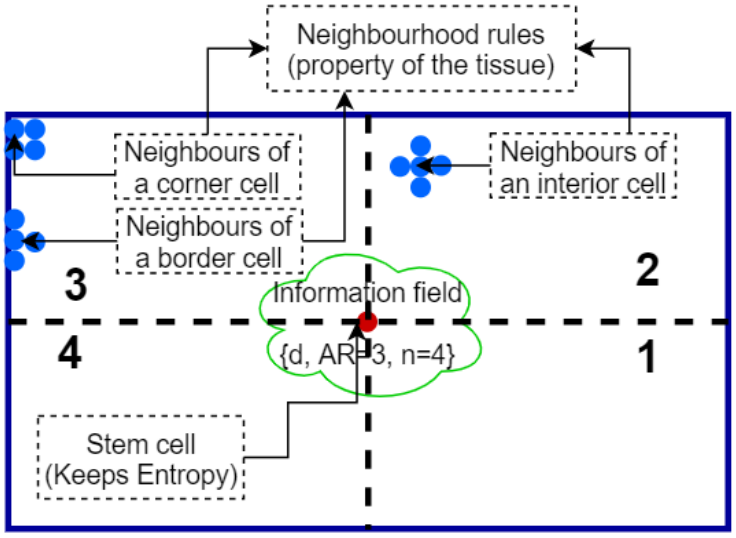


Figure 4.5.Rectangular tissue repair model. Tissue model consists of a stem cell and information field and tissue cells (AANN and neighbourhood rules); Tissue is divided into four regions (1, 2, 3 and 4) for entropy

$$\text{Interior cells:} \quad u = \sum_{i=1}^{4} C_i * w_i \; ; \quad y = \begin{cases} 1 & if\ u = 4 \\ 0 & otherwise \end{cases}$$

$$\text{Border cells:} \quad u = \sum_{i=1}^{3} C_i * w_i \; ; \quad y = \begin{cases} 1 & if\ u = 3 \\ 0 & otherwise \end{cases} \qquad (4.2)$$

$$\text{Corner cells:} \quad u = \sum_{i=1}^{3} C_i * w_i \; ; \quad y = \begin{cases} 1 & if\ u = 3 \\ 0 & otherwise \end{cases}$$

Two cases of regeneration of rectangular tissue

We apply the tissue repair model to 2 cases of damage; damage where the tissue loses one side (Figure 4.6A), and severe damage where the whole exterior region is lost leaving only a tiny fragment with the stem cell (Figure 4.6B).

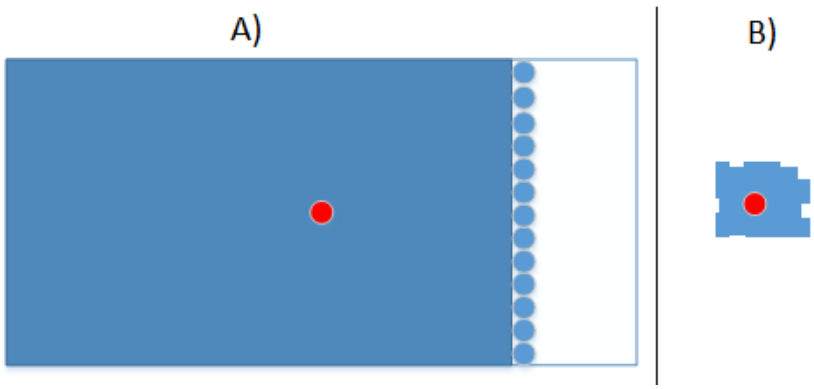


Figure 4.6. Two cases of rectangular tissue damage - (A) damage to one side and (B) severe damage leaving a small tissue fragment with the stem cell

As in the triangular case, the system identifies the damage region with Global Sensing using entropy and finds the location and size of damage through Local Sensing of missing neighbours in the AANN and regenerates completely with the above neighbour rules and pattern information. As a side has received damage, the system taps into the field for required pattern information. Figure 4.7 shows the damaged tissue, segmented tissue and entropy before and after damage, as well as AANN detected damage border and the regenerated tissue for the first damage case.

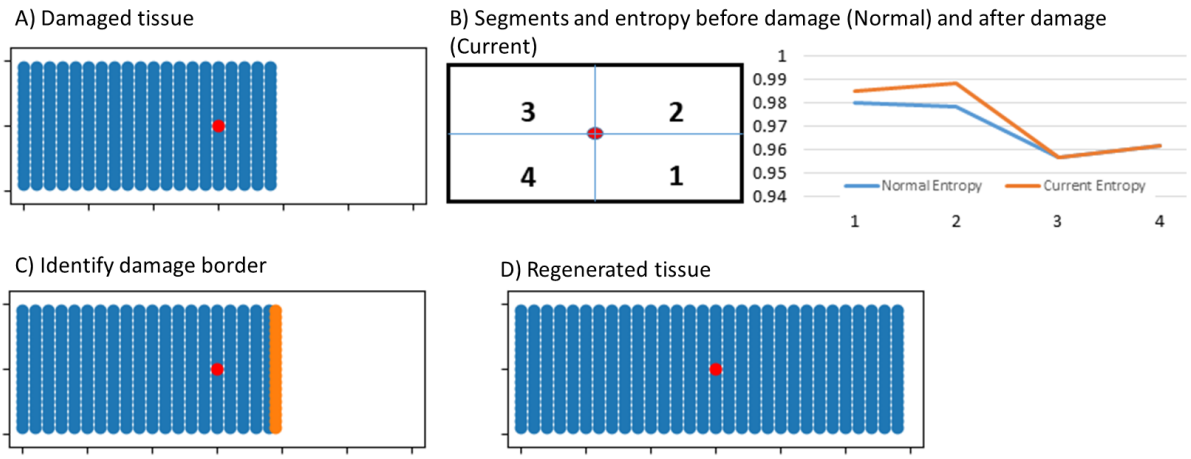


Figure 4.7. Rectangular tissue regeneration after damage: (A) Damaged tissue as in Figure 4.6A, (B) Segmented tissue and identification of damaged region by Global Sensing based on entropy, (C) Identification of damage border by AANN and rules, (D) Regenerated tissue

Figure 4.8 shows the stages of progression of regeneration until complete recovery for the second damage case shown in Figure 4.6B. As the whole exterior has received damage, pattern information is accessed from the field. Starting from regenerating a scaled version of the tissue (Figure 4.8B), it is grown until complete recovery (Figure 4.8C-D).

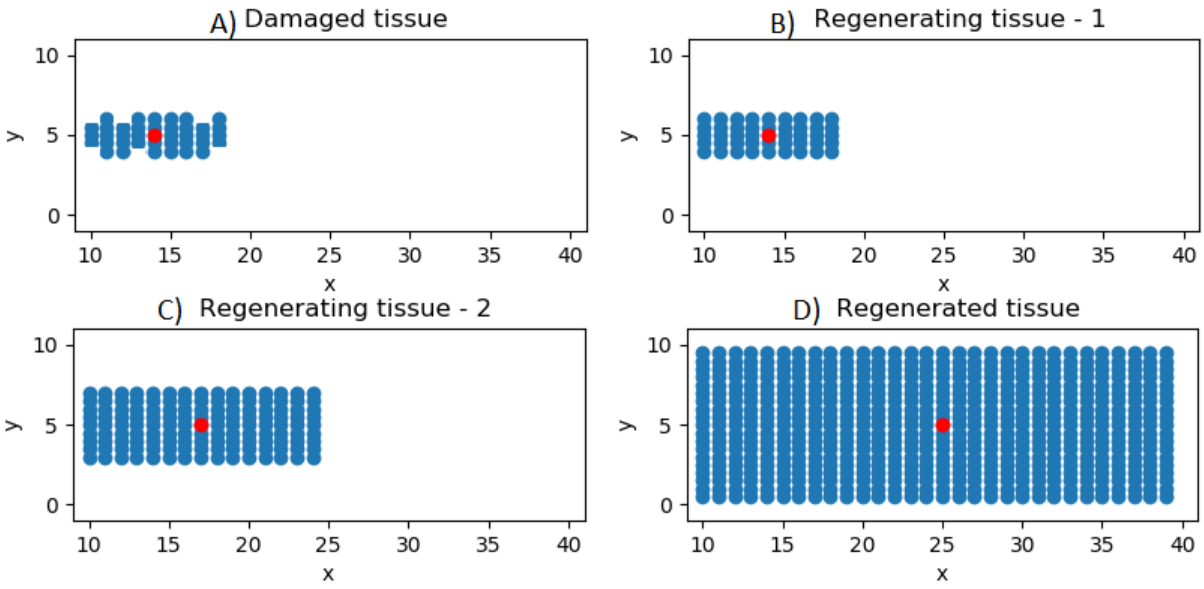


Figure 4.8. Regeneration of a rectangular tissue from a small fragment left after damage as in Fig. 4.6B. A) Damaged tissue, B-C) incremental tissue regeneration guided by the shape information d, AR and n, and D) Regenerated tissue

4.4.2 Creating a virtual organism from individual tissues and its core operation

We assemble two triangular and one rectangular tissue representing head, body and tail to form a simple planarian-like worm as shown in Figure 4.9A. It shows the shared information field (with the information presented in vector form) as applicable to the three tissues. The shared field enables regeneration from any stem cell or tissue damage anywhere in the system as long as one (any) stem cell remains. Figure 4.9B shows the stem cell network consisting of head (C1), body (C2) and tail (C3) stem cells and network connections representing a flow of information weighted according to stem cell location identifiers (1, 2 or 3). (As will be presented later, these location identifiers help stem cells establish A/P polarity of the organism). Each stem cell is represented as a neuron with a linear transfer function (Figure 4.9C) and the three stem cells together form a fully connected neural network that produces a numerical output (Figure 4.10 & Eq. 4.3-4.5) depending on the activation signal each stem cell receives. This helps decide the extent of the damage, whether one or more stem cells have been damaged.

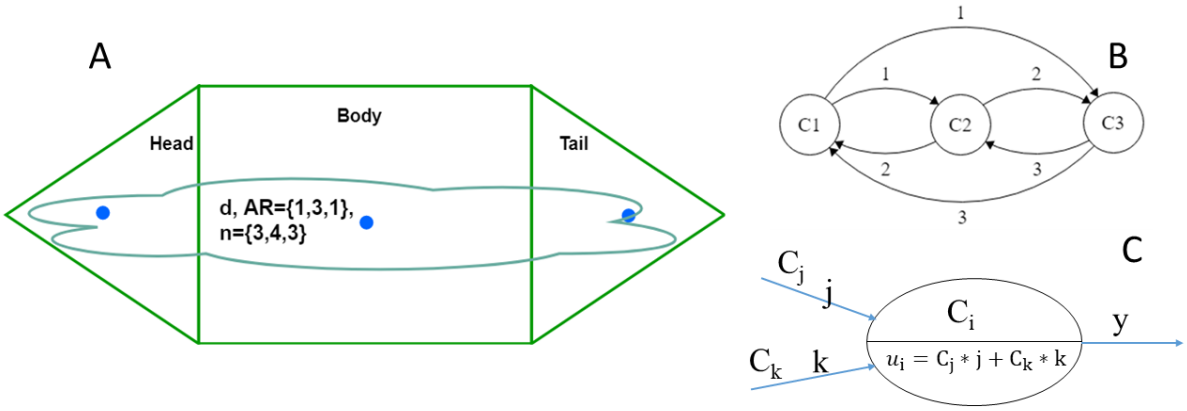


Figure 4.9. The whole system regeneration model. A) Form of the virtual organism with head, body and tail tissues and shared collective information field containing pattern information- n, d, AR; and B) Stem cell network of head (C1) body (C2) and tail (C3) stem cells. Stem cell connections represent the flow of signals weighted according to stem cell location identifiers (1, 2 or 3). (C) Linear Neuron model of a stem cell.

Head stem cell

$$y = \begin{cases} body\ \&\ tail & if\ u = 0 \\ tail & if\ u = 2 \\ body & if\ u = 3 \\ normal & otherwise \end{cases} \quad (4.3)$$

Body stem cell

$$y = \begin{cases} head\ \&\ tail & if\ u = 0 \\ tail & if\ u = 1 \\ head & if\ u = 3 \\ normal & otherwise \end{cases} \quad (4.4)$$

Tail stem cell

$$y = \begin{cases} body\ \&\ head & if\ u = 0 \\ body & if\ u = 1 \\ head & if\ u = 2 \\ normal & otherwise \end{cases} \quad (4.5)$$

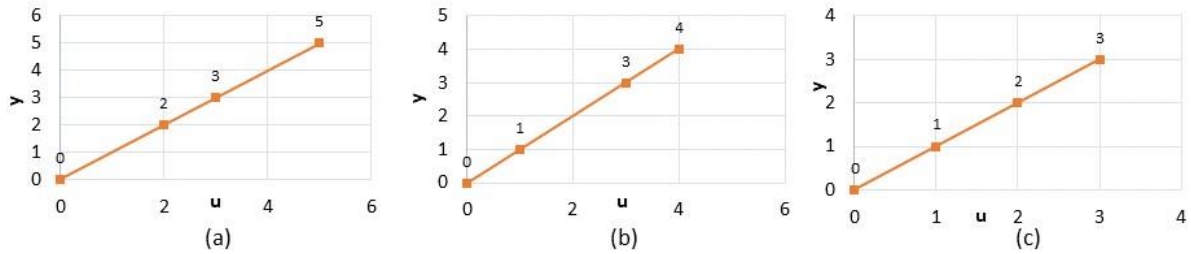


Figure 4.10. Neural network output of individual stem cells: (a) head, (b) body and (c) tail. From the output, each neuron (stem cell) identifies the extent of stem cell damage that can be either individual or combined stem cell damage using Eqs. 4.3 -4.5.

4.4.3 Algorithmic assembly of the conceptual framework for whole system regeneration

The overall framework, with its operational flowchart, is shown in Figure 4.11. It operates as a tissue-wise modular, largely distributed memory, intelligent neural feedback control system that achieves complete regeneration through two levels of operation:

Level 1: This level represents individual (triangular and rectangular) tissue self-repair models and gets activated in cases where stem cells are intact, and only the tissues receive partial damage (blue box in

Figure 4.11). Algorithmically, it encompasses Global Sensing (through entropy) and Local Sensing (through AANN with perceptrons and neighbourhood rules) for damage identification. The communication involved is that between stem cell and tissue cells to obtain the distance to tissue cells, from which the stem cell extracts tissue plan (only once in the life of the organism) and computes entropy for the tissue segments and compares with stored entropy pattern in damage. The other communication involved is that between tissues cells in their local neighbourhood to identify the exact damage location and border.

Level 2: This represents the stem cell network with overlapping information fields for recovering from the loss of stem cells and whole tissues (head, body or tail) (purple region Figure 4.11). Algorithmically, it encompasses the neural network shown in Figure 4.9B with outputs shown in Figure 4.10 and Eq.4.3-4.5 to identify and regenerate missing stem cells and whole tissues. Amount of communication in the network is only that among the three stem cells. Another communication at this level is stem cells accessing the information field only in extreme damage cases.

The idea of the framework is that the two levels operate in three ways to accomplish complete and correct recovery:

(i) When there is only partial tissue damage leaving stem cells intact, only Level 1 of the model is activated where the stem cell senses the damage through entropy change, detects the general damage region and triggers the AANN to find the exact damage location. Then the stem cell migrates to the damage region and repairs it with the help of the AANN as shown previously for individual tissues (red arrows in Figure 4.11). This system taps into the information field only when the whole outer boundary receives tissue damage.

(ii) For any damage involving loss of stem cells and *whole* tissues, Level 2 is activated where stem cells communicate with each other through the stem cell network, and senses missing stem cell(s) and regenerate them using the information in the shared information field (green arrows in Figure 4.11). The shared information (e.g., minimum pattern information for all tissues) can be accessed by all stem cells. This idea is used in the production of new stem cells. Specifically, a lost stem cell is replaced with a new stem cell with similar characteristics as before by the neighbour stem cell that transfers the shape information from the shared field into the new stem cell. The new stem cell then carries out complete regeneration of the damaged tissue using the received pattern information, and a new tissue AANN is re-established along with neighbour rules. The stem cell also restores tissue entropy. When more than one stem cell is lost, new stem cells are produced by the remaining stem cell using the pattern information in the field for concurrent and seamless regeneration of the missing parts and the whole organism.

(iii) The two levels collaborate in the case of partial tissue damage with lost stem cells, as shown by the green arrows flowing through both Levels in Figure 4.11, where after replacing the missing stem cell(s) in Level 2, the corresponding tissue AANN model(s) in Level 1 is activated where entropy-based global sensing (GS) and AANN based local sensing (LS) detect damage and its border and the stem cell executes full regeneration of the damaged tissue.

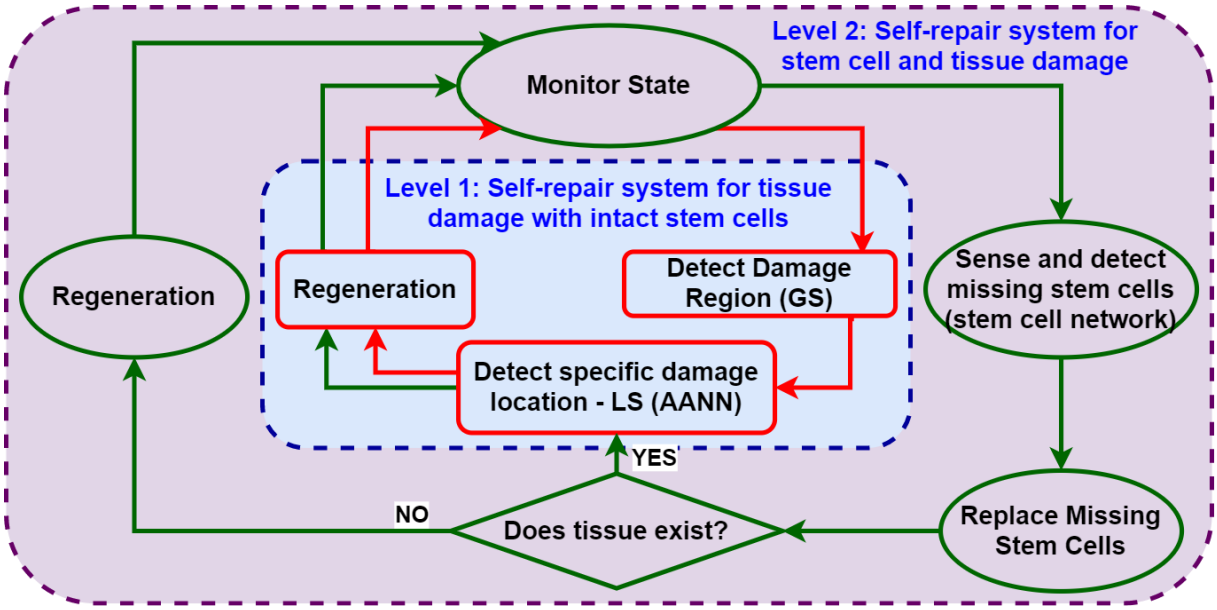


Figure 4.11. A conceptual framework with algorithms for autonomous whole system regeneration after tissue and/or stem cell damage; Level 1 (blue region and red arrows) represents individual tissues with a stem cell self-repair models for the cases where tissues receive partial damage leaving the stem cell intact. Level 2 (the purple region with green arrows) represents the stem cell network for recovering lost stem cells and whole tissues.

4.5 Implementation of the regeneration framework

In this section, we demonstrate the efficacy of the whole system regeneration framework in recovering from any damage by implementing it on the simple *in silico* worm-like organism assembled in the previous section. We present results from major damages that invoke most repair capabilities of the framework.

4.5.1 Only tissue damage with intact stem cells: system regenerates completely

This type of damage is simple in that only a part of the organism is missing, but stem cells are intact. An example is where a group of differentiated cells in a tissue is cut off while the stem cell of this tissue remains as in Figure 4.12 (Recall that (x, y) are coordinates and red and blue dots are stem cells and differentiated (tissue) cells, respectively). In this case, the damaged organism with intact stem cells regenerates the head fully and accurately. The small part of the tissue that was separated from the head tissue does not contain a stem cell so it cannot regenerate.

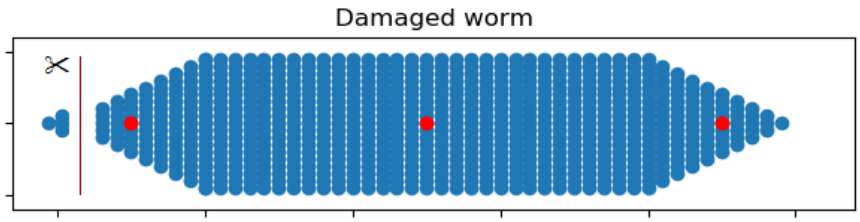


Figure 4.12. Virtual worm with a missing fragment of the head

This damage case does not require accessing pattern information from the field and regeneration proceeds as follows using only Level 1 of the framework that operates only the relevant tissue models. In this example, the damaged cells in the head tissue cease sending signals to the nearest stem cell that senses the resulting entropy change and activates the head tissue repair model (the blue part with red arrows in Figure 4.11) to initiate repair using the following steps: (i) calculate entropy change, (ii) activate AANN of the head tissue, (iii) identify damage borders and (iv) repair damage. The first step is that the stem cell compares the stored and new value of entropy of each segment to identify general damage region. The entropy graph in Figure 4.13b shows that the damage occurred in two segments 3 and 4 (i.e., at the front of the head). Then, the stem cell activates the part of the AANN of the head tissue corresponding to these segments to identify the border of the damage (Figure 4.13c) using perceptron rules. The last step is that the stem cell migrates to the damaged border and regenerates missing cells using rules for the border. The regeneration proceeds until the damaged part is regenerated returning the worm to the original form, as shown in Figure 4.13d.

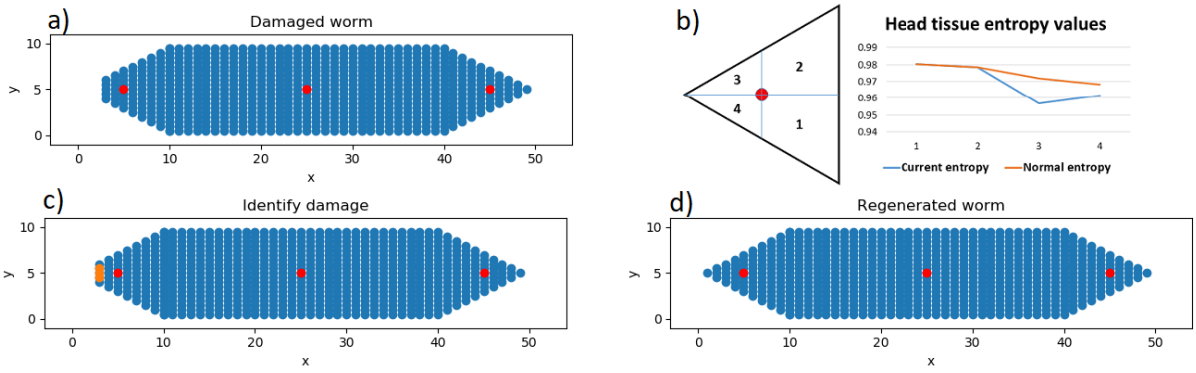


Figure 4.13. Regeneration after damage to head tissue: a) Damaged worm, b) Segmented tissue and entropy, c) Identification of damage border, and d) Regenerated worm. (x and y are coordinates)

4.5.2 Organism receives both tissue and stem cell damage

The main idea for this case is that the stem cells can be amputated with the surrounding tissue. We consider two damage situations for this case with an organism fragmenting into two parts: (i) along a tissue boundary; and (ii) encompassing tissue and stem cell damage in the body interior.

A. Organism fragments into two parts along a tissue boundary

Here we consider damage along the head-body boundary that fragments the organism into two parts, as shown in Figure 4.14. The main question is: how does each part regenerate into a whole organism as the original one, resulting in two identical worms? We explain the process of regeneration below.

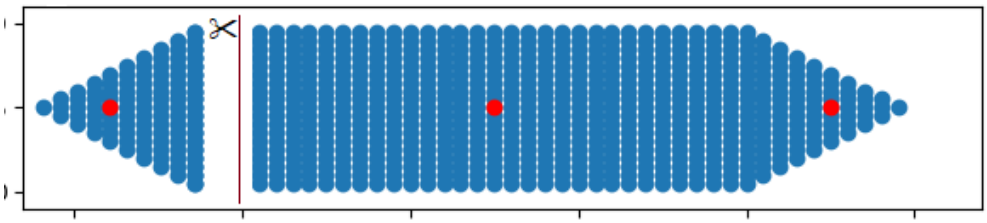


Figure 4.14. Virtual worm damaged along the head-body boundary (x and y are coordinates)

As the damage has left an open-ended boundary, the system taps into the field for minimum pattern information. Both parts regenerate by activating Level 2 of the framework as follows (follow the green arrows in Figure 4.11): After amputation, stem cells in both parts check for any local damage as described in the case above for tissue damage. If there is no local damage, as is the case in this example, it activates the stem cell repair model (the purple part with green arrows) in Figure 4.11 (Level 2) and follow three steps: (i) identify missing stem cell(s), (ii) replace missing stem cell(s), and (iii) regenerate missing tissues concurrently and re-establish AANN. We explain the regeneration of each missing part below.

a. Head regenerates body and tail

(i) Identify missing stem cells (Level 2)

Here, the loss of stem cell activates the stem cell neural network to detect missing stem cells. From the operation of the stem cell network, the head should recognise that the *body and tail stem cells are missing* in the organism. Specifically, as head stem cell receives no signal from body and tail stem cells (C2 and C3), the output y of the model is 0 (Figure 4.10 & Eq. 4.3) indicating that body and tail stem cells are missing**.**

(ii) Replace missing stem cells (Level 2)

After identifying missing stem cells, the head stem cell produces a new body stem cell and a new tail stem cell and transfers the required information to each new cell from the shared information field (Figure 4.15b). It means that the newly formed stem cells now have the shape information of the representative tissues.

(iii) Regenerate missing tissues (Level 2)

The new body stem cell produces a new rectangular body tissue while the new tail stem cell produces a new triangular tail tissue. The regeneration proceeds from a small body and tail shape attached to the head and the two new tissues grow concurrently, as shown in Figure 4.15b&c, maintaining the overall shape guided by the shape information d, AR and n (number of corners) accessed from the field. After regeneration, the two levels of the self-repair system return to normal in that each stem cell returns to normal operation and maintains tissue entropy, the newly regenerated tissues establish neighbourhood relations (as in Figure 4.2A and Figure 4.5) and recognise themselves and the stem cell network gets reestablished and resumes its normal operation where each stem cell receives the correct signals from the other stem cells.

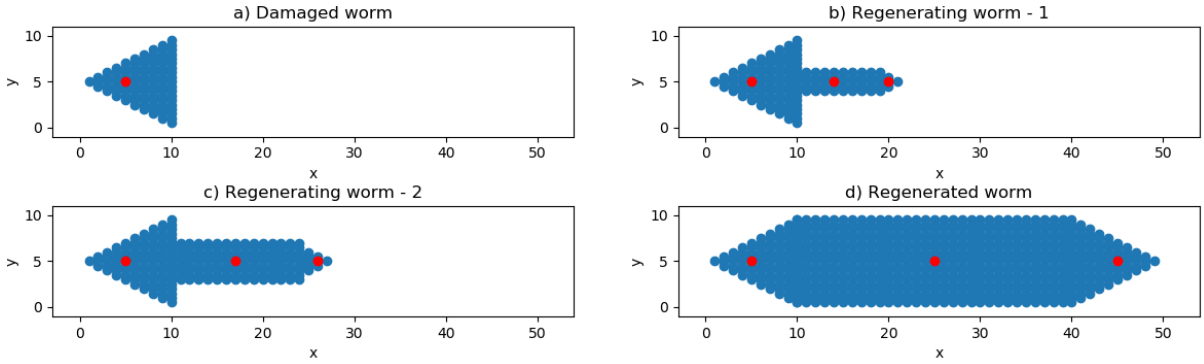


Figure 4.15. Progression of regeneration of body and tail from the head

b. Separated part containing body and tail regenerates head

Steps involved in head regeneration are similar to those in body and tail regeneration. Body and Tail recognize the missing head stem cell from the stem cell network output using the same process as above. Body stem cell output y=3 (Eq. 4.4) and tail stem cell output y=2 (Eq. 4.5) indicate that head stem cell is missing. The body stem cell regenerates a new head stem cell and transfers the required shape information. Then, the new head stem cell starts producing new triangular tissue, and the

regeneration continues until the sides become equal to d (as in Figure 4.16). In this regeneration, pattern information for the triangular tissue (d, AR, n) is applied and the AANN and the neighbour relations get re-established. After regeneration, the two levels of the system return to normal operation.

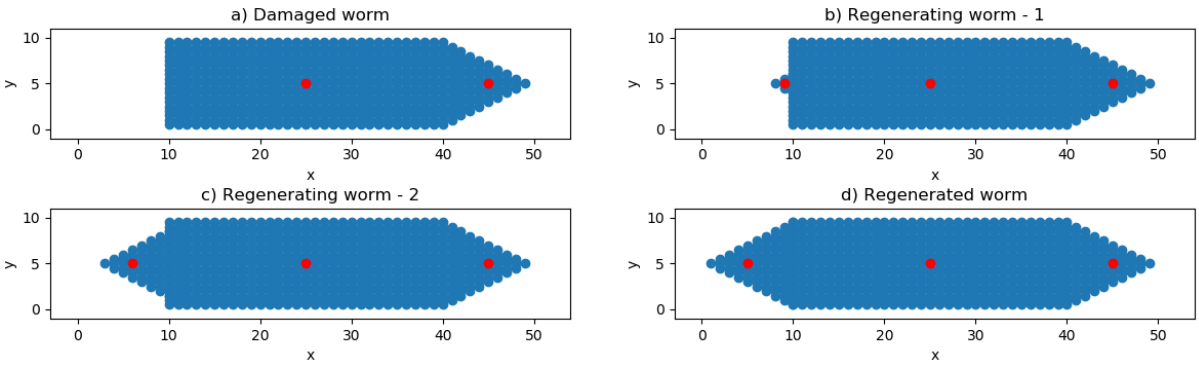


Figure 4.16. Progression of regeneration of head from the separated part containing body and tail

B. Organism fragments into two parts encompassing damage to the interior

In this case, the organism fragments into two parts - one small fragment of the body tissue containing the body stem cell is removed from the worm leaving 2 damaged parts as in Figure 4.17. Both parts will regenerate by activating the repair framework differently.

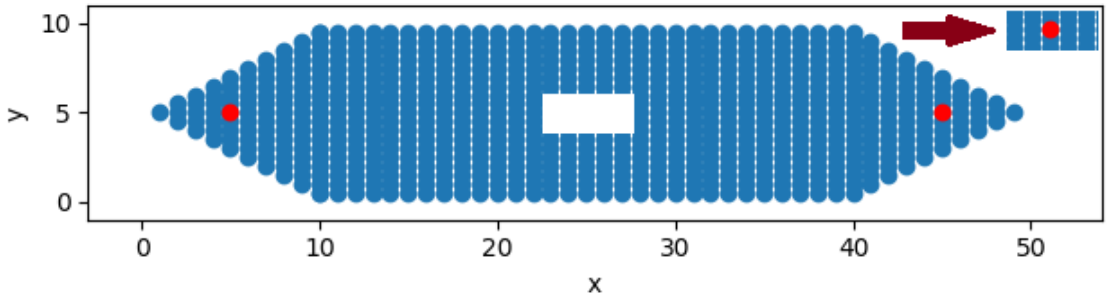


Figure 4.17. Interior damage to the worm removes a fragment of tissue with stem cell leaving two damaged parts

a. The organism with the missing stem cell regenerates the original form

Regeneration of the larger part (with missing stem cell and a small tissue fragment) (Figure 4.17) requires communication between both repair levels (Levels 1 and 2) as there is both partial tissue damage and missing stem cell. Damage signals activate the stem cell repair network (Level 2). The process is as follows: (i) identify the missing stem cell (ii) replace the missing stem cell, (these two steps belong to Level 2), (iii) activate AANN model to identify the damage border; (iv) regenerate the missing

tissue fragment (latter two steps belong to Level 1). As the damage is interior, the new stem cell repairs the damage without tapping into the information field as it finds cues from the remaining tissue for regeneration. The repair process is described below.

i. Identify missing stem cells (Level 2)

Here, the loss of stem cell activates the stem cell neural network to detect missing stem cells. From the operation of the stem cell network, the head should recognise that the *body stem cell is missing* in the organism. Specifically, as head stem cell receives a signal from a tail stem cell (C3) and not from a body stem cell (C2), the output y of the model is 3 (Figure 4.10 & Eq. 4.3) indicating that body stem cell is missing. Similar to the head stem cell, the tail stem cell receives a signal from a head stem cell (C1) only, producing an output y of 1 (Figure 4.10 & Eq. 4.5), which also indicates that the body stem cell is missing.

ii. Replace missing stem cells (Level 2)

After identifying missing stem cells, either the head or tail stem cell reproduces a new body stem cell (Figure 4.18B).

iii. Activate body tissue repair model (Level 1)

As a large part of the body tissue remains in the organism, the new body stem cell does not need to regenerate the whole body tissue. Instead, it only needs to identify the border of the damage and repair the damage. It activates the AANN model of the body tissue to find the damage border. This case does not require tapping into the information field for pattern information.

iv. Regenerate missing tissues (Level 1)

After identifying the damage border, the new body stem cell migrates to the damage site (Figure 4.18C) to regenerate the missing tissue until full recovery (Figure 4.18D). After regeneration, the two levels of the framework in the worm resume normal operation.

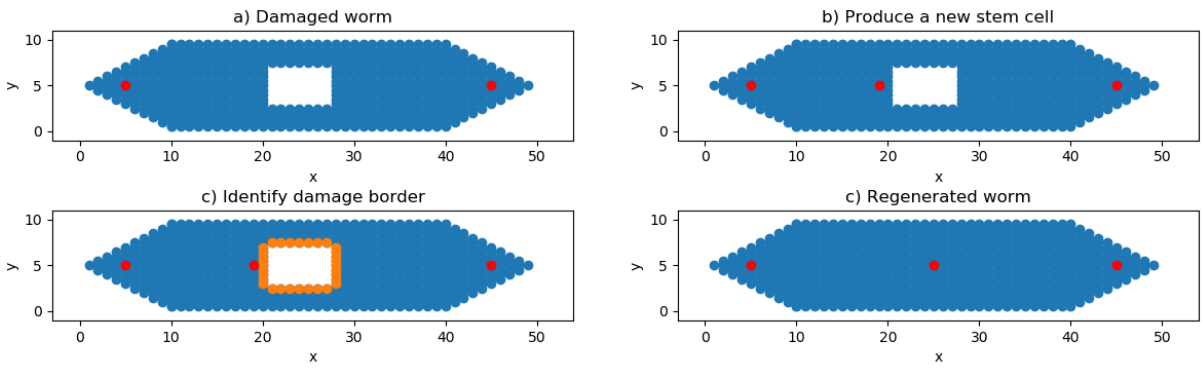


Figure 4.18. Process of regeneration of missing interior tissue and stem cell. A) Damage to Body tissue interior; B) Regeneration of a new Body stem cell by the stem cell repair network; C) Damage border identified by AANN Body tissue repair model, and D) Fully recovered organism

b. Small tissue fragment with body stem cell regenerates into a full organism

This is major damage involving loss of stem cells and the whole outer regions (tissue loss) of the organism. Here only the Level 2 of the framework applies. In this case, the damage signals activate the stem cell repair network, and the process is as follows: (i) identify missing stem cells, (ii) replace missing stem cells, (iii) regenerate missing tissues concurrently. As this damage leaves an open-ended boundary, the system accesses pattern information from the collective information field.

The first and second steps are similar to the case in the previous section. The separated tissue fragment with the body stem cell identifies and replaces the head and tail stem cells and transfers the required shape information to each new stem cell. Following the shape information, the head stem cell produces a new triangular head tissue while the tail stem cell produces a new triangular tail tissue on the corresponding sides of the body tissue. However, how do new stem cells know the direction to regenerate the head and tail tissues? Here, we assume that the stem cell network polarises each stem cell (+,-) depending on their relative position indicated by the cell identifier (1, 2, and 3 in our example) where "+" and "-" show the direction of the head and tail, respectively, as shown in Figure 4.19A. A stem cell with a smaller identifier relative to a neighbour cell indicates "+" direction. This property of the stem cells helps them identify and grow in the correct direction as in the original. (Recall that these identifiers were used as weights in the stem cell neural network). The three stem cells then self-organise to become a new small worm (Figure 4.19B), and then the three parts grow concurrently while maintaining the overall shape. Regeneration continues, as shown in Figure 4.19C-D until the sides of the head and body become equal to *d,* and the body reaches Aspect Ratio of 3. After regeneration, the two-levels in the organism resume normal operation.

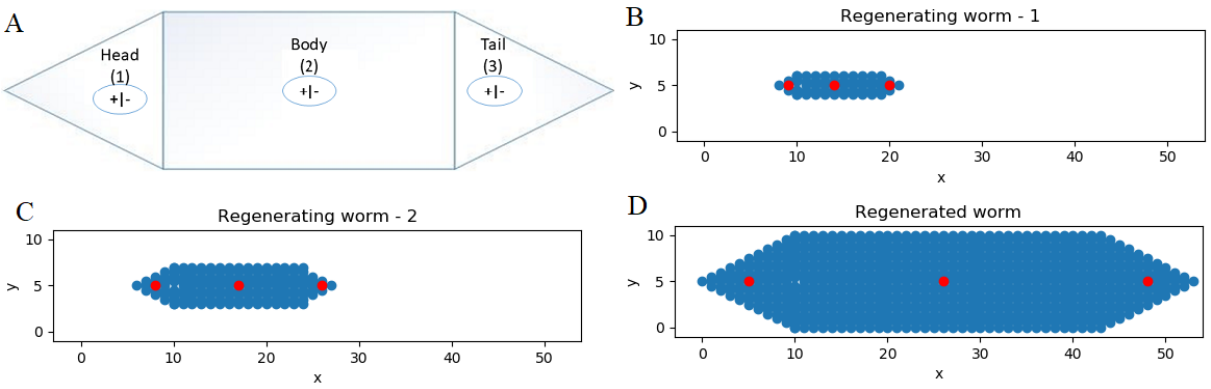


Figure 4.19. Regeneration of the whole worm from the fractured tiny 'interior fragment with the body stem cell'. A) Identification of stem cell polarities; "+" and "-" show the direction of the head and tail, respectively. B) Initiation of new tissue regeneration with new head and tail stem cells; (C-D) Stages of concurrent regeneration of the tissues into a complete organism

4.6 Discussion

This study developed a conceptual framework with mechanisms and algorithms of regeneration that mimic some essential aspects of planarian regeneration. The framework is based on the collective operation of tissue and stem cell regeneration models to completely and accurately recover from any damage anywhere in an *in silico* planarian-like organism as long as just one stem cell remains in the damaged tissue. We demonstrated its efficacy in recovering from small and severe damages to tissues and stem cells, mimicking the extreme regeneration capabilities of planaria, including body-wide immortality.

Our model makes a number of conceptual and algorithmic contributions to advancing the capability of regeneration models and offers a hypothetical framework for generating new ideas for improving the understanding of regeneration. From a conceptual perspective, our framework introduces new concepts of tissue and stem cell collaboration to mimic observed regeneration in planaria. From an algorithmic perspective, it introduces new but simple algorithms with the minimum computational burden. Specifically, it extended our previous circular tissue self-repair model to multiple tissues and integrated them into a modular regeneration framework. Additionally, we introduced an information field to stem cells where minimum pattern information collected from the tissue by the respective stem cell is stored for use in the case of complete loss of tissues or exterior borders. The other novelty is the stem cell network, represented by a neural network, that monitors the stem cell state and produces new stem cells after stem cell loss to regenerate new tissues. These tissue and stem cell network models formed a 2-Level regeneration framework for efficient pattern restoration after any damage. These advancements have made the capability of our framework far exceed that of past biological regeneration models.

To highlight and contrast the novel aspects of our framework, we make the following comparison to previous models of regeneration. Researchers in (Bessonov et al., 2015), assumed that all cells in a single tissue communicate with each other through signals that decay with distance. Each cell remembers the total signal it receives, and after amputation, remaining cells compute the difference between the current signal and old signal to stimulate regeneration of a correct cell structure using some rules of regeneration. There is a high computational burden due to extensive communications between cells to maintain the pattern and to calculate the total signal received by each cell. In our model, only the stem cell communicates with all tissue cells in a limited fashion to calculate entropy to monitor the system while tissue cells only communicate with their (≤ 4) neighbours. The tissue network (AANN) and stem cell network do limited computation as and when needed and, importantly, only the required region of the AANN is activated where the damage is as guided by the stem cell, thus keeping computation to a minimum.

In Tosenberger et al. model (Tosenberger et al., 2015), a circular tissue is regenerated by stem cells that do not get damaged. In our model, stem cells can get damaged, and the stem cell network replaces missing stem cells. In our model, the regeneration of individual tissues is different from their tissue model in some aspects. We use entropy to detect general damage region, AANN to detect the exact location and border of damage and neighbourhood rules for regeneration while they assumed that a stem cell could produce and maintain a circular tissue based on the assumption that the stem cell has a survival region. A newly produced tissue cell located beyond this region dies as it receives a signal smaller than a threshold. This also implies killing cells to achieve the required shape, which our system avoids. Moreover, their local model only applied to a simple circular tissue while our model works successfully on other shapes such as triangular and rectangular shapes and a multiple tissue system.

The model of De et al. (De et al., 2017) involves interactions between a neural network representing the nervous system of an organism and non-neural cells representing a tissue to coordinate tissue growth using physiological variables, and it has achieved reasonable regeneration success (55-80%). Limitations of this model are that it does not recognise damage and generates and kills many cells before reaching a partially recovered form. Our framework detects damage and recovers the complete form using simpler and more efficient computations. A stem cell monitors the integrity of its tissue through entropy, and the tissue maintains neighbourhood and border rules. Minimum pattern information is stored in the stem cell information fields that are accessed only in extreme damage cases; for the majority of damages, it uses cues from the remaining part for regeneration. As long as a single stem cell exists, it can regenerate the whole organism correctly, while in De et al. (De et al., 2017) model, the percentage of accuracy of regeneration decreases with the severity of the injury.

The agent-based models of (Ferreira et al., 2016; Ferreira, Scheutz, et al., 2017b; Ferreira, Scheutz, et al., 2017c) need to store a lot of information (paths of packets) in stem cells and require a lot of communication between cells in discovering cell states. The information our model carries for regeneration is high-level and generic - minimum information on shape, entropy and neighbourhood rules; therefore, it achieves efficient regeneration with minimum computation. These agent-based models are also limited when it comes to regenerating larger sized organisms due to the inability of the packets to go too far or signals decaying with distance. In contrast, our model allows correct regeneration of an organism of any size. Further, as our system can sense the changing entropy due to even a single missing cell, it can autonomously regenerate even dead or aged (senesced) cells to maintain pattern integrity.

Not many nature-inspired *form* self-repair systems exist due to the challenge in producing spare parts on demand. Most carry few spare parts which define the limit of repair capability of these systems. In biological regeneration, stem cells produce new cells on demand, and our framework has captured this feature to represent perpetual regeneration. Moreover, the 2-level framework where stem cells and tissues together maintain whole pattern homeostasis could be among the most robust and efficient self-repair systems, in general, considering the range of functionality that the framework achieves with limited information and communication. Our framework received inspiration from function repair in non-biological fields, especially with regard to accessing the internet for complex repairs in software systems (Park et al., 2005).

4.7 Conclusions

In this study, we developed a comprehensive conceptual framework for autonomous whole organism regeneration and modelled its computational dynamics that enable regeneration in a simulated biological system. It is a flexible, high-level, modular, distributed memory, neural intelligent feedback control system for dynamic morphological homeostasis under ageing and damage conditions similar to body-wide immortality in planaria. The proposed framework is very robust in returning the system to the normal state after any damage. Importantly, our model is consistent with many observations of planarian regeneration, including complete and accurate regeneration of any part or whole organism from any damage. The minimum requirement for regeneration is that only a single stem cell remains after damage. These novel features of the framework make its regeneration capability far exceed that of existing biological regeneration models that have achieved limited regeneration success on simple tissues with much greater information or computational burden. Our framework also provides a perspective on the role of local (short-range) interactions (between tissue cells), long-range interactions (between stem cells as well as between tissue and stem cells) and potential information fields (encoding minimal pattern map) in regeneration that presents a novel hypothetical paradigm for

understanding the regeneration processes. It would be of interest if biological experiments could reveal these short and long-range communications and the possibility for bioelectric or morphogenic fields to carry patterning information. The success of the model suggests the possibility for a biological system to autonomously sense, detect and repair damages completely. Our work was inspired by the body-wide immortality of planaria which is an extreme example of regeneration. As fundamental regeneration processes are generally conserved in diverse organisms, our framework could inspire potential avenues in bioengineering for regenerating damaged organs or limbs in humans and in synthetic biology for designing bio-engineered robots. Further, our framework contributes to nature-inspired computing that could advance artificial self-repair systems and robots. Considering the versatility and extent of the framework, it could be one of the most advanced and comprehensive nature-inspired self-repair frameworks that closely mimic a repertoire of biological regeneration processes within a single framework.

Although our model covers some important aspects of regeneration in biology, it still has limitations that need to be considered. First, there are only three stem cells in the proposed model that seem unrealistic for an organism. In the next stage, we plan to increase the number of stem cells to about 20% of the total cells (like in planaria). Also, AANN tissue models are perceptrons that identify the presence or absence of neighbours. In the next stage, the model will be extended by investing the neurons in the AANN with greater functionality in terms of further information processing to enhance collective intelligence and decision-making; and incorporating properties of living cells such as bioelectric signals for communication. In particular, communication within the AANN will be bioelectric via Gap-junctions. In the current format of the AANN, weights are fixed; however, with bioelectric communication, these can be trained to represent Gap junctional properties. Similarly, the stem cell network has fixed weights, and these may also be trained to represent features of long-range bioelectric communications, including A/P polarity. Further, the information field could either be formed by the bioelectric signalling within the tissue or inform a body-wide bioelectric pattern. These new features will be incorporated into the framework in the next chapter to make it more resemble the communication and regeneration processes in living systems and transform it into a framework that enables restoration of both anatomical and bioelectric homeostasis in regeneration. The aim will be to exploit the new modelling framework to provide insights into general self-recognising, self-organising and self-stabilising cell communities that are intelligent decision-making systems with an increasingly greater resemblance to living cell systems.

Chapter 5

A Comprehensive Conceptual and Computational Framework for Autonomous Regeneration and Bioelectric Homeostasis in Biological Systems

Abstract

Regeneration is a collective process whereby cells in an organism communicate to achieve an anatomical set point (target pattern of organ, body etc.). Another important aspect of regeneration is the restoration of the original function in the regenerated tissue or the organism. In this regard, restoration of body-wide bioelectric homeostasis required for regular physiological functioning of cells is an important functional requirement of regeneration. Moreover, bioelectricity is also the predominant means of cellular communication in regeneration. In the previous chapter, we proposed a conceptual framework and algorithmic structures that mimic the observed structure and capacity of regeneration in living organisms and achieves body-wide immortality, much like the planarian, and implemented it on a synthetic (*in silico*) organism.

In this chapter, we extend the framework developed in chapter 4 to enable it to fully restore bioelectric homeostasis along with anatomical homeostasis (form and function) during regeneration in a system that even more resembles planarian anatomy than our previous worm-like organism. To achieve this goal, we extend our framework in a number of ways: increasing the number of stem cells to 4% of total cells to better reflect planarian anatomy; introducing bioelectricity as the format of communication between cells; modifying the stem cell network and its operation to accommodate the increased number of stem cells; and introducing a high-level network that maintains bioelectric homeostasis. Accordingly, the proposed framework consists of three levels that cooperate to fully recover the anatomical pattern and bioelectric homeostasis in a simple simulated worm with three tissues (head, body and tail) and a greater number of stem cells (4% stem cells distributed among tissue (somatic) cells). The first level of the framework consists of three tissue models where thousands of somatic cells in individual tissues collaborate to identify its own pattern and tissue damage. Here individual tissues are represented as locally recurrent networks of perceptrons as in our previous framework but they communicate through bioelectricity. The second level is a body-wide stem cell network which is also a locally recurrent perceptron network with bioelectric communication. Due to the larger number of stem cells, this network is overlaid and distributed across tissue models, and it communicates with the tissue models to repair and maintain the whole anatomical pattern under any damage condition. The third level is a body-wide macro-level network that connects larger segments

(i.e., groups of stem cells and somatic cells) of the whole organism to restore and maintain body-wide bioelectric homeostasis. It is in the form of an Associative Memory (AM) neural network that learns the bioelectric gradient and stores the body-wide homeostatic bioelectric pattern in its attractor. The AM network uses this information to not only automatically restore bioelectric homeostasis from perturbations due to regular/normal physiological functioning but also be activated by the stem cell network under conditions of damage to restore bioelectric homeostasis upon full recovery from damage.

Using a number of perturbed bioelectric states we show that the AM accurately restores bioelectric homeostasis under normal function. Using a number of example damage cases ranging from small to medium and extreme, we show that the proposed framework is very robust in returning the system to the normal anatomical and bioelectric homeostasis after any damage. Specifically, our model is consistent with observations of planarian regeneration, including complete and accurate regeneration of any part or whole organism from any damage (body-wide immortality) and restoration of body-wide bioelectric homeostasis. Apart from the above-mentioned implications for biology, this framework could also be useful in engineering for building self-repairing and self-sustaining biobots and artificial robots.

5.1 Introduction

Regeneration in biology primarily refers to morphological processes that characterise the plasticity of the phenotype of traits that allow multi-cellular organisms to self-repair and maintain the integrity of their physiological state (function) and their morphology (form or anatomical pattern). Hydra and planarian flatworms have long been models of regeneration for their highly adaptive regenerative abilities that make them body-wide immortal (Alvarado, 2000). After being injured, their cells are activated to restore organs to their pre-existing state of anatomy and physiology. Regeneration of organs is widespread in animals such as snails, axolotls, zebrafish (Rabinowitz et al., 2017). In a related context, several animals can reproduce asexually through fragmentation (e.g., starfish, some worms, fungi, plants, lichens), budding (e.g., Yeast, Hydra) or fission (e.g., Bacteria, Protists, Unicellular Fungi) (Brockes & Kumar, 2008). For example, a planarian mother will narrow, split in the middle, and each half creates a new head or tail to form two copies of the original. The rationale of how animals are able to reproduce and maintain their physiological state (e.g., bioelectric) and morphology (pattern) remains an open question. There is a need for testable models to explain what methods are used to maintain the correct physiological state and morphology of the organisms.

A 6 mm long planarian (as shown in Figure 5.1) has approximately 0.6 million cells (Takeda et al., 2009) where adult stem cells comprise about 20 to 30% of all cells and are distributed throughout the planarian body (Ivankovic et al., 2019). A single stem cell introduced after damage can reproduce new stem cells that regenerate the whole pattern in an irradiated animal where no stem cells were remaining without the possibility of regeneration (Wagner et al., 2011a). With such amazing observations, the discovery of the mechanisms and algorithms of regeneration in organisms has attracted the interest of many researchers, but many questions about regeneration still await answers.

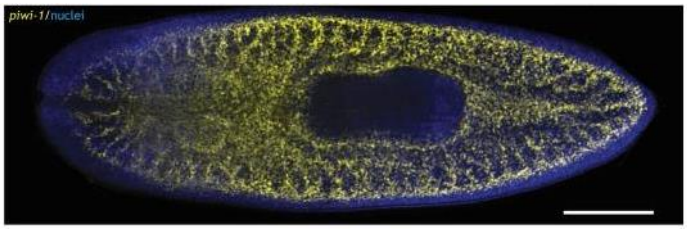


Figure 5.1. Planarian stem cell system. The distribution of stem cells (yellow). Scale bar: 500 µm. (Sourced from (Ivankovic et al., 2019) with permission)

Repair of damaged organs or even replacement of missing limbs in humans may benefit from the understanding of how regeneration happens in other species. In particular, understanding complete mechanisms and algorithms of regeneration are very important because it is not enough to produce new cells only, but it is necessary to restore the correct anatomy (size, shape) and function of the organs and the whole organism. For example, many physiological functions in an organism are

supported or coordinated by bioelectricity (McLaughlin & Levin, 2018). Therefore, maintaining body-wide bioelectric states after regeneration is important. However, we do not yet understand the mechanisms that coordinate cells into tissue and organ organisation in regeneration and how bioelectricity modulates these responses as well as how bioelectric equilibrium (homeostasis) is restored by an organism after regeneration. This work aims to propose a high-level conceptual framework for the regeneration of a simple organism where stem cells and tissue cells communicate with each other through bioelectric signals in restoring both anatomical and bioelectric homeostasis. Currently, no computational models of bioelectric maintenance and restoration exist.

As shown in Chapter 1, planaria are also capable of identifying anterior-posterior (A/P) (along the body) and dorsal-ventral (D/V) (across the body) polarity so that a small piece of planaria can locate the new head and tail correctly as in the normal planarian (Figure 1.1A) (Lobo et al., 2012). It has been shown that bioelectric signalling can carry anterior-posterior polarity and patterning information in planarian regeneration (Beane et al., 2011; Nogi et al., 2005). However, how the remaining cells, in a worm split into two segments, for example, re-establish polarity in the two segments in the process of regeneration is still unclear. It is plausible that bioelectric signals communicated through GJs and ion fluxes are controlled by as yet unknown mechanisms that promote collaboration within the cell collective to maintain the correct polarity, geometry and bioelectric pattern of the planaria.

In the previous chapter, we raised some fundamental questions about regeneration and provided hypothetical answers to them. These questions included: How do stem cells and tissue cells corporate to repair local damage? How do stem cells work together in large-scale repair? How do stem cells know what to recover and when to stop? Where is such morphological information stored? How do stem cells share morphological (pattern) information? And importantly, how does a small body fragment regenerate into a fully formed organism with correct polarity? In our previous chapter, we provided a framework to address all these issues as a collective intelligence problem in achieving complete and accurate regeneration of the anatomical structure. This framework was based on simple binary communication between cells indicating their presence or absence and a simple stem cell arrangement where there was only one stem cell per tissue. Further, it was not designed to restore the original function after regeneration. In this chapter, we address the issue of making the framework more biologically realistic with respect to planarian anatomy with a larger number of stem cells, bioelectricity as the form of communication between cells, and restoration of bioelectric homeostasis. This raises some new questions: how does a large number of stem cells dispersed through a tissue (as in Figure 5.1) accomplish regeneration in collaboration with tissue cells? What communication structures are involved in this process? How does a cell collective maintain bioelectric homeostasis in the form of a body-wide voltage gradient? And how does the body restore bioelectric homeostasis under normal perturbations due to physiological functioning as well as in regeneration?

In order to progress towards a holistic understanding of regeneration, this study aims to develop a conceptual framework for an autonomous regeneration system- incorporating what is known and what is required - that recovers from any damage to accurately restore the original anatomical pattern (pattern homeostasis) and body-wide bioelectric pattern (bioelectric homeostasis) to support the continuation of physiological functioning- resembling the body-wide immortality in planaria even more fully than our previous framework. In the new framework, developed to capture some essential aspects of planarian regeneration including body-wide immortality, a change in the bioelectric state due to either normal physiological functioning or damage triggers appropriately mechanisms of regeneration as well as mechanisms that restore bioelectric homeostasis, as demonstrated on a simple artificial (*in silico*) worm-like organism. The framework could serve as a platform for advancing hypotheses to better understand the mechanisms and algorithms of regeneration. It could also promote advancements in regenerative medicine in solving biomedical problems such as congenital disabilities, injury from trauma, cancer and ageing (Levin, 2011). Further, it could contribute to bioinspired computing for engineering self-repairing biobots, artificial robots and synthetic living machines (Doursat, 2013; Doursat & Sanchez, 2014; Doursat et al., 2012, 2013; Fernandez et al., 2012; Kamm et al., 2018).

A novel aspect of our proposed framework is concerned with the maintenance of the endogenous body-wide voltage gradients (bioelectric homeostasis) under normal perturbations of V_{mem} due to physiological functioning as well as its restoration after physical damage. The other part of the framework is concerned with the mechanisms of cell communications through bioelectric signalling to recover the exact original geometric pattern (pattern homeostasis). In the current research, our previous framework is brought even closer to the cellular makeup and organisation of planaria and integrated with the new aspect of bioelectric homeostasis so that the integrated framework autonomously maintains pattern and bioelectric homeostasis under any small or large disturbance.

5.2 Objectives

The aim of this chapter is to present a new conceptual framework that greatly extends the previous framework in chapter 3 to provide a basis for both pattern and bioelectric homeostasis in regeneration for an organism containing a larger number of stem cells. Specifically, the new framework addresses all the above questions and proposes some new mechanisms and algorithms to mimic planarian regeneration involving communication between a large number of stem cells and their local tissue cells using bioelectric signals to maintain both pattern and bioelectric homeostasis under any perturbation or damage.

5.3 Summary of the framework

The new framework consists of three levels that cooperate to fully recover the anatomical pattern and bioelectric homeostasis in a simple simulated (*in silico*) worm-like shape with three tissues (head, body and tail). Stem cells, which account for 4% of total cells, produce tissue cells in damage recovery. The first level consists of three tissue models where a large number of somatic cells in individual tissues collaborate locally to identify their own tissue pattern and damage. The second level is a body-wide stem cell network. Both tissues and stem cells are represented as locally recurrent networks of perceptrons that communicate through bioelectricity. These two networks collaborate to repair and maintain the whole anatomical pattern under any damage condition. The third level is a body-wide macro-level network that connects segments (i.e., groups of stem cells and somatic cells) of the whole organism to restore and maintain body-wide bioelectric homeostasis. It is in the form of an Associative Memory Network (AMN) that learns the bioelectric gradient and stores the body-wide homeostatic bioelectric (voltage) pattern in its attractor. AMN uses this information to automatically restore bioelectric homeostasis from perturbations and after full recovery from damage.

In the next section, we describe the new conceptual framework followed by its computational structures in the subsequent two sections. We then present the implementation of the framework for bioelectric restoration and pattern regeneration in a simple worm-like structure under small to large damage conditions. The last two sections are devoted to a discussion and conclusions.

5.4 Conceptual framework for a regeneration system that maintains both pattern and bioelectric homeostasis

In this work, we extend the previous framework with several new features to greatly enhance its capabilities for collective decision making in autonomous regeneration as in planaria. As in the previous work, we hypothesise that the system is capable of maintenance and regeneration of the whole pattern. Damage can occur anywhere and be of any size from a few cells or tissues to the almost entire organism leaving only a small fragment. In the new framework, however, with a large number of stem cells, regeneration requires an even more flexible framework and effective algorithms to recover from any damage. Besides the pattern regeneration in this new condition, the system is also able to self-regulate the bioelectric state of homeostasis. Our aim is to propose a conceptual and computational framework for regenerating the entire pattern even from a small fragment and subsequent restoration of bioelectric homeostasis that more closely mimic regeneration in living planaria in nature.

Our new system contains the following new extensions. First, we assume that the organism maintains a longitudinal bioelectric gradient from head to tail and a transverse bioelectric gradient across the body. Briefly refer to Figure 5.5 for a view of the bioelectric (voltage) pattern across our model

organism where colours indicate the magnitude of the membrane voltage of the corresponding tissue cells. Bioelectric pattern refers to this distribution of membrane voltage of tissue cells across the whole organism and living planaria continuously restore this bioelectric pattern as it is vital to the proper continuation of functions that sustain its life. Second, the organism consists of a larger number of stem cells (4% of total) distributed throughout the body. Figure 5.1 shows the distribution of stem cells in a living planarian. Third, all communication is according to the body-wide bioelectric gradient. Three simple communication formats exist in the framework, as shown in Figure 5.2 that demonstrates an example local neighbourhood of stem cells (we discuss this figure in detail later in this section). Specifically, tissue cells communicate with each other locally and directly through physical Gap junctions (GJ). Stem cells communicate with (neighbour) somatic cells through ion fluxes released to the environment. This is because there are no fixed GJs between somatic cells that are mobile and migrate on demand to various damage sites in the tissue. Stem cells communicate with each other long-range and indirectly through intermediate somatic cells that carry through their GJs ion fluxes released to the environment by one stem cell and released into the environment of the receiving stem cell. Importantly, due to the prevalence of stem cells in a tissue, communication of stem cells with tissue cells, in general, is limited to the local neighbourhood (i.e., signals travel through only a few somatic cells via GJs before a stem cell receives this information through ion fluxes released from its neighbour somatic cell). Therefore, in the new framework, stem cells do not employ the concept of entropy to maintain tissue integrity. However, they do employ the information field.

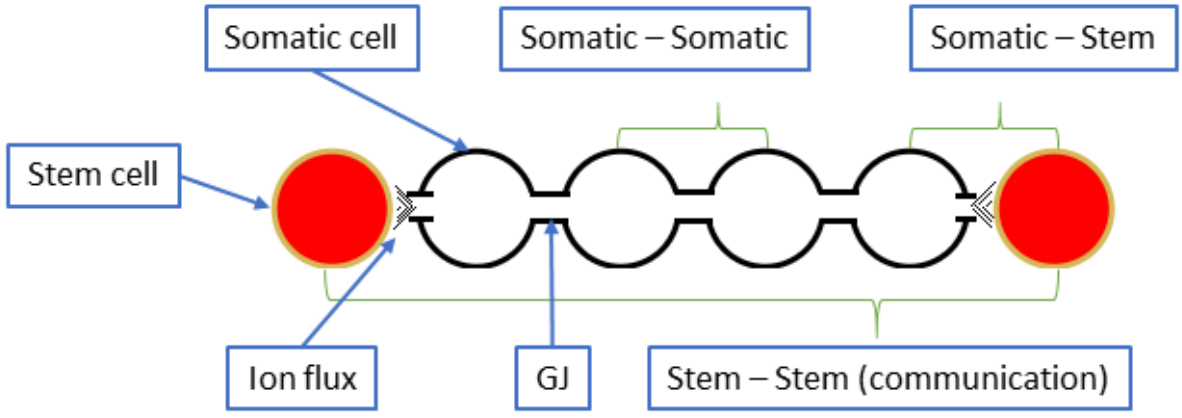


Figure 5.2. Three types of communication between cells (red and white cells are stem cell and somatic cells, respectively): Communication between somatic cells through GJs; between stem cell and somatic cell through ion fluxes; between stem cells through GJs of intermediate somatic cells and ion fluxes.

We present the framework in two parts: conceptual basis and Functional description (Figure 5.3). In part one (in Section 5.4.1), we elaborate on the conceptual basis of the framework shown in Figure Figure 5.3A. Main elements of the framework are presented in Table 2 and they are shown in full view in Figure 5.4 and described in detail in this section. In part 2 (section 5.4.2), we elaborate on the

functional description of the framework shown in Figure 5.3B with algorithmic/computational structures as it applies to a simple synthetic worm containing head, body and tail tissues.

5.4.1 Conceptual basis and organisational view of the framework

To accommodate the above forms of communication and maintain bioelectric homeostasis, we introduce three new concepts in the framework: somatic cell network, stem cell network and an Associative Memory Network (AMN) - that communicate through bioelectricity as an integrated whole (Figure 5.3A). These are expanded in Figure 5.4 to show the networks in detail and elaborated in Table 2, showing the configuration, properties and activities of these networks. Specifically, it proposes a high-level (macro-level) body-wide associative memory neural network as a mechanism for maintaining and restoring voltage homeostasis (Level 3 in Figure 5.4). This is a neural network trained to retain the body-wide bioelectric gradient in its attractor state. Purpose of AMN is to: (1) produce a simple model that maintains the body-wide bioelectric pattern; (2) be a flexible and fault-tolerant system, without excessive micromanagement of bioelectric state. Since the total number of cells is very large (about 3750 cells), an effective way to represent them in a flexible and fault-tolerant way is to group the cells into clusters, each consisting of a group of stem cells and surrounding somatic tissue cells, and use clusters as nodes of the AMN (Figure 5.4E).

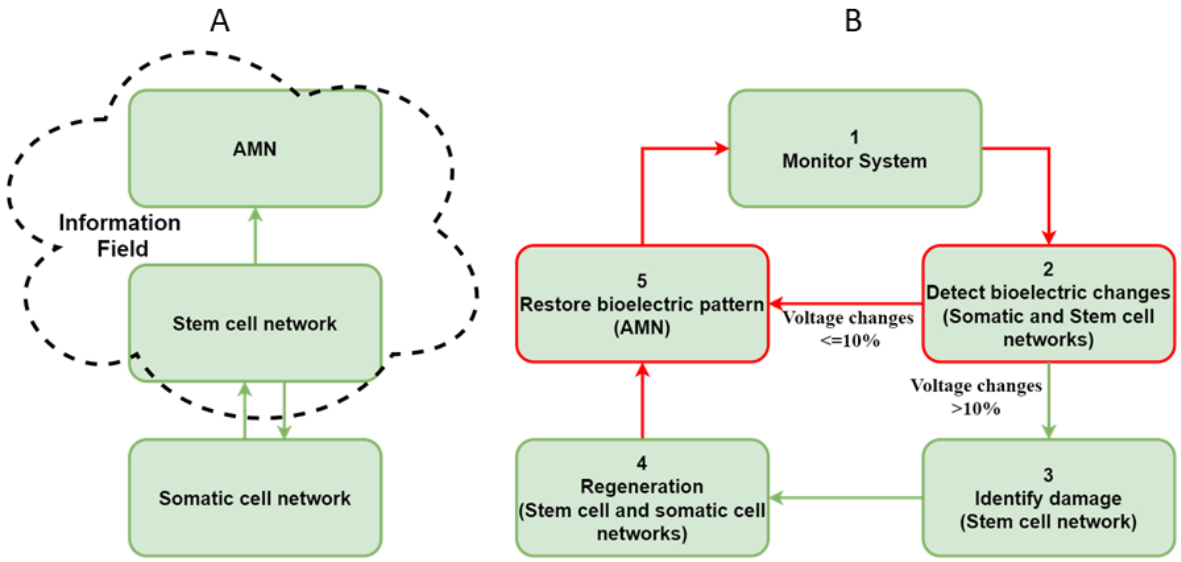


Figure 5.3. Overview of the framework for anatomical and bioelectric homeostasis. (A) Conceptual basis involving the organisation of three networks. (B) Functional aspects of the framework.

For pattern regeneration, the framework employs somatic cell networks (one for each tissue) and a stem cell network. Stem cell network is the driver or engine of regeneration. Specifically, we design regeneration mechanisms through bioelectric communication between a large number of stem cells

and somatic tissue cells. In particular, stem cells in this new framework form an extensive network distributed throughout the organism (Figure 5.4B). Both stem cell and somatic cell networks (Figure 5.4C) are represented by perceptron networks with only local neighbourhood communication (Table 2 column 2&3). This drastically reduces the amount of computation carried by the system in self-repair. Further, as the voltage varies along and across the organism but there are only a few patterns of perceptron communication (interior, border etc.), we introduce motifs (repeating patterns) for communication within the somatic cell networks and stem cell network (Table 2 bottom of columns 2&3). These motifs, presented in detail in the next section, further reduces the computational burden. As can be seen, we retain the configuration of the somatic cell network (Figure 5.4C) as in the previous framework, but communication now is through bioelectricity. The configuration of the stem cell network is new; in the previous framework, there were only three stem cells, and they formed a fully connected network of linear neurons. The new stem cell configuration is a network of a large number of only locally communicating perceptrons.

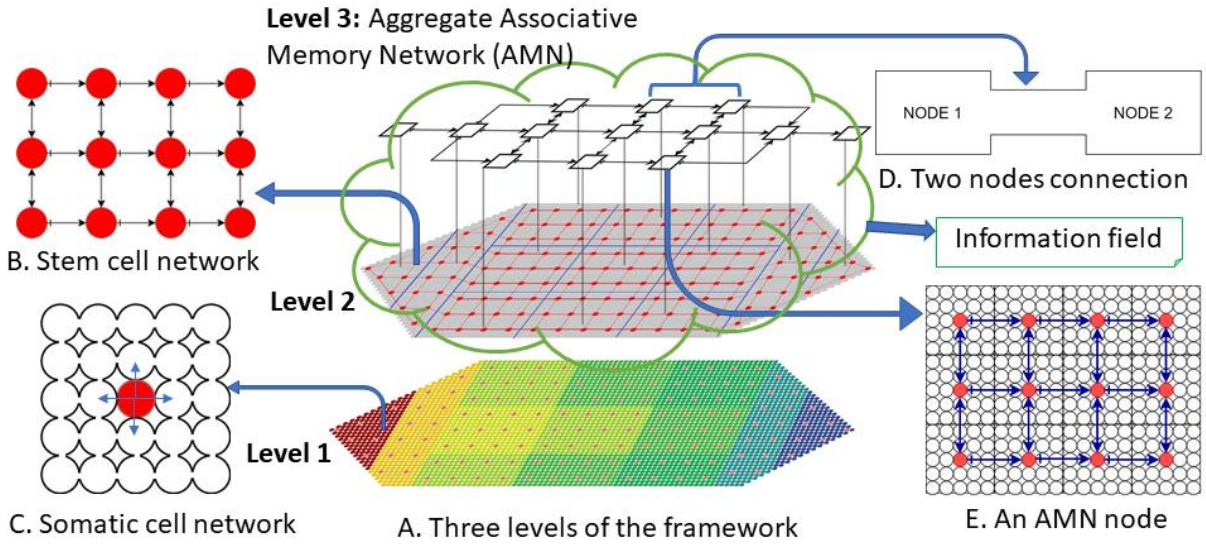
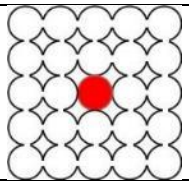
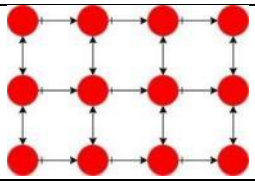
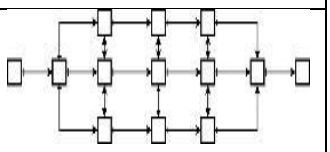
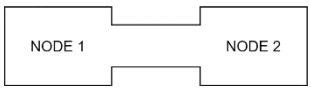
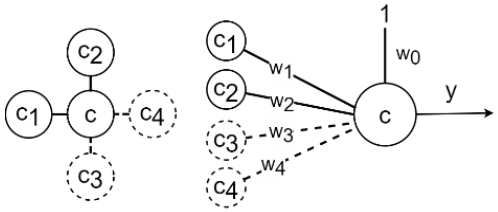


Figure 5.4. Detailed view of the framework for anatomical and bioelectric pattern homeostasis. (A) Three levels of the framework: Level 1- somatic cells structure (bottom – colours indicate voltage and there are about 150 stem cells (red dots) distributed through the organism and 3750 somatic cells surrounding stem cells (tiny dots filling the whole organism)). Level 2- stem cell structure (middle) with information field and Level 3- global nodal structure of AMN (top), that communicate together to restore pattern and bioelectric homeostasis. All three levels form computational networks. (B) Stem cell network showing connections between stem cells. (C) Somatic cell network in a segment of a tissue consisting of a stem cell surrounded by somatic cells. These segments repeat to form the head, body and tail tissues that make up the whole pattern. (D) Two nodes of the AMN with a connection that represents four gap junctions connecting the somatic cells in respective nodes. (E) Detailed view of an AMN node that comprises a segment of a tissue (square blocks) with about 300 cells- stem cells (red dots) and somatic cells (white dots). Stem cells communicate with their neighbours by indirect interactions through GJs of somatic cells

We keep some additional features of our previous framework presented in Chapter 3. First, somatic cells store neighbourhood rules which help them assist the stem cells in regeneration. These neighbourhood rules refer to local neighbourhood geometry and are the number of neighbours of interior cells, border cells, and corner cells. In the current model, we assume that stem cells also keep simple neighbourhood rules similar to somatic cells (i.e., rules for border, corner and interior cells). Second, stem cells store some minimum pattern information for the tissues in the information field for the case where all exterior boundaries are gone without leaving any traces of the original pattern boundary. For the example case of our simple worm, the stored pattern information contains information for the 3 tissues: length of the body tissue (d), aspect ratio (AR) (length to width ratio) of the head, body and tail (AR=1, 3, 1), and the number of corners of the head, body and tail (n=3, 4, 3).

Table 5.1. The three cell networks in the framework and their structure, properties and activities

<table>
<tr><th></th><th>Somatic Cell Network</th><th>Stem Cell Network</th><th>AMN</th></tr>
<tr><td>Network Structure</td><td>  </td><td>  </td><td>  </td></tr>
<tr><td>Network Type</td><td>Perceptron network with local communication</td><td>Perceptron network with local communication</td><td>Auto-Associative (Associative Memory) network with local communication</td></tr>
<tr><td rowspan="2">Communication</td><td>Somatic – somatic cell (bioelectric, directly through GJs)</td><td><u>Stem – stem cell</u>
(bioelectric, indirectly through somatic cell GJs and ion fluxes released to the environment.
(i.e., stem cell i ->fluxes-> GJs -> fluxes -> stem cell j)

<u>Stem – somatic cell</u>
(bioelectric, through somatic cell GJs and ion fluxes released to the environment
(i.e., somatic cell i -> GJs -> fluxes -> stem cell j)</td><td>Node – Node (directly through node connections</td></tr>
<tr><td>  </td><td> 
</td></tr>
<tr><td>Variables</td><td colspan="2">Cell Status (damaged or not)
Cell Voltage</td><td>Node Voltage
Network Weights</td></tr>
<tr><td>Properties</td><td colspan="2">Three neighbourhood rules for cell location (interior – 4 neighbours, border – 3 neighbours, corner – 2/3 neighbours)
Three perceptron communication Motifs (for 2, 3, or 4 neighbours) trained to represent the three neighbourhood rules for identifying the presence or absence of neighbours (i.e., damage). Solid circles represent one motif and the other two are obtained by adding dashed circles one by one. w_i represent weights of the motifs.
 
</td><td>-Whole Bioelectric pattern
-Communication through Network Weights</td></tr>
<tr><td>Information field</td><td></td><td>Minimum tissue pattern information (d, AR, n)</td><td>Bioelectric pattern stored in the attractor</td></tr>
</table>

5.4.2 Functional overview of the framework

The three neural networks operate collectively in sensing damage, detecting where the damage is and what is missing, and repairing the damage and finally restoring bioelectric state as presented in the high-level view of the system in Figure 5.3B. The logic of the framework is highlighted in five States that operate in two modes. The first mode is about restoring bioelectric homeostasis under normal physiological fluctuations in the undamaged organism where the system monitors (State 1), detects bioelectric (membrane voltage) changes (state 2) and if the change is smaller than a threshold (10%) that is indicative of no damage, then restores bioelectric state using AMN (State 5). The second mode is about damage repair followed by restoration of bioelectric homeostasis. If the voltage change is greater than the threshold, this indicates damage and the framework activates stem cell network for damage identification (State 3) upon which stem cell and somatic cell networks together repair the damage (State 4) and subsequently, the bioelectric pattern is restored by the AMN (State 5).

In order to prepare the framework to perform the above functions, the components in it need to be invested with the capability to do so as follows. The somatic cell network recognises the intact pattern of individual tissues of the organism through local bioelectric communication (using neighbourhood rules for the border, corner, and interior cells). The AMN gets trained to store the bioelectric homeostasis pattern in the attractor. The stem cell network also recognises its pattern using neighbourhood rules for the border, corner, and interior cells, similar to somatic cell networks. Details of the preparation and the operation of the three networks are presented in Section 5.5. At the end of this, the system monitors its pattern and bioelectric state (State 1). When there is a voltage change, it is first sensed by the somatic cells, and the information quickly reaches the neighbouring stem cells due to the close proximity of somatic cells to stem cells. The nodes of the affected AMN thus also sense the change as the voltage of an AMN node is the average voltage of all the cells that constitute it. This way, all three networks of the system sense the local changes in the bioelectric state triggers one or the other of the two modes of operation described above. Next, we elaborate on the operation of the network through the 5 States after sensing bioelectric changes.

a) Bioelectric restoration under the normal physiological function

When there is a perturbation in the bioelectric state, the first task of the system is to check if the voltage change is due to variations under normal physiological functioning or damage (State 2). This decision is made by the stem cell network as the engine of regeneration. For damage cases, the change in voltage in somatic cells which are nearest to the damage can be above 10%. We assumed an increase of 10% in normal operation as in (Reid, Song, McCaig, & Zhao, 2005). In State 2, the somatic cells that have experienced a change in their voltage send this information to other cells through GJ and the

surrounding environment (ion fluxes). The action of sending information to the outside of a cell corresponds to the release of ion and chemicals into the environment. The stem cells receive this information and decide whether the change is due to damage or not based on the threshold. Using the stated threshold voltage level (set at ±10%), stem cells determine whether the change is due to damage or not. If there is no damage, the stem cell network informs the normal state to the AMN when the voltage changes are below 10%. Then, the AMN works autonomously to restore the bioelectric homeostasis pattern in the organism by iteratively reaching the stored attractor pattern (State 5).

b) Anatomical pattern and bioelectric restoration under damage

When there is damage (indicated by >10% voltage change), the stem cell network informs the presence of the damage to the AMN. The AMN holds on while the stem cell network identifies whether stem cells also have received damage (State 3). The stem cell network with the knowledge of its pattern checks for and detect any stem cell damage and restores the lost stem cells. The somatic cell networks with the knowledge of their tissue patterns check for and detect the damage location and boundary and assist neighbouring stem cells that migrate to the damage location to regenerate the exact pattern. This way, somatic cells and stem cells corporate with each other to maintain the whole pattern from any damage (Step 4). For extreme damages involving the loss of all exterior perimeters of tissue(s), stem cells access the information field and get the basic pattern information to recover the original pattern. After regenerating, the AMN is reactivated by the stem cell network to update the voltage in cells in the restored region and restore the bioelectric pattern of the organism (State 5) upon which the organism returns to the normal anatomical and bioelectric homeostasis (State 1). With the AMN, we introduce the possibility of maintaining the bioelectric state as a global system property that represents the normal physiological or functional state of the organism.

This framework presents a system that mimics planarian regeneration from various damages from simple (single-cell damage) to complex large scale tissue damages and regenerates from even a tiny fragment with a single stem cell left after damage. Further, the proposed framework is robust with high flexibility, adaptability and fault tolerance as in real planaria. In the next section, we present the functional details of the framework.

5.5 Computational (algorithmic) structures of the framework for regeneration and bioelectric homeostasis

In this section, we present the algorithmic structures that constitute the functional aspect of the framework. It presents the model organism and its innate bioelectric state, somatic cell networks (tissues) and their operation, organisation of stem cells and stem cell network operation, and the

structure and training of the AMN for restoring bioelectric state. Finally, how these 3 levels are combined into an integrated framework is presented.

5.5.1 The model worm and its original geometric pattern and bioelectric state

The model artificial organism is a simple worm with head, body and tail tissues consisting of stem cells and somatic cells (Figure 5.5A). There are about 150 stem cells (red dots) distributed through the organism and 3750 somatic cells surrounding stem cells (tiny dots filling the whole organism) (shown with details in Figure 5.4). The organism consists of repeated patterns (square blocks) of stem cell-centred somatic cell neighbourhoods of size 5 × 5 (Figure 5.4C) (24 somatic cells per stem cell). Arrows in Figure 5. 4C show the closest neighbours of the stem cell. The colours in Figure 5.5A show the body-wide bioelectric gradient indicating a decreasing cell membrane voltage from head to tail and from mid-body to border regions. A/P and D/V indicate the two main axes, Anterior-Posterior and Dorsal-Ventral, respectively (Figure 5.5B).

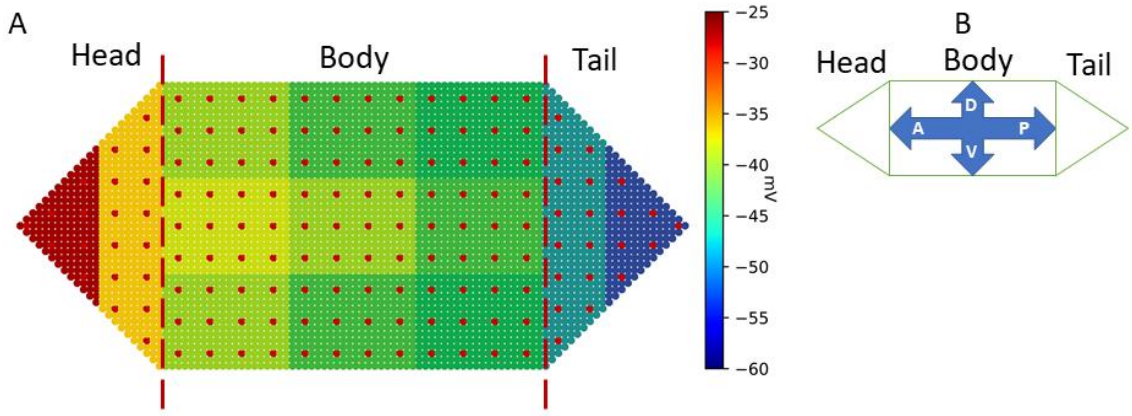


Figure 5.5. Planarian structure, innate bioelectric pattern and body axes. (A) Structure of the artificial model organism in the form of a simple worm – stem cells are denoted by red dots distributed throughout the organism; others are somatic cells (tiny dots filling the whole organism) with different colours indicating their membrane voltages. Colours indicate the voltage gradient along the length and across the body. Voltage increases from tail to head and decreases from mid-body to exterior regions. (B) The two main axes of the planarian structure: Anterior-Posterior (A/P) and Dorsal-Ventral (D/V).

5.5.2 Structural and functional integration of the framework

The proposed conceptual framework integrates three structural levels of the organism - somatic cell structure, stem cell structure and the global cluster/node structure of the Associative Memory Network (Figure 5.4A)- and their mechanisms of interaction to restore anatomical pattern and bioelectric homeostasis. It represents these structures and mechanisms within a neural computing paradigm as follows:

At the bottom level (bottom image of Figure 5.3A and Figure 5.4C), somatic cells form a network with local neighbourhood communications. It is represented by a perceptron neural network with local interactions for all three tissues of the organism. Each cell only communicates with adjacent cells that form a distributed memory of the location and bioelectric state in a local sense.

In the middle level (middle image of Figure 5.3A & Figure 5.4B), the stem cell network – the engine of regeneration- also forms a distributed memory network and is represented by a perceptron neural network with local interactions and spanning the whole organism. This network combines individual stem cells into a network for identifying and regenerating both missing stem cells/whole tissues and somatic cells.

At the top level (top image of Figure 5.3A and Figure 5.4D&E), global (aggregated) nodes form a network represented by an Associative Memory neural Network (AMN) that recognises the body-wide bioelectric state of the nodes.

The information field surrounds the organism and is accessed by the stem cells and AMN.

The high-level view of the framework with five major states was depicted in Figure 5.3 in the previous section. Here we present the operational aspects of the framework corresponding to these five states in terms of how it achieves complete regeneration and bioelectric restoration through the operation of the three networks: stem cell network, somatic cell network and AMN.

5.5.3 Algorithms of regeneration in the framework

In this section, we discuss the specific algorithms of computation in the three levels of the framework. We start with the somatic (tissue) cell networks (Level 1) followed by the stem cell network (Level 2) and identification of tissue and stem cell damages by these networks. Then we present the global level (Level 3) where the Associative memory network maintains bioelectric homeostasis.

A. Level 1: Somatic cell network (Perceptron network with local communication)

The somatic cells form a network with local neighbourhood communications (as shown in Figure 5.4C, extracted from Figure 5.4C and presented below for clarity). Table 2 also presented this format of the network). As stated before, it is represented by a perceptron neural network with local interactions for all three tissues of the organism. Each cell only communicates with adjacent cells that form a distributed memory of its location (interior, border etc.) and bioelectric state in a local sense. In our framework, cells communicate through bioelectricity, for which voltage is used as a proxy (Figure 5.2). It means somatic cells communicate directly with adjacent somatic cells through electrical synapses (GJs through which ions flow between adjacent cells) and they communicate with stem cells through ion fluxes released to the environment by the somatic cells adjacent to stem cells.

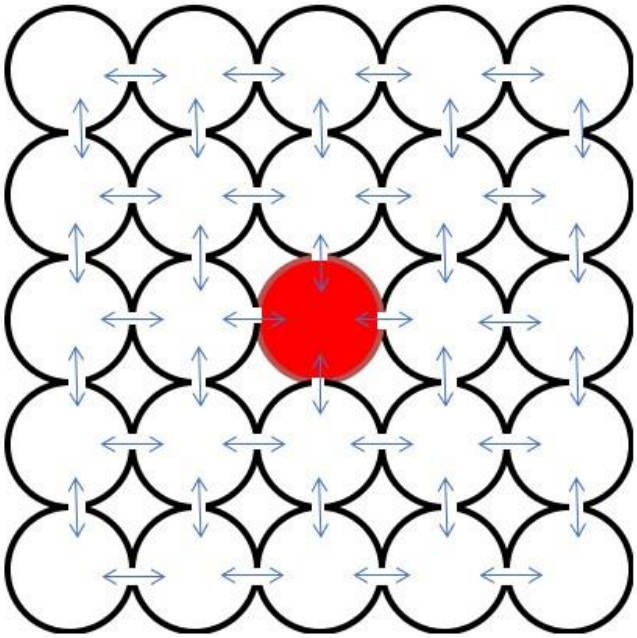


Figure 5.4C Somatic cell network (extracted from Figure 5.4)

The somatic cell network recognises its structure (and the damaged state) through structural motifs (Figure 5.6). Each somatic cell has either 2, 3 or 4 neighbours depending on its location in the stem cell network (interior, border or corner) as in our previous framework. It means that a somatic cell receives 2, 3 or 4 inputs from its neighbours. This results in three communication motifs in the somatic cell network, as shown in Figure 5.6 and described below with the respective Eqns 5.1 to 5.3. Table 2 also provided the format of these motifs. We call these motifs because they can be applied to all (corner, border, interior, respectively) somatic cells regardless of where they are in the organism. As voltage varies throughout the body, only the inputs need to be standardised to 0 or 1 before presenting to the corresponding motif. As the voltage is negative throughout the system (See Figure 5.8), an input of 1 refers to the presence of a negative voltage and 0 to the absence of any voltage.

The three perceptron motifs are:

Motif 1: Two inputs [c_1, c_2] are normal when [c_1, c_2] are non-zero values, and other input combinations indicate damage.

$$y = \begin{cases} 1 & if\ c_i > 0;\ i = 1,2 \\ 0 & otherwise \end{cases} \tag{5.1}$$

Motif 2: Three inputs [c_1, c_2, c_3] are normal when [c_1, c_2, c_3] are non-zero values, and other input combinations indicate damage.

$$y = \begin{cases} 1 & if\ c_i > 0;\ i = 1,2,3 \\ 0 & otherwise \end{cases} \tag{5.2}$$

Motif 3: Four inputs [c_1, c_2, c_3, c_4] are normal when [c_1, c_2, c_3, c_4] are non-zero values, and other input combinations indicate damage.

$$y = \begin{cases} 1 & if\ c_i > 0;\ i = 1,2,3,4 \\ 0 & otherwise \end{cases} \tag{5.3}$$

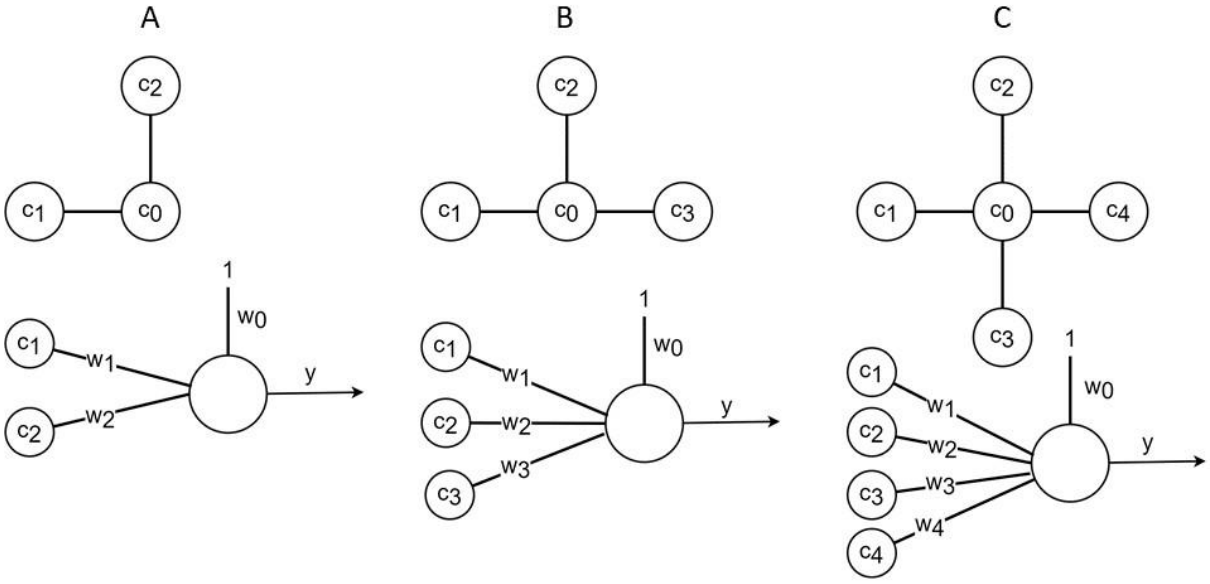


Figure 5.6. Perceptron motifs. Three communication motifs (A, B and C) depending on the number of neighbours 2, 3 or 4, respectively, in the somatic cell network.

In the normal state, each somatic cell knows the number of neighbours (neighbourhood rules extracted from the motifs). The inputs to the perceptron are the voltage of the neighbour somatic cells communicated via GJs; if the voltage is not zero, then the input is one; otherwise, it is zero. For example, in motif 2 below, a somatic cell receives three signals from neighbours in the normal state, but when this somatic cell receives only two signals, then the third input value is zero.

Unlike in our previous framework, here we allow somatic cells to learn to recognise their neighbours dynamically through the training of perceptrons. For training, each perceptron motif starts with random weights, as depicted in Figure 5.6. These weights reflect the electrical conductance of Gap Junctions between somatic cells in a real planarian tissue. Bias weight stabilises the outputs. Inputs 0/1 represents presence or absence of somatic cells based on their voltage. The motifs were trained using Hebbian rule until convergence to a stable state such that each motif can classify whether a neighbour is normal or damaged. The training was quick and completed successfully and the weights for the three motifs are presented in Table A1 in Appendix A.

Tissue damage identification by the somatic cell network

In the damage state, individual somatic cells, which are neighbours of damage cells, sense the damage through a change in bioelectricity and identify damage through the corresponding motif. The set of somatic cells which recognise the missing neighbours becomes the border of damage. This way, the somatic cell network can identify borders of any damage. To illustrate this, refer to Figure 5.7A showing a small tissue damage with a missing stem cell (black square). Red dots are stem cells and green dots are somatic cells with a specific voltage (voltage scale was given in Figure 5.5). (For now, please refer only to the tissue damage part (green dots) of this figure; other aspects shown in it will be discussed in the next section). Due to the damage, tissue cells next to the damage receive an increase in voltage (yellow cells indicated by the blue arrow in Figure 5.7A). Applying the motifs, these tissue cells in the somatic cell network identify that their neighbours are missing and they become the damage border of the tissue.

B. Level 2: Stem cell network – Operation of the regeneration engine

The stem cell network structure is shown in Figure 5.4A&B (extracted and presented below for clarity). It is represented by a locally interacting Perceptron network similar to the somatic cell network and is activated by sensed voltage signals exceeding the threshold communicated through somatic cells in damage. As stated before, stem cells communicate with neighbour stem cells through somatic cells by means of a combination of direct connections through GJs of intermediate somatic cells and indirect connections through ion fluxes released in the neighbourhood of somatic and stem cells (Figure 5.2). Further, these stem cell communications are either activation (+) or inhibition (-) signals depending on their position (from posterior to anterior cells inhibit neighbours, from other directions they activate neighbours as shown in Figure 5.4B. (This activation and inhibition pattern follows the optimum pattern of nodal interactions found for the AMN network that is described in detail in the next section. As stem cells are within AMN nodes, stem cells were made to follow the same interaction format of the AMN). Stem cells communicate with each other by activation of the stem cell network only when needed (e.g., in damage). Stem cells also communicate with somatic cells as described in the previous section. Depending on the information received by stem cells, it can trigger stem cells to do specific actions.

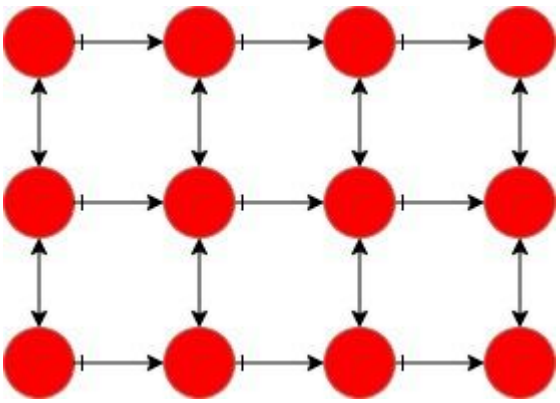


Figure 5.4B. Stem cell network and interactions (extracted from Figure 5.4). Blunted arrows denote negative (inhibitory) signals and solid arrows denote positive (activation) signals. Signals from left to right or tail to head direction are negative (inhibitory) and signals in all other directions are positive (activation)

The stem cell network recognises its structure (and the damaged state) through three perceptron motifs with the same structure and weights as in the somatic cell network. The inputs to the perceptron are the voltage of the neighbour stem cells communicated via somatic cell GJs and ion fluxes released into the environment to be picked up by stem cells; if the voltage is negative (e.g., neighbour exists) then the input is one; otherwise, it is zero.

Stem cell and large scale tissue damage identification by the stem cell network

As the driver of regeneration, the stem cell network is the primary entity in directing damage detection. When some stem cells in the stem cell network sense increased voltages beyond the threshold, it has to first identify if there is stem cell damage, and if so, whether it is large scale global damage involving completely missing head, body etc. (as in Figure 5.7C) or less destructive damage (local damage) involving one or many stem cells and associated tissue damage (as in Figure 5.7A).

Basically, it has to identify the extent of damage to the stem cell network and from this figure out the type of damage to the organism. To establish if stem cells have been damaged, the stem cell network applies perceptron motifs shown in Figure 5.6 to the cells that experienced changes to their voltage status sensed through GJs of the intermediate tissue cells. If all affected stem cells report no missing neighbour stem cells, then the damage involves local tissue only without stem cell damage. In this case, the somatic cell network identifies the border of the damage in the affected tissue(s) (as described previously for the tissue damage example in Figure 5.7A) and the nearest stem cell migrates to the damage border and repairs it. This damage does not involve tapping into the information field as relevant pattern information is contained within the remaining tissue.

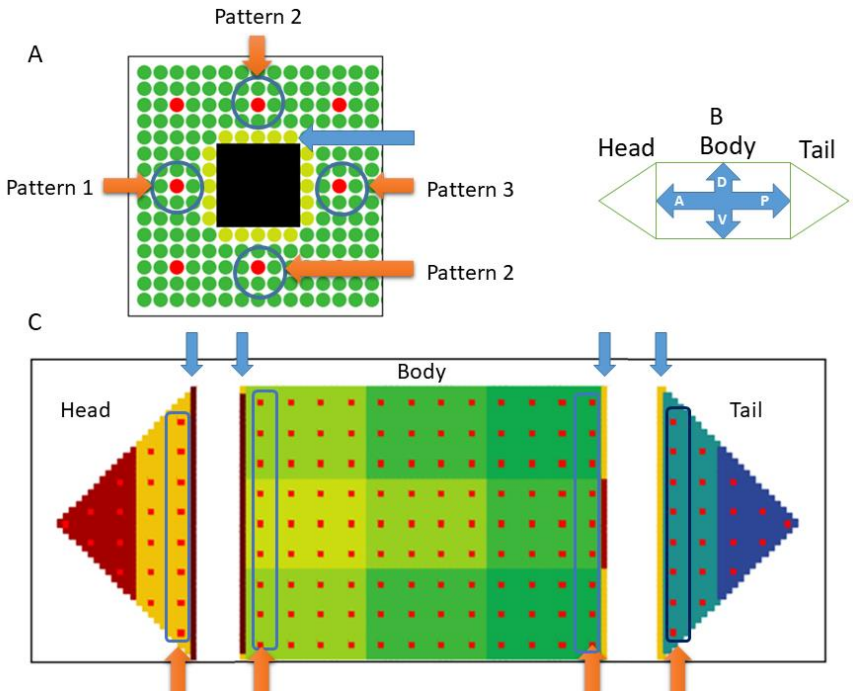


Figure 5.7. Examples of damage. (A) A stem cell with its tissue damage. (B) The A/P and D/V direction polarities in the worm (C) Large damage cases – head, body, and tail missing completely. In A and C, red dots are stem cells; other colours are somatic cells with corresponding voltage. Orange arrows indicate the patterns of stem cells in the damage border identified by the stem cell network ; Blue arrows indicate the border of tissue damage identified by the somatic network

As for detecting damage to stem cells, although forms of damage can be many, the stem cell network uses few specific rules for detecting all types of damage. These rules are based on the nature of the pattern of the damage border created by neighbours of missing stem cells in the stem cell network. We explain these rules under two broad categories of: local stem cell damage involving one or many stem cells (without whole tissue loss) (e.g., Figure 5.7A); and large scale damage involving loss of whole tissues causing damage to large segments of stem cells (e.g., Figure 5.7C).

With respect to local stem cell damage, refer again to the example damage shown by the black square in Figure 5.7A depicting one missing stem cell (and the surrounding tissue fragment). In this type of damage involving one (or more) stem cell, there still remain stem cells to the left, right, above and below of the damage, indicative of local (in this case encased) stem cell damage. In other local damages, there may be stem cells only above, below, to the left or right of damage or any combination of these arrangements creating many damage scenarios. To identify all such local damage scenarios, Figure 5.7A shows three generic patterns (primitives) (refer to orange arrows and defined below) depending on whether the stem cell damage border is in A/P or D/V directions (Figure 5.7B). Any local damage would encompass all three generic patterns or primitives and thus the primitives help identify the nature of all local damages without whole tissue loss. For example, in the one stem cell damage case in Figure 5.7A, all three patterns apply, as there are stem cells to the left, right and above and below the damage, indicating local (i.e., not whole tissue) damage. Figure 5.7C shows stem cell border

patterns for whole tissue damages. Such damage is identified by the absence of one or more of the three primitives in defining the stem cell damage border. These patterns are specified below.

- ***Identification of local stem cell damage through primitives applied to pattern of stem cell border (Figure 5.7A): All three primitives below should apply***

  Pattern 1: stem cell damage border has one (or more) stem cells to the left of the damage in AP (along the body axis) direction.

  Pattern 2: stem cell damage border has stem cells above and/or below the damage (in DV direction).

  Pattern 3: stem cell border has one (or more) stem cells to the right of the damage (in AP direction).

- ***Identification of whole tissue damage through primitives applied to pattern of stem cell border (Figure 5.7C): At least one or more primitives should not apply***

  When one or two pattern primitives described above are absent in a damage border, it indicates whole tissue damage as in *Figure 5.7*C. For example, in the case of severed head tissue in Figure 5.7C, the stem cell pattern has all stem cells on the side of the damage; therefore, only one primitive applies and 2 are absent.

In summary, local or large tissue damage anywhere in the organism is identified by the pattern of stem cells surrounding each damage (i.e., stem cell border surrounding the damage). This is the pattern of organisation of stem cells that have recognised that some (or all) of their neighbours are missing by applying the perceptron motifs to the stem cell network where a change in voltage beyond the 10% threshold has been sensed. There are three such stem cell border pattern motifs (1, 2, and 3) for local damages depending on whether the stem cell in damage border are in A/P or DV directions, and applying these rules, the local damage is identified. When these rules do not apply, it indicates whole tissue damage (Figure 5.7C). We describe in some details how these rules apply to identify local stem cell damage in the next section. Then we explain the identification of large-scale stem cell damage involving loss of tissues in the following section.

Identification of local stem cell damage by the stem cell network

When the stem cell damage is local, the stem cell network finds the border of stem cell damage by applying perceptron motifs and the nature of local stem cell damage by applying the three pattern rules described above. Specifically for the damage case shown in Figure 5.7A, the neighbours of the missing stem cell in the stem cell network sense the voltage increase, and by applying perceptron motifs, the stem cell network identifies these four neighbours (circled in blue). These are the stem cells that have recognised that their neighbour stem cell is missing from the changes in their voltage status sensed through GJs of the intermediate tissue cells. These then form the border of stem cell damage. Then the above pattern rules are applied to the damage border and it is evident that all three rules apply as there are border stem cells to the left, above and below, and right of the damage. This indicates local stem cell damage inside the stem cell network. Then the stem cell network regenerates new stem cells to replace the missing ones. Then, repair of damage to any tissue(s) is accomplished in collaboration with the somatic cell network of the affected tissue(s) that identify the damage border of somatic cells in the tissue(s).

Identification of large-scale stem cell damage involving loss of whole tissues by the stem cell network

When the damage involves whole tissues, the stem cell network identifies the damage border and recognises that there is only one border pattern - all stem cells are on either side of the damage (Figure 5.7C). The three border patterns are applied to the damage with the result that not all three are valid for this damage. From this, the stem cell networks recognise that a whole tissue(s) is missing. Now the question is how to identify which tissue is missing, head, body or tail, upon which stem cell network regenerates missing tissues while tapping into the information field for the minimum pattern information of lost tissues.

For identification of the type of missing tissues, we define few simple rules based on the communication between stem cells in the stem cell network with activation (positive) and inhibition (negative) signals as shown in Figure 5.4B. In this network, signals from tail to head (P/A direction) are negative (inhibitory) and in all other directions, signals are positive (activation). We use these two features to identify the type of missing tissue(s) as follows:

<u>Rules of identifying missing whole tissues:</u>

- Stem cells in the damage border pattern receive **positive signals** but **no negative signals**. This means there is no communication in the tail to head direction (left to right or A/P direction). Therefore,
    - If the border stem cells are in the head, then body is missing.

- If the border stem cells are in the body, then tail is missing.

- Stem cells in the border pattern receive **negative signals** and **no positive signals**. This means there is no communication from head to tail (right to left or A/P direction)

    - If the stem cells are in the body, then head is missing.

    - If the stem cells are in the tail, then body is missing.

Summary of rules to identify local and global damages by the stem cell network

Below we illustrate in summary form how the stem cell network applies the three generic stem cell border patterns and two rules for identification of missing whole tissues to recognise local and global damages, respectively.

Example 1: *Local damage*: A stem cell with a surrounding tissue fragment is missing, as shown in Figure 5.7A. First, the stem cell network determines the nature of damage from the pattern of border stem cells. In this border pattern, the network identifies all three generic patterns of stem cells described in the previous section. Thus the stem cell network recognises that it is **local stem cell damage** inside the network.

Example 2: *Whole tissue loss (body missing)*: The head tissue separates after damage as shown in Figure 5.7C. First, the stem cell network identifies the border of stem cell damage in the head tissue, which contains all stem cells on the head side. The network recognises that, of the three generic border patterns, only one stem cell pattern applies. This indicates large scale tissue damage. Then, it identifies the missing tissue by using the above rules for identification of lost tissues. As these border stem cells are in the head tissue, they **receive positive signals and no negative signals.** From this, the network identifies that the **body tissue missing**. The stem cell network remaining in the head tissue regenerates body and tail by tapping into the information field upon which it re-establishes the stem cell network and somatic cell network. Similarly, the body-tail part regenerates the head tissue produces another worm.

Example 3: *Whole tissue loss (head missing)*: Let's consider the body tissue remaining after damage in Figure 5.7C. There are two damage regions: head side and tail side. For each region, the stem cell networks identify the damaged stem cell border and recognise that there is only one stem cell pattern on either side. As not all three generic patterns apply, the damage is due to tissue loss. Then, by applying the rules of identification of lost tissues to the stem cell border pattern in each damage region, the network identifies that one pattern **receives negative signals but not positive ones** (head) and vice versa for the tail side. Thus it identifies **head missing on the left side and tail missing on right side of**

damage. Similarly, the stem cell network in the tail tissue recognises that body is missing and head tissue recognises that the body is missing. The remaining stem cell network fragments then regenerate three worms from the three split segments.

C. Level 3: Global level Associative memory network (AMN) maintains and restores bioelectric homeostasis

This section proposes a hypothetical concept for maintaining bioelectric homeostasis in general functioning and restoring it after damage in an organism like the planarian. The question addressed is what minimal and generic approach could allow the remembrance and restoration of the bioelectric pattern represented by the membrane voltage of thousands of cells in the organism. We propose a global Associative memory network sitting on top of the stem cell network in our framework (Figure 5.3A). We assume that the body-wide bioelectric pattern is fault-tolerant in that small scale anomalies such as small deviations of voltage at the cell level (much below 10% threshold) does not impact it significantly. Therefore, considering the large number of cells in the organism, a more aggregate form of monitoring at a macro level that does not involve all cells in the required computations is justifiable.

As a proof concept, we divide the organism into 13 segments according to membrane voltage (Figure 5.8A) as basic units or nodes that form a global Associative Memory Network (AMN) (Figure 5.8B and also Figure 5.4A and Table 2) involved in monitoring and restoring the body-wide bioelectric pattern. This not only makes the monitoring system fault-tolerant (failure in a single cell does not fail the system) but also drastically reduces the amount of computation necessary in maintaining and restring bioelectric homeostasis. The downside is that the voltage is assumed constant within a node in this study to keep it simple and test the concept of AMN. However, in future studies, the constant voltage can be considered as the average of progressively changing voltage values across the node in A/P and D/V directions. Thus the average values can be used to derive intermediate values of membrane voltage to be assigned to individual cells within nodes, that seamlessly meld with the voltage of cells in neighbour nodes to preserve in a more granular sense the bioelectric gradients in the A/P and D/V directions.

The global level Associative Memory Network (AMN) is a recurrent network, which is modified form of the fully connected Hopfield Neural Network (HNN) (Hopfield, 1982) that stores specific state vectors in one or few attractors. We aim to find a network with minimum connectivity (as opposed to full connectivity of all nodes as in HNN) that is sufficient to remember the bioelectric pattern. The benefit of these networks, in general, is that for any distorted input vector (state vector), the network finds

the original undistorted form of the same input vector by recalling the closest state vector stored in the attractors. The AMN operates over the whole anatomical pattern of the organism and learns and memorises the body-wide bioelectric gradient shown in Figure 5.8A.

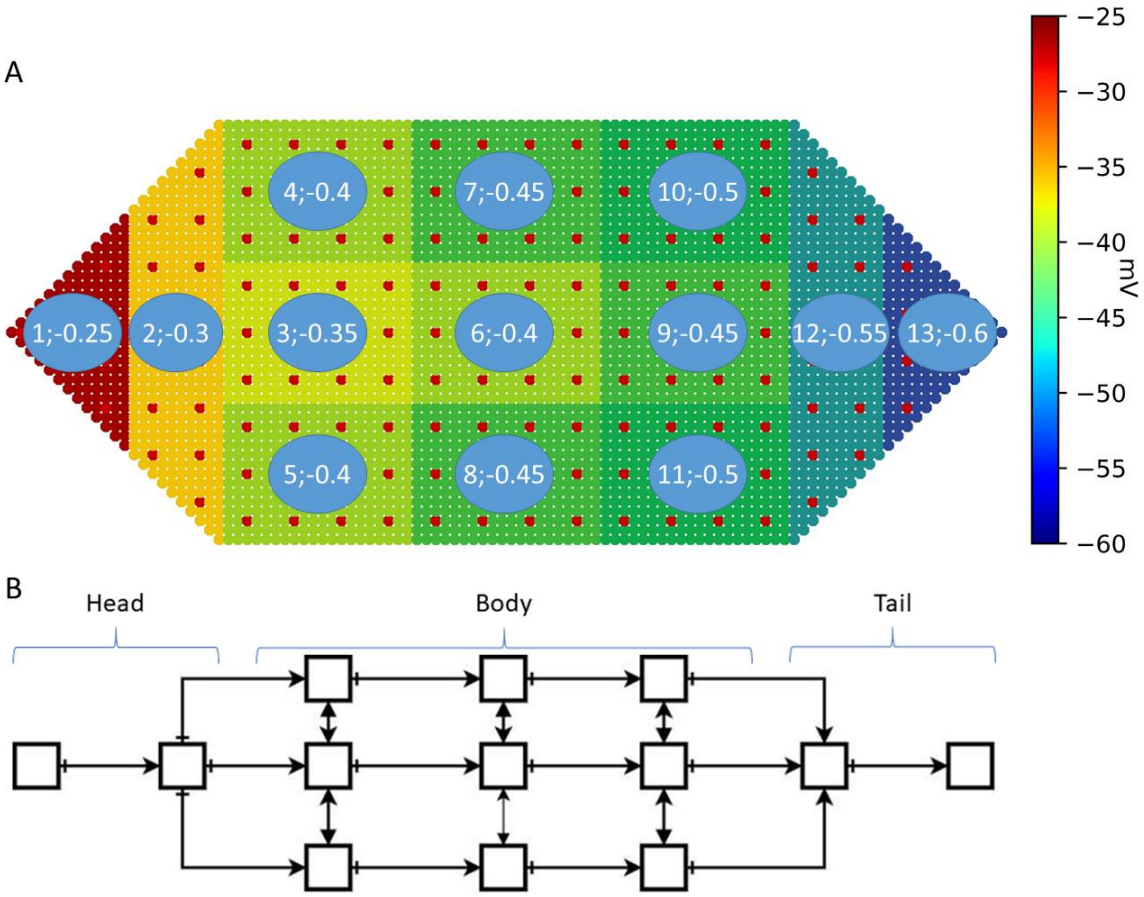


Figure 5.8. An organism with the original homeostasis bioelectric pattern and the Neural Network representing it. A) Innate body-wide bioelectric pattern and 13 tissue segments of the organism used to monitor it. The two numbers within a node indicate the node number and the corresponding membrane voltage (normalised). Colours indicate the magnitude of voltage and whole voltage pattern across the organism denotes the homeostasis bioelectric pattern; B) Associative Memory Network (AMN), consisting of the 13 segmented nodes, corresponding to the whole organism and proposed to learn and remember the body-wide bioelectric pattern

The AMN has 13 macro-level nodes representing a group of stem cells and somatic cells, as shown in the top layer in Figure 5.4A, Figure 5.4D&E and Figure 5.8B. The voltage pattern shown in Figure 5.8A (i.e., 13 voltage values) is the pattern we wish to store in the AMN attractor. When this pattern changes due to normal perturbations or damage, the AMN should recall the original pattern and re-establish bioelectric homeostasis in collaboration with the other two networks. We do not know if the planarian uses a mechanism such as this to keep the bioelectric pattern stable in the body as in this AMN. However, we know that the worm does maintain a stable pattern, and we assume that the approach

it uses has the attributes of flexibility and fault tolerance, presumably achieved without excessive micromanagement and extensive memory requirements.

Our proposed AMN have the following attributes. Each AMN node represents a group of cells (300 tissue cells and 12 stem cells in a node; the head and tail nodes have a slightly different make of these cells). Each node or group represents a constant voltage, and the voltage of all clusters together form the body-wide bioelectric pattern. This arrangement allows the AMN nodes to sense voltage changes in their constituent stem cells and tissue cells in a global or average sense and react accordingly to either to restore the bioelectric pattern under normal function or wait for the stem cell network to initiate repair after injury and complete regeneration. Within a node, neighbour somatic cells are connected by Gap junctions (GJs) with a particular conductance. A node containing these cells communicate with neighbour nodes through pseudo GJs connections reflecting an effective or average conductance of the GJs connecting the somatic cells in the neighbour nodes, as shown in Figure 5.4D. This makes the AMN accurately resemble the cellular structure and interactions without loss of information due to aggregation, which helps it to accurately preserve the average membrane voltage profile throughout the body. These pseudo-GJ conductances are represented by the weights of the AMN. The proposed macro-level nodal configuration allows the AMN to represent the whole worm in a smaller representative network with grossly similar bioelectric properties to that of thousands of somatic cells. This greatly simplifies the required computation to achieve the end results of maintenance of homeostasis where all somatic cells contribute to the process. Moreover, by exploring sparse but meaningful connections between nodes in the AMN, we further reduce the computational burden on the network substantially. This may even provide insights into the potential existence of efficient and high-level organism-wide networks in an organism. Basically, we are not eliminating anything but simplifying the system. Since individual somatic cells are not nodes in the network, the network is flexible and fault-tolerant. For example, the AMN gets damaged only if all cells in a node get damaged. Until then, the AMN keeps functioning collaborating with the stem cell network within the affected nodes to quickly restore bioelectric homeostasis after repair of any damage to cells within a node. When the perturbation is not due to damage, it automatically restores the original bioelectric pattern.

a. AMN training

The original HNN is a fully connected (symmetric) recurrent network with two discrete values for states ($s_i = \pm 1$). When trained with Hebbian learning, these networks converge to at least one attractor. Our voltage input pattern vectors, however, are not discrete values of ± 1 but a number of real (continuous) values. In (Zarco & Froese, 2018), the authors used continuous state HNN with $s_i \in [-1, +1]$ and a fully connected asymmetric connection matrix. (asymmetric means weights for forward and backward interactions between two nodes are not the same). This continuous-state

network also trained well with Hebbian learning and converged into attractors from any inputs. Therefore, in our model, we use real-valued inputs, and we further explore beneficial connection arrangements as an alternative to fully connected networks to reduce the computational burden of the network in having to work with a large number of weights.

Inputs in our model are real voltage values corresponding to the states of the 13 nodes in the network and scaled to $s_i \in [-1, 0]$. (minus value represent a hyperpolarised (more negative potential inside the cell than outside) voltage state of the cell membrane)). Figure 5.8A shows the original homeostasis bioelectric pattern in planaria represented by the 13 AMN nodes. The desired bioelectric gradient pattern is in the range from -25mV to -60mV (milli Volts) which has been reported as the pattern of bioelectric homeostasis in planaria (Cervera, Pietak, Levin, & Mafe, 2018; Pietak & Levin, 2017). In our model, the desired homeostasis bioelectric pattern of our organism consists of the 13 scaled voltage values of (-0.25, -0.3, -0.35, -0.4, -0.4, -0.4, -0.45, -0.45, -0.45, -0.5, -0.5, -0.55, -0.6) shown in Figure 5.8A and they correspond to the voltage values of the 13 macro-level nodes and represent the desired network attractor. We trained the AMN with a large number of voltage patterns (approximate 600 patterns) randomly perturbed from the original voltage pattern in Figure 5.8A. This corresponds to voltage patterns that can result from the perturbation of parameters in a set of Differential Equations (Eq. 5.4) modelling bioelectric communication between somatic cells via gap junctions as in (Cervera, Alcaraz, & Mafe, 2016).

$$C_i \frac{dV_i}{dt} = -I_{in}(V_i) - I_{out}(V_i) + \sum_{j\ nn\ i} G_{ij}(V_j - V_i) + \sum_{j\ nn\ i} C_{ij}\left(\frac{dV_j}{dt} - \frac{dV_i}{dt}\right) \qquad 5.4$$

where $V_i$is the voltage of cell *i*, G_{ij} is the conductance of GJ between cells i and j (C_{ij} is capacitance), nn is the number of nearest neighbours, $I_{in}(V_i)$ is the current-voltage curve, and $C_i$is a constant; for details of these attributes refer to (Cervera et al., 2016).

We simply highlight this ODE model to point out that the AMN in functionality represents existing bioelectric models of cell networks and in future, this connection can be exploited to integrate cellular bioelectric and even molecular models into the framework (i.e., not only bioelectric models but also models of slow diffusion of molecules carrying signals for cell division in regeneration).

In training, the AMN starts with random connection weights that allow for learning the desired bioelectric pattern. Basically, we wish the AMN to store the target homeostasis bioelectric pattern in its attractor and invoke this pattern in response to any (altered) input voltage pattern vector. Therefore, all input patterns generated by perturbing the original voltage should form the basin of attraction of the attractor. The learning algorithm goes through the following steps: (1) assignment of the initial states of the neurons (from the generated input patterns), (2) convergence of the network

for a certain period (network simulation) and (3) application of Hebbian learning that adjusts weights incrementally until the network learns (stores) the target voltage pattern in an attractor and correctly recalls it from any input voltage condition that the organism may encounter.

The model dynamics use asynchronous neuron state updates with a rectilinear function (ReLU) in the nodes (Figure 5.9) that calculates the output (voltage) s_i of i^{th} node as follows:

$$s_i = \theta\left[\sum_j^N w_{ij}s_j + b_i\right];\ \theta(a) = \begin{cases} 1 & if\ a \geq 1 \\ -1 & if\ a \leq -1 \\ a & otherwise \end{cases} \tag{5.5}$$

where w_{ij} is the connection weight between node *i* and *j*, θ is the transfer function (ReLU) and b_i is the bias weight of node *i*. In order to be able to store the desired pattern, we set random values for the connection weights (*w, b*) and adjusted them using Hebbian learning rule:

$$w_{ij} = w_{ij} + e * \delta; \qquad b_i = b_i + e * \delta; \qquad e = T_i - I_i \tag{5.6}$$

where δ is the learning rate, *e* is the difference between target pattern *T* and current input *I*.

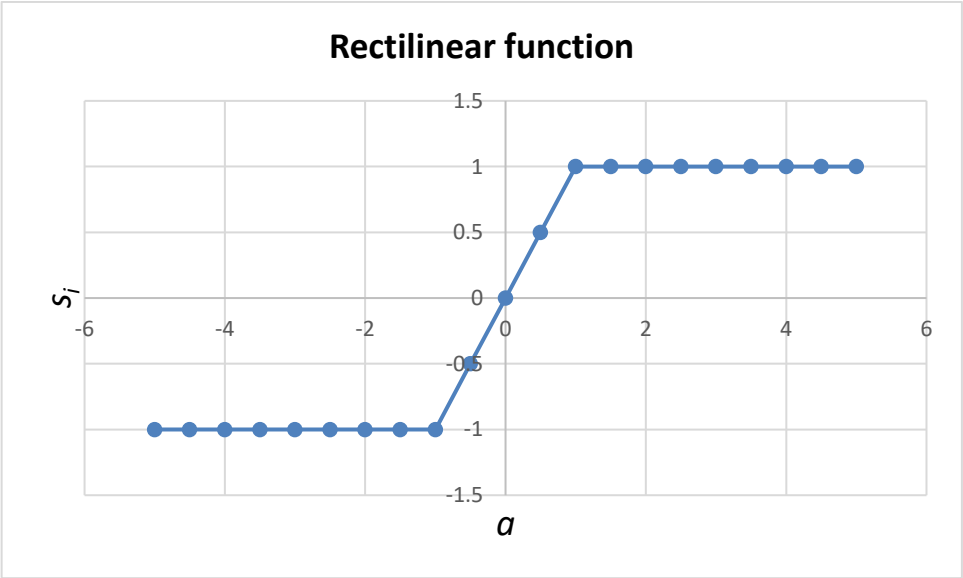


Figure 5.9. The rectilinear function for output (voltage) s_i of the AMN

We designed the structure of the network by iteration. We first used a fully connected network as in HNN where all nodes communicate with each other. As this requires a massive amount of calculation to find an attractor with a large number of connection weights, we then reduced the connectivity in a number of stages to explore a simpler yet meaningful connection configuration that stores and recalls the pattern with minimum computation. We found a minimal connection configuration where local communication prevails as in Figure 5.10 and where both the computation and memory storage requirements are drastically reduced. This resulted in 40 connection weights, a reduction of 87% from

312 connection weights for the fully connected network. This means that the reduced network has only 12% of the connections of the full network. The trained weights of the AMN are given in Table A2 in Appendix A. All networks trained easily within few minutes.

The minimal network topology thus found can be described as follows:

- Connections from head to tail and in the transverse (from the midline to edges) directions are positive (activation signals).
- Connections from tail to head are negative (inhibitory signals).

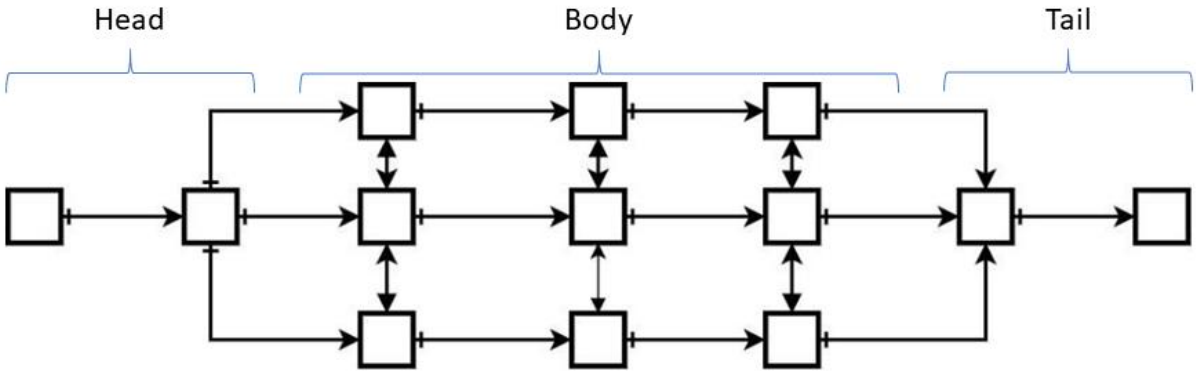


Figure 5.10. A minimal AMN structure that stores the original bioelectric pattern

The fact that such a simplified and organised structure emerged was intriguing in itself. Specifically, this communication structure means that a node only receives signals from adjacent cells as follows:

- **For Anterior-posterior (AP) axis (longitudinal direction of the body):**
- Positive signals: flow from head to tail
- Negative signals: flow from tail to head
- **For the Dorsal -ventral (DV) axis (transverse direction of the body):**
- Positive signals flow in both directions from mid-body to edge

Recall that this communication structure between nodes was adopted for the stem cell network as well as described in the previous sections. Further, the above signal flow structure along and across the whole organism was used to derive the rules for identification of missing whole tissues by the stem cell network as was also presented in the previous section.

b. AMN maintains bioelectric homeostasis under regular perturbations

In this section, we first discuss the algorithms and computations involved in the restoration of bioelectric homeostasis under normal physiological conditions and then present those involved in its restoration after recovery from damage.

The voltage of each AMN node $\boldsymbol{V_k}$ is calculated by the following formula:

$$\boldsymbol{V_k} = \frac{\mathbf{1}}{\boldsymbol{n}} \sum_{\boldsymbol{i=1}}^{\boldsymbol{n}} \boldsymbol{v_i} \tag{5.7}$$

where n is number cells in a node (e.g., n=300 cells), $\boldsymbol{v_i}$ is the voltage of cell *i*, and *k* is the node number $k \in [1,13]$. Figure 5.12A shows the unperturbed original bioelectric pattern. Figure 5.12B shows an example of perturbed voltage in a single node where one or few of its cells experience voltage change, and Figure 5.12C shows the perturbed voltage in all nodes where all cells experience voltage changes.

A single cell in a node receives perturbation to its voltage

Cells may be affected by their surroundings, such as temperature, light, chemicals inside the cells, during normal physiological function, causing the voltage to change. According to the literature, membrane voltage can change up to ± 10% during normal operation (Reid et al., 2005). We assume a threshold of ±10% for normal perturbations of membrane voltage of a cell.

Let $\boldsymbol{\epsilon}$ be the voltage change in a single cell. The updated voltage in node k is presented as:

$$V_k = \pm\epsilon + \frac{1}{n} \sum_{i=1}^{n} v_i \,; \qquad \epsilon = \frac{vp}{n} \tag{5.8}$$

where, $\boldsymbol{p}$ is the percentage of voltage change, e.g. -10% to 10 %, and v is the original voltage of a single cell.

Many cells receive voltage perturbations

In the case where many cells undergo changes in voltage (Figure 5.12C), the total voltage change in a node N_k (k=1, 2, .., 13) is computed as follows:

$$\epsilon = \sum_{i=1}^{j} \frac{v_i p}{n} \tag{5.9}$$

where j is the number of cells in node N_k receiving voltage perturbations

The algorithm for AMN recovery of the original voltage pattern from perturbed voltage vector is as follows:

- Get the current altered voltage vector for the nodes (using Eq. 5.8 and 5.9).
- Input to AMN
- AMN iterates a number of times until it reaches a steady state which is the original bioelectric pattern stored in the attractor.
- In normal physiological functioning, the network is assumed to make affected nodes (specifically, cells within nodes) incrementally alter the bioelectric state until they return to the normal state.

c. AMN maintains bioelectric homeostasis after regeneration

The AMN restores the bioelectric pattern after the stem cell network completes regeneration. Recall that AMN nodes consist of 300 somatic cells and 12 stem cells. In case of small damages where any single AMN node is not completely damaged (i.e., not all cells within a node get damaged), the AMN can restore bioelectric state after regeneration of missing somatic or stem cells in individual nodes. When a network node gets damaged, the spatial topology of the network is broken. The network will then lose the voltage value of the lost node and all connection weights to this node. After completing regeneration, the connections from this node to others need to be re-established to restore lost connection weights. Further, the voltage of repaired nodes will not be the same as the required and therefore, the nodal voltage also needs to be updated. We assume that after the initial training of the AMN, the original bioelectric pattern (bioelectric gradient) captured by the attractor is stored in the Information Field along with the connection topology. After nodes are repaired following injury, the AMN (Figure 5.10) retrains itself by accessing the bioelectric gradient from the field until the network learns to store it in the attractor and the connections and weights to repaired nodes are re-established. The algorithm is below:

- Initialise connections: Connections from the new node to others are re-established based on the topology structure and corresponding weights are initialised with random values (e.g., 0.1 for positive, -0.1 for negative signals).
- Retrain the AMN: The network retrains with the target bioelectric pattern accessed from the Information Field (since the network has to train only the damaged connection weights, it is trained faster than the original network)
- Restore weights to repaired nodes.

- Adjust voltage: Cells in the affected node(s) alter the bioelectric state incrementally until they return to the normal equilibrium state.

  In the next section we describe the operation of the whole framework integrating the three levels.

5.6 Operation of the framework

The functional flow chart of the regeneration framework is shown in Figure 5.11, giving an expanded view of the 5 states previously shown in the overview Figure 5.3. We use this flow chart to show the operation of the network in normal conditions and damage repair; specifically, to demonstrate the maintenance of bioelectric gradient under normal physiological conditions and recovery of both anatomical and bioelectric patterns after very small or very large damages.

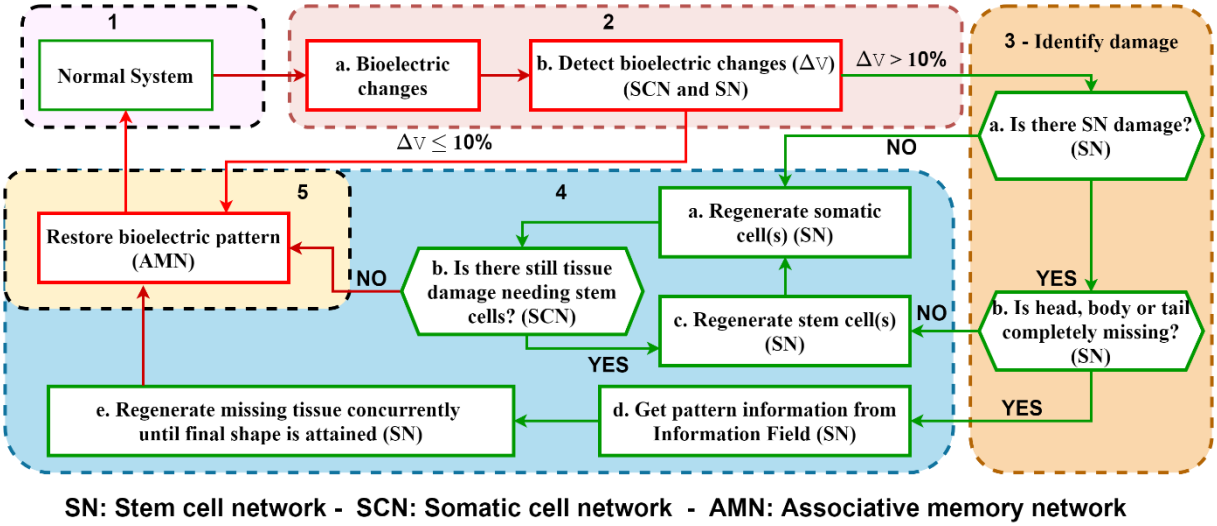


Figure 5.11. Expanded view of the functional flow chart of the regeneration framework

A. Normal system operation: Maintaining bioelectric homeostasis in regular function (States 1 --> 2 --> 5)):

State 1: Monitor system

In the intact system, the three networks are in standby mode when everything is normal, and the organism is in pattern and bioelectric homeostasis. Further, each cell (stem cell or somatic cell) has, as it is property, the knowledge of how many neighbours it has and its position in the system (interior, border, or corner). AMN has captured in its attractor the spatial pattern of the voltage distribution through the voltage of its nodes. Its connection weights depict representative gap junctional conductance between nodes.

State 2: Detect bioelectric changes (somatic cell (SN) and stem cell (SCN) networks)

The bioelectric state can get perturbed due to external (damage) or internal factors. The perturbation causes the flow of bioelectric signals from the affected somatic cells through their GJs and then released into the surrounding environment (as ion fluxes) in the vicinity of stem cells. Stem cells receive this signal, and if a change in voltage is equal or smaller than 10%, they activate the AMN to restore the bioelectric pattern (State 5). Otherwise, if the change in voltage is equal to or higher than 10%, they move to the identification of damage (State 3).

State 5: Restore bioelectric pattern (AMN)

If there is no damage, the AMN adjusts the voltage of cells to return the system to the equilibrium state as described in the previous section. Specifically, starting from the current bioelectric state, it iteratively updates the voltage of the affected nodes until the whole bioelectric pattern converges to the homeostasis voltage vector stored in the AMN attractor. From the perspective of physiology, this process involves activation of molecular pathways that operate to adjust membrane voltage of individual somatic cells through modulation of relevant ion channels and other proteins, which is beyond the scope of this thesis.

B. Damage detection and regeneration (States 3 and 4)

The damage recovery is covered by States 3 and 4 in Figure 5.11. As shown, there are many types of damage covered in these 2 States ranging from damage involving one or more somatic cells (tissue damage), one or more stem cells (stem cell and local tissue damage), and multiple tissues or large parts of the organism including those damages where only a tiny fragment of the body remains.

State 3: Identify damage (stem cell network)

After an injury, somatic cells around damaged cells increase their voltage as they pick up ions released by the damaged cells into the surrounding and their bioelectric status change to "*damage*" indicating they have been disconnected from the damaged cells resulting in loss of GJs. The increased voltage signals then spread from these somatic cells through GJs and ion fluxes until they reach the closest stem cell(s).

All stem cells that receive damage notification from somatic cells start identifying the damage. First, they start to check if the stem cell network has received damage and lost stem cells (State 3a). These stem cells communicate with their neighbours using the applicable perceptron communication motif depending on their location in the network as described in the previous section. After some iterations, all stem cells update their status. If no stem cells are missing (State 3a and NO), there is only local tissue cell damage. If any stem cells are missing (State 3a and YES), then the stem cell network has to establish whether the damage involves completely missing tissues (State 3b and YES) or more contained tissue damage (State 3b and NO). When stem cells are missing, their neighbour stem cells

will change the status to "*damage*". The stem cells with "*damage*" status will define the stem cell damage border and then the stem cell network applies the rules presented in the previous section to identify whether the damage is tissue contained (State 3a and NO) or involves missing whole tissues (head, body etc.) (State 3b and YES).

State 4: Regeneration

Depending on the type of damage, the corresponding processes are executed to recover from damage. If the damage involves only somatic cells leaving stem cells intact, then closest stem cell moves to the damage location and produces new somatic cells (State 4(a)). If the damage involves a stem cell with small surrounding tissue damage (State 3b and NO), then the networks find the stem cells making up the damage border, and by applying the three generic patterns discussed in the previous section, it recognises that it is a local stem cell damage. Then the closest border stem cell with "*damage*" status moves to the somatic cells with "*damage*" status and produces a new stem cell (State 4c) upon which the new stem cell produces new somatic cells to recover the tissue from damage (State 4a). The voltage of the new stem cell and somatic cells is not identical to that of the original cell and could be either similar to the voltage of the stem cell that produced it, or between the original voltage and that of somatic cells in the damage border.

In the case of relatively larger tissue damage (e.g., an AMN node consisting of multiple stem cells) (state 3b and NO again), the remaining stem cell network identifies the stem cell border pattern and, by applying the three generic patterns to the border, recognises the nature and extent of multiple stem cell damage. Then the stem cells on the border reproduce new stem cells (State 4c) and somatic cells (States 4a). However, due to each new stem cell being responsible for producing only a square block of tissue (24 somatic cells), the repair can require more new stem cells to complete regeneration (State 4b). Further, if the damage involves tissue exterior leaving a segment of the original border, new somatic cells use the remaining tissue geometry as cues to completely recover the damage tissue parts. If the damage completely removes one or more sides of the tissue without leaving any cues, then the minimum tissue pattern stored in the Information field is extracted by the stem cells to fully regenerate the tissue.

In the case of multiple tissue damage (missing head, body etc.) (State 3b and YES), stem cell border becomes open separated into two parts. As this does not conform to the three generic border patterns, the two rules for identifying the missing whole tissues described in the previous section are applied to the stem cell border pattern. From this which tissue(s) is missing is established. Then, new tissues are generated by using the minimum tissue pattern information stored in the information field by the stem cells (State 4d) and the tissues are regenerated concurrently so that they grow incrementally to the required size (States 4e). The voltage of the new stem cells and somatic cells

could be either higher or lower than the voltage of the stem cells that produced them due to the bioelectric gradient decreasing along A/P direction (from head to tail).

5.7 Implementation of the framework for bioelectric restoration and pattern regeneration

In this section, we demonstrate the implementation of the framework on a simple synthetic worm to show that it efficiently and robustly recover from very simple to very complex damages. We consider two main types of damage: partial tissue damage with intact stem cells; and severe damage associated with loss of stem cells and tissues or whole body parts (head, tail etc.). We also show that the framework efficiently restores the bioelectric pattern due to normal perturbation or damage.

5.7.1 Restoration of body-wide bioelectric voltage pattern due to normal perturbation (without damage)

In the resting condition, the original body-wide bioelectric pattern has the form of Figure 5.12A. When a cell or few cells experience a change in voltage, the corresponding AMN node k that the cells reside in also undergoes a change in its voltage $\boldsymbol{V_k}$ (Figure 5.12B). This triggers the AMN network to restore the original bioelectric pattern of nodes. The cells that underwent a change, thus restore their original voltage (Figure 5.12D). The AMN acts as a mechanism to help nodes communicate with fixed (already trained) connection weights so that they can collectively maintain the voltage in the constituent cells after any change. Specifically, starting with the altered voltage pattern as input, AMN retrieves the original voltage pattern stored in the attractor in a series of steps. This process of the gradual restoration of voltage in steps can allow cells to incrementally produce the required changes in voltage. As stated earlier, physiologically, this return to the original state happens through the altered bioelectric state activating the corresponding molecular signalling networks involving ion channels and other signalling proteins in the affected cells. The AMN will keep them activated until the system returns to the original bioelectric state. If a great number of (or all) cells change their voltage from the equilibrium values as shown in Figure 5.12C, the AMN similarly restores the original bioelectric pattern (Figure 5.12D) in a series of steps. This way, the framework restores bioelectric homeostasis under any disturbance.

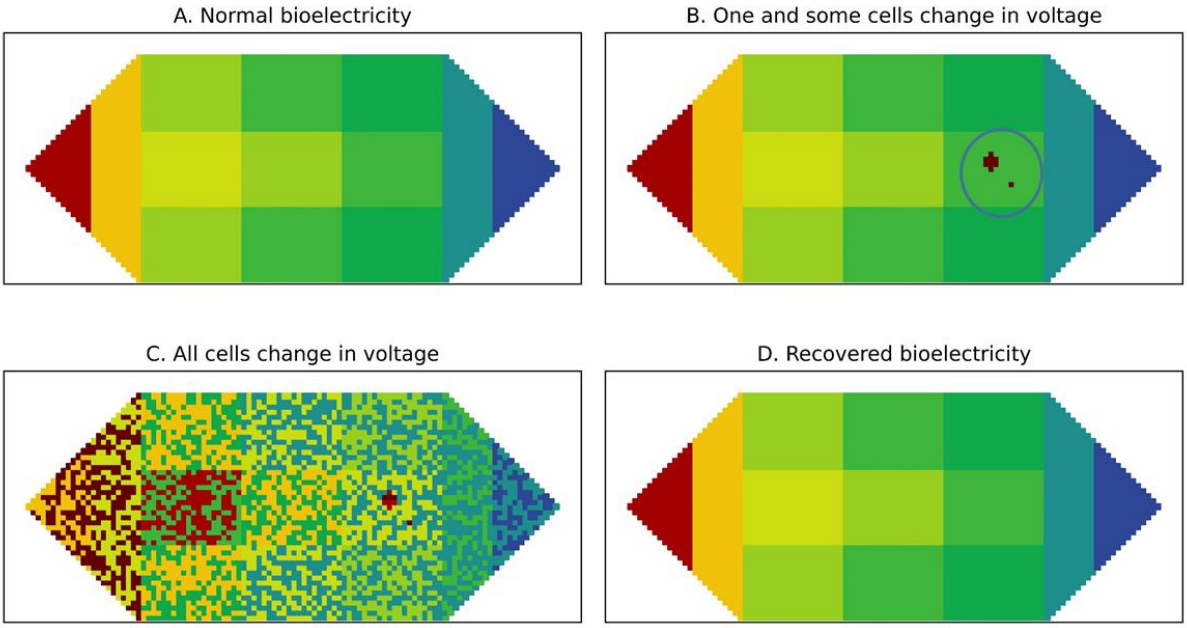


Figure 5.12. Perturbation and restoration of body-wide bioelectric voltage pattern. (A) Normal equilibrium voltage pattern (homeostasis); (B) One cell or few cells in a node change voltage up to 10%; (C) All or most cells in all nodes change voltage up to 10%; (D) Worm after recovering the original voltage pattern through the activation of the AMN

5.7.2 Recovery from Damage

A. Simple damage with one somatic cell missing

This is a case of cell death due to ageing or harmful factors such as trauma or toxic chemicals (as shown in Figure 5.13A and enlarged in Figure 5.13B). Damaged cell membranes lead to cell death. This process is called Necrosis.

Description:

This is a simple damage type where only one somatic cell is missing anywhere in the organism. When a somatic cell dies, connections between the damage cell and its neighbours (somatic cells) through GJs are lost. The voltage of its neighbour cells increases by >10% (Reid et al., 2005) due to chemical signals released from the dead cell.

Detect bioelectric changes (State 2):

Somatic cell network recognises the change in voltage and then send bioelectric signals through GJs and ion fluxes to stem cells. Stem cell network senses the changes in voltage through ion fluxes.

Damage identification (State 3):

Due to voltage change being greater than 10%, there is damage, and the stem cell networks start to identify damage. Specifically, after sensing the increased voltage indicative damage, the neighbour

(somatic) cells apply the motifs to confirm the damage and change their status to "*damage*". As illustrated in Figure 5.13B, somatic cells with "*damage*" status (yellow colour cells) are neighbours of the damage cell (black cell) and become the damage border. These somatic cells release bioelectric signals (status and voltage) to the surrounding. The nearest stem cell (dark red cell in Figure 5.13B) receives the chemical signal from the damage cell and bioelectric signals from its neighbour somatic cells that confirm the damage. The stem cell conducts a quick check on its neighbour stem cells by applying the relevant perceptron motif and senses that all its neighbour stem cells are normal (State 3a). In this case, only one somatic cell is lost.

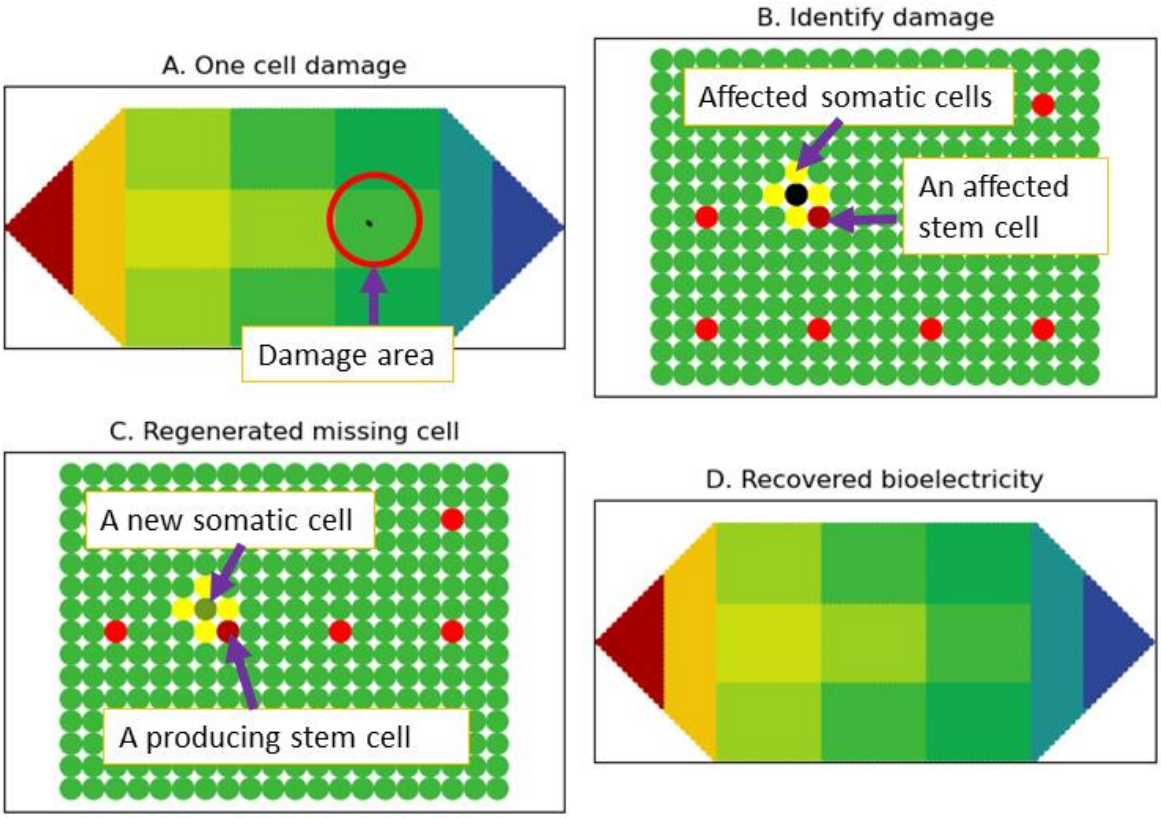


Figure 5.13. An example of a somatic cell damage – (A) One damage somatic cell (black cell); Solid line circular is highlight the affected cell area (B) Damage identification: neighbours (yellow colour cells) of the damage cell. The red cells are stem cells in this tissue; A dark red cell is a stem cell which is affected by the damage cell (C) Regeneration of missing cell by the affected stem cell (the producing stem cell); the new cell with voltage as the producing stem cell; (D) AMN recovers the equilibrium bioelectric pattern.

Regenerate (State 4):

As the stem cell is already near the damage border, it moves to the border and divides to produce a new somatic cell (State 4a). The voltage of the new cell is similar to the voltage of the stem cell that produced it. After regenerating, the new cell establishes connections with its neighbours and accordingly establish the relevant neighbourhood rules and motifs (Figure 5.6). All cells in the damaged border re-check their neighbours and update their status. As all these cells have the correct number

of neighbours as in the initial state, their status changes to “normal” (State 4b). Then, the stem cell returns to its original place in the tissue and activates the AMN to restore the bioelectric state (State 5).

Recovery of voltages (State 5):

After successful damage recovery, the stem cell activates the AMN to recover the voltage similar to the case without damage. The increase in voltage in the cells in the damaged area (yellow and dark red cells in Figure 5.13B and C) has resulted in a slight increase in the voltage of the corresponding AMN node. After activation, AMN uses the bioelectric state after recovery as input and returns the voltage of the altered nodes to the normal state in a series of steps (Figure 5.13D).

B. Number of somatic cells missing

The case of more than one somatic cell missing is similar to one cell missing (Figure 5.14). However, more than one stem cell may check for damage and implement recovery. Figure 5.14 illustrates an example case where five somatic cells (black) have been lost. Somatic cells determine the border of damage (yellow colour cells in Figure 5.14B. Here, three stem cells recognise that there constituent somatic cells have been damaged (Figure 5.14C) and recognise what/where the damage is. Here, only somatic cells are gone, and each stem cell repairs the tissue in its neighbourhood (Figure 5.14C), and then AMN restores the original voltages (Figure 5.14D).

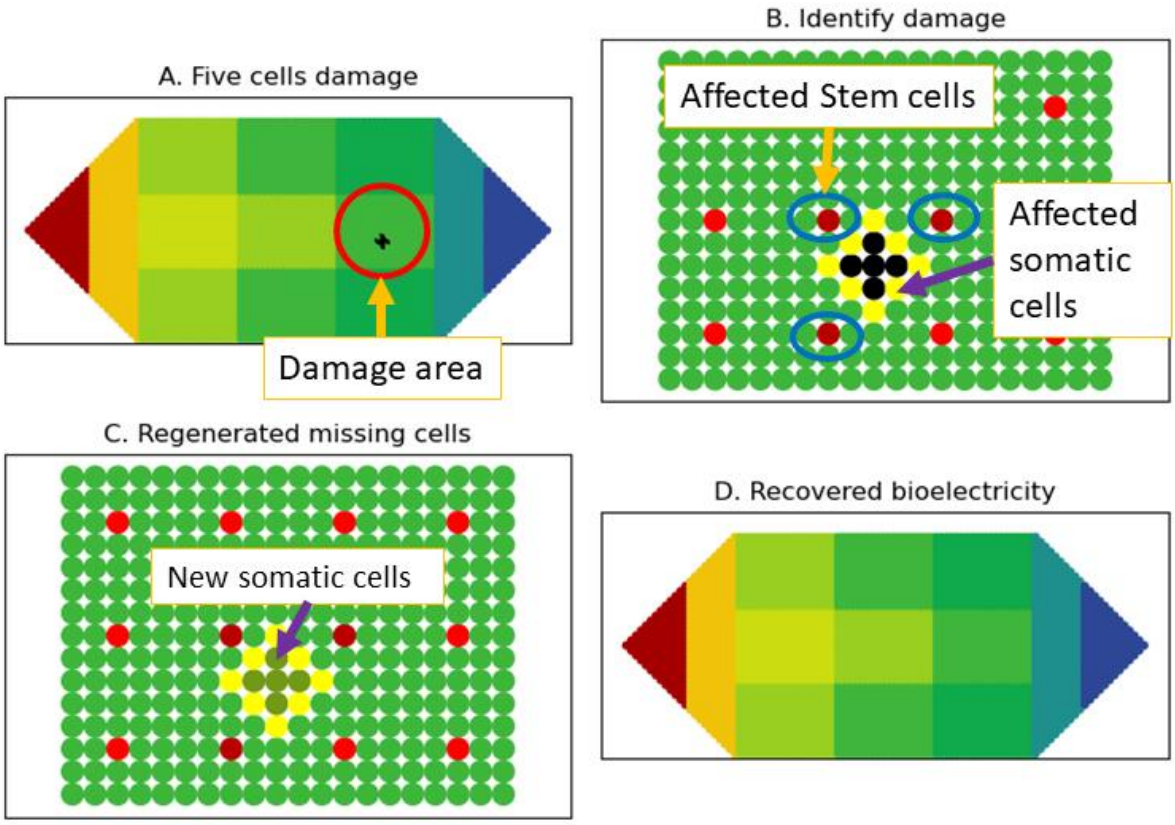


Figure 5.14. An example of five somatic cell damage – (A) Five damaged somatic cells (black cells); (B) Damage identification: damaged cells and their neighbours (yellow colour cells); burgundy cells and red cells are stem cells in the affected area and unaffected stem cells; (C) Regeneration of missing cells; the new cells (with increased voltage) are among yellow cells, depicted by green colour; (D) AMN recovers the original bioelectric state

C. A stem cell with its surrounding tissue damage

Description:

This is a more complex damage case where stem cells and a considerable number of somatic cells (about 24 cells) are gone (Figure 5.15A). An example damage region is shown in Figure 5.15B where a stem cell and its surrounding tissue are missing (black square).

Detect bioelectric changes (State 2):

Stem cell network senses the damage as described previously since the voltage change is greater than 10%. It then attempts to identify the damage.

Damage identification (State 3):

As in the previous case of only somatic cell damage, somatic cells which are neighbours of damage cells sense the damage (yellow border in Figure 5.15B) and send signals to the surrounding. As shown in Figure 5.15B, four nearest stem cells (four dark red cells in Figure 5.15B) receive the bioelectric signals and quickly check their stem cell neighbours by using the perceptron motifs. This establishes

that a neighbour stem cell is missing (State 3b). To identify the nature and size of damage, the four stem cells encompass all three previously defined generic pattern primitives for borders. This means that the damage is local damage and is surrounded by these stem cells.

Regenerate (State 4):

The stem cell in the pattern 1 or 3 migrates to the damage region and repairs it (State 4c). It first produces a new stem cell with the same voltage as the producing stem cell. The new stem cell then produces a new tissue with 24 somatic cells guided by the damage border (State 4a). In this case of interior damage, new somatic cells fill the damage region at which point regeneration stops (Figure 5.15C) (State 4b). For similar tissue damages extending to the boundary of the tissue, the new cells are produced until they come into alignment with the existing boundary cells to achieve the correct final shape of the tissue.

Restore voltage (state 5):

After successful damage recovery, the stem cells activate the AMN to recover the bioelectricity pattern (Figure 5.15D) as in the previous example.

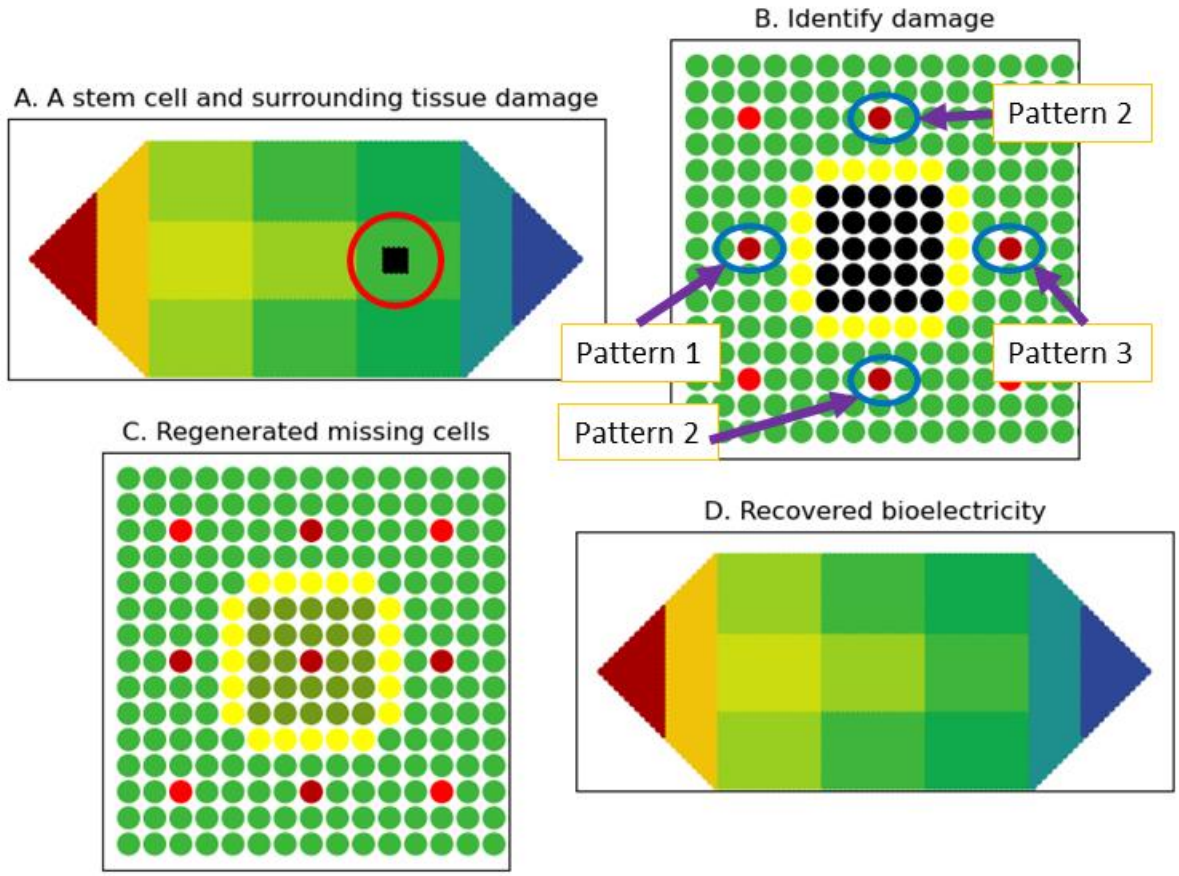


Figure 5.15. A stem cell with tissue damage and regeneration. (A) A stem cell and surrounding tissue damage; (B) Identification of damage- black square (yellow cells show the damage border of the somatic cell network and red dots show the damage border of the stem cell network; (C) After regeneration of missing stem cell and somatic cells; (D) AMN restores body-wide bioelectric pattern.

D. Many stem cells with surrounding tissue missing – an AMN node damage

Description:

A relatively large damage (about 300 cells) involving 14 stem cells and surrounding somatic cells in the interior of the body is shown in Figure 5.16A and in the magnified view of damage region (black square) in Figure 5.16B. This is damage to a node in the AMN. The methods of sensing, identifying and repairing the damage are similar to the methods of the previous case (a stem cell and surrounding tissue damage). Specifically, the stem cell network first identifies missing stem cells and the damage border in the stem cell network (dark red dots (stem cells) surrounding the black square in Figure 5.16B), and somatic cell network identifies missing neighbour somatic cells and identifies the damage border in the tissue (yellow cells surrounding black square), using the perceptron motifs. Figure 5.16B-D show the regeneration steps for this damage case.

Restore voltage (state 5):

As a node in the AMN is gone, we apply the algorithm in section 5.5.3.C. above to restore bioelectric homeostasis. This algorithm is shown again below for convenience.

<u>The algorithm for recovering the voltage:</u>

- Initialise connections: Connections from the new node to others are re-established based on the topology structure, and corresponding weights are initialised with random values (e.g., 0.1 for positive, -0.1 for negative signals).
- Retrain the AMN: The network retrains with the target bioelectric pattern accessed from the Information field
- Restore weights to repaired nodes.
- Adjust voltages: Cells in the affected node(s) alter the bioelectric state incrementally until they return to the normal equilibrium state.

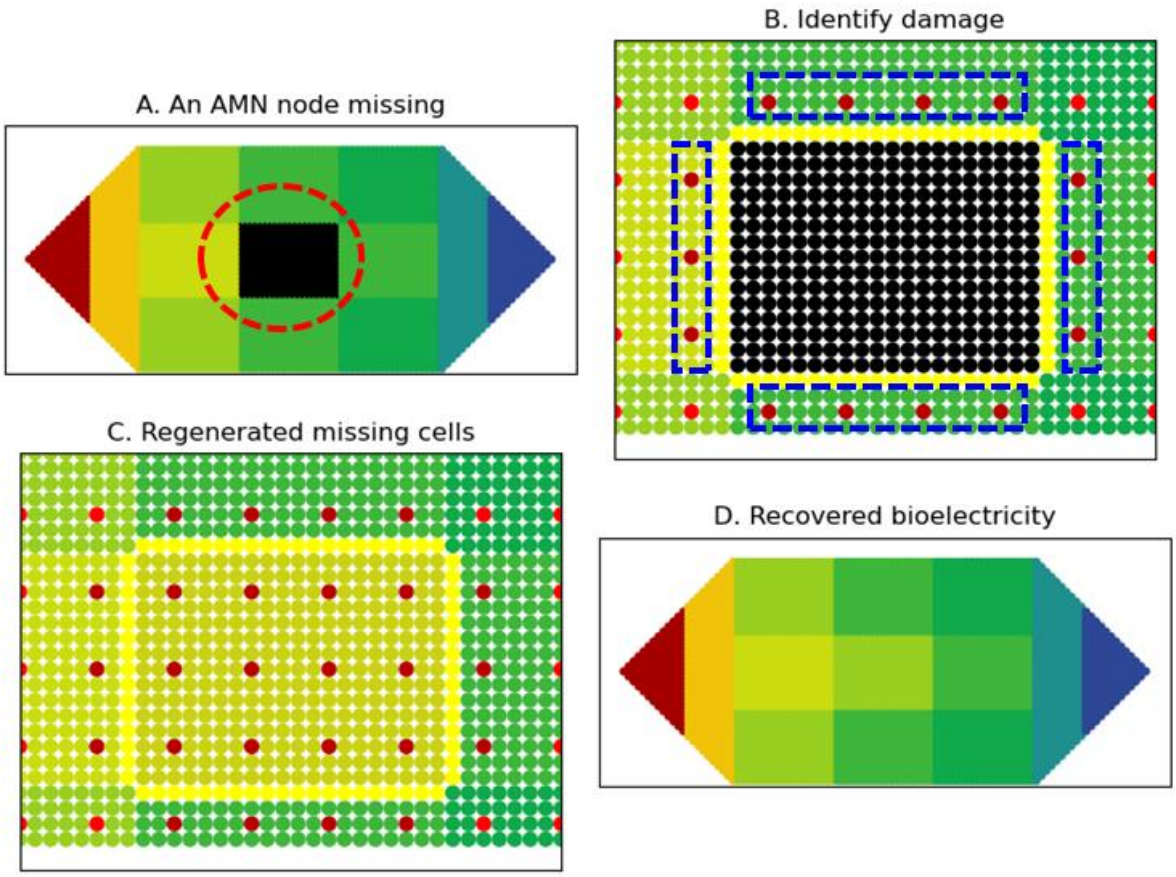


Figure 5.16. Large damage of the size of an AMN node, including 12 stem cells and 300 somatic cells. (A) Damage region (black square) where cells are missing; Dash lines indicate the affected cell area (B) Somatic cell network identifies border surrounding tissue damage (yellow border) and stem cell network identifies its damage border (dark red dots surrounding the damage); (C) damage area and surrounding tissue after regeneration (yellow indicates that voltage in the region is higher than normal (D) AMN restores the body-wide bioelectric pattern.

E. Damage to whole body parts – head, body or tail completely missing

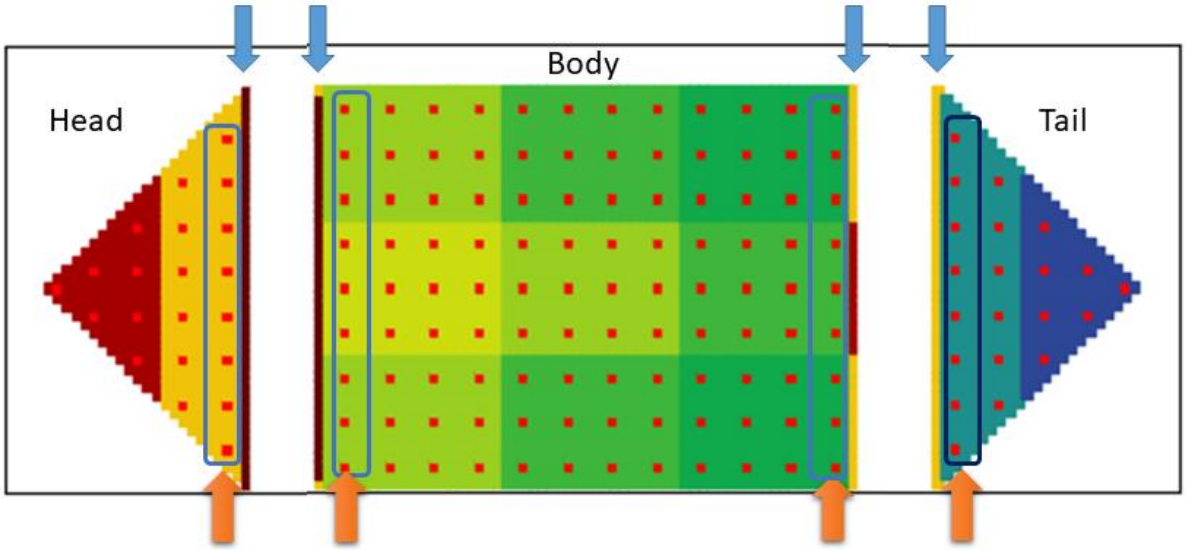


Figure 5.17. Damage to whole body parts – the worm is cut into three tissue: head, body and tail completely separating each other. Blue arrows indicate the borders of damage identified by somatic cell network. Orange arrows indicate the stem cell borders identified by the stem cell network.

Description:

The worm is cut into three parts: head, body, and tail, as shown in Figure 5.17.

Detect bioelectric changes (state 2):

Stem cells and somatic cells sense the voltage change as described in the previous example cases.

Damage identification (state 3):

In each tissue, stem cells identify missing stem cells by applying perceptron motifs (State 3a&b) and then identify the type of damage as whole tissues missing. Specifically, first, somatic cells identify the border of damage (blue arrows in Figure 5.17) and send signals to the surrounding. The stem cells close to damage borders receive the signals and quickly check their neighbours (stem cells) by using the perceptron motifs. This establishes the borders of stem cells. Now the task is to identify the severity of damage. By applying the three generic border patterns to the actual stem cell borders, it is found that these border stem cells encompass only one generic pattern. It means that this is whole tissue damage. Then, these stem cells identify the type of damage using the tissue damage rules defined at the end of section 5.3b as follows.

Head tissue is severed:

The border stem cells in the head tissue do not receive any inhibitory signals along AP direction (from body side) while they still receive normal activation signals (from the head side), so it recognises that the **body is lost**.

Body tissue is severed:

The stem cells on the border of the stem cell damage identify the type of damage as head tissue missing. Specifically,

- Damaged Side on the Left: Stem cells do not receive activation signals along AP direction (from the head side) while they receive inhibitory signals (from the tail side); therefore, it recognises that the **head is lost**.

- Damaged Side on the Right: Stem cells do not receive inhibitory signals along AP direction (from the tail side) while they still receive normal activation signals (from the head side), so it recognises that the **tail is lost**.

Tail tissue is severed:

The stem cells close to the damage side identify the type of damage as head and body missing. These stem cells do not receive activation signals along AP direction (from the head side) while they still receive normal inhibitory signals (from the tail side), so it recognises that the **body is lost**.

All three separated tissues regenerate into three separate worms, as described below (Figure 5.18 and Figure 5.19).

Regenerate (State 4):

Head regenerates body and tail:

Stem cells in the damaged border produce new stem cells for body and tail, transfer pattern information from the information field (AR, d, n) to them (State 4d), and then they produce their corresponding tissues (body and tail tissues) concurrently (Figure 5.18B) (State 4e). Voltage values of new stem cells are smaller than that of producing stem cell due to the bioelectric gradient decreasing continually from head to tail. Body and tail regenerate concurrently starting from small tissues and will then grow into the exact original form following shape information taken from the Information field (State 4d), as shown in Figure 5.18B-E. Finally, the AMN restores the network configuration and retrains the weights using the original bioelectric pattern stored in the field, and this way restores the voltage of the repaired nodes (State 5) and brings the whole worm to the equilibrium voltage pattern (Figure 5.18F) (State 1).

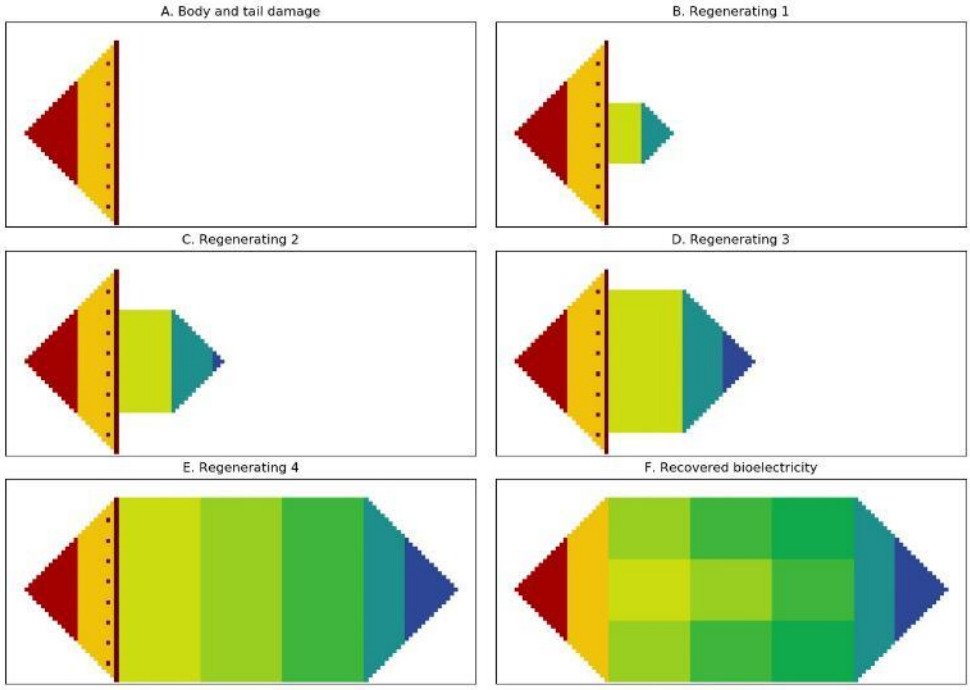


Figure 5.18. Head regenerates body and tail. (A) Severed head tissue showing stem cell border close to the damage site; (B-E) Production of body and tail stem cells by the stem cells in the stem cell border and concurrent regeneration of head and tail tissues until full recovery of anatomy and (F) AMN restores body-wide bioelectric pattern.

The body regenerates head and tail:

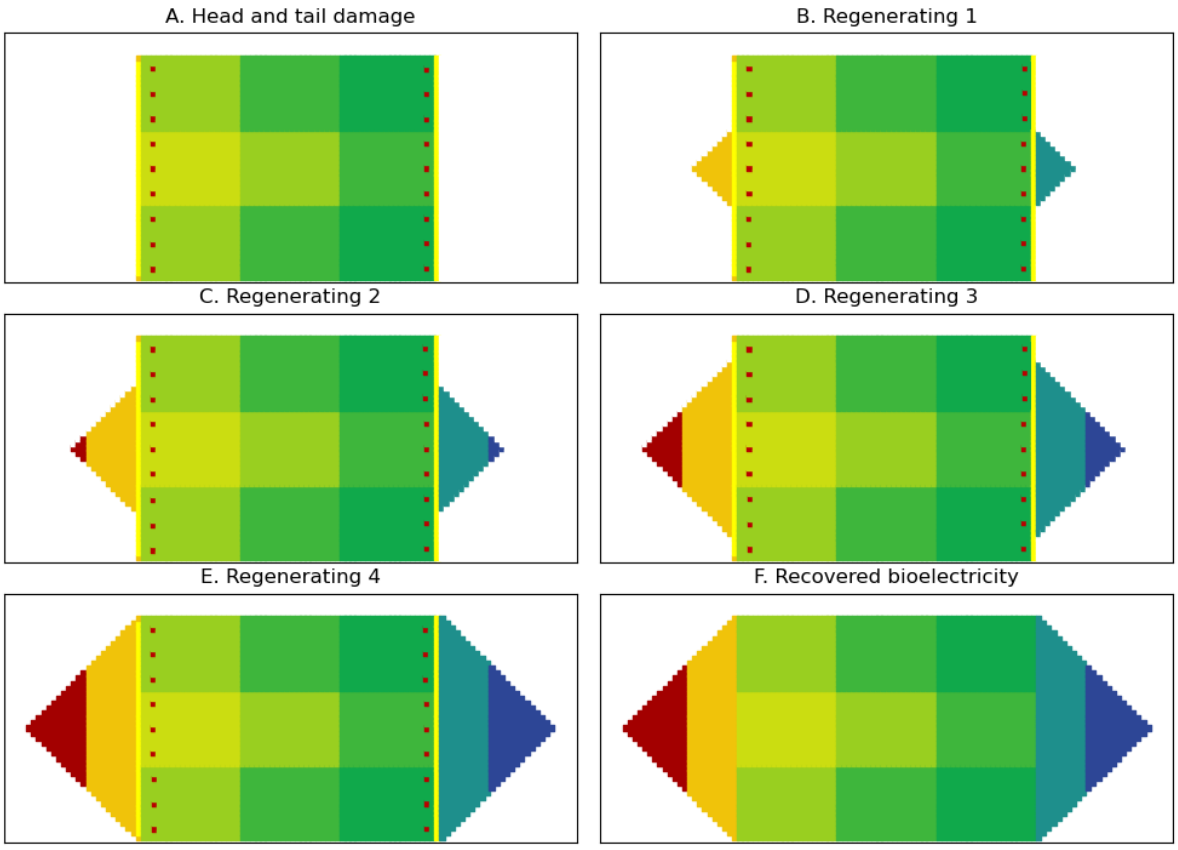


Figure 5.19. Body regenerates head and tail. (A) Severed body tissue; (B-E) production of new head and tail stem cells from the stem cells on the respective stem cells borders to regenerate head and tail tissues that grow incrementally until the full form is achieved; (F) AMN retrains the network and restores the original bioelectric pattern.

Left side damage:

Stem cells at the border produce new stem cells for head regeneration and transfer pattern information (State 4d) to them as in the previous case. Here, the voltage of the new stem cells is higher than that of the producing stem cell due to bioelectricity increasing from tail to head. The new stem cells produce a small head tissue (Figure 5.19B) at the centre of the damage border. The head increases its size and number of stem cells based on neighbourhood rules and pattern information (d, AR, n) (Figure 5.19B-E) (States 4).

Right side:

The mechanism is similar to the methods on the left side. Here, a tail is regenerated with a lower voltage. Regeneration steps are as in the (Figure 5.19B-E) (States 4d&e).

After regenerating the head and tail, the AMN restores the voltage in the whole worm by reconfiguring and retraining the AMN (Figure 5.19F) (State 5).

The tail regenerates body and head: The process is similar to head regeneration steps.

We also simulated some complex and severe damage cases which are presented in Appendix A.

5.8 Discussion

In this Chapter, we asked the question of whether it is possible to conceptualise the mechanisms and algorithms of regeneration of an amazing model organism like planarian that achieves body-wide immortality and maintains bioelectric homeostasis that allows the organism to continue functioning. We proposed an autonomous computational self-repair framework for a simple *in silico* worm that displays body-wide immortality and restoration of bioelectric homeostasis much like the planarian. The model combines three levels: somatic cell networks and stem cell network for damage identification and full and accurate recovery from diverse forms of injuries, and a high-level Associative Memory neural Network (AMN) for the restoration of body-wide bioelectric homeostasis. The efficacy in recovery from several very small to very complex damage cases was demonstrated in the implementation of the framework. The three networks work collectively as an integrated whole to operate the system to maintain the normal anatomical pattern and bioelectric homeostasis. We believe that this is the first work that provides a conceptual basis and algorithms explaining how an organism like the planarian can maintain and restore morphology and the bioelectric pattern.

The somatic cell network mainly focuses on detecting and identifying the border of tissue damage and transfer bioelectric signals to the stem cell. Stem cells as a network are the engine of regeneration. They sense the tissue damage, recognise and repair any stem cell damage, and repair any amputated parts as necessary. The AMN, which is a modified form of Hopfield network in this proposed model, mimics an unknown mechanism in planaria that maintains the voltage in each cell and the bioelectric gradient along and across the worm. In this model, bioelectricity is a fundamental property of the cells that helps cells communicate with each other to keep the original pattern and bioelectric gradient. Through bioelectric signalling, the stem cells can detect, identify, and locate the damage that guides them to know where to move to reach the location of the injury.

Our model contributes several concepts and algorithms to advance computational models of regeneration and a potential framework to generate hypotheses about the mechanisms of regeneration to advance the knowledge of regeneration. In this regard, we compare our model to the state-of-the-art computational regeneration models to demonstrate its novelty and efficacy. We have made a number of improvements to our previous model in Chapter 4.

Firstly, we proposed a greater number of stem cells, about 4% of the total. In the previous model, only three stem cells represented three tissue. In the new framework, a large number of stem cells are distributed within tissues. Secondly, we introduced two types of bioelectric communications- through

gap junctions (GJ) and through ionic fluxes released to the cell surrounding. Somatic cells use physical GJs to connect to and communicate with adjacent cells, while stem cells maintain long-range bioelectric communications propagated through the intermediate somatic cells (through GJs and then released into the surrounding). Compared to our previous model (Chapter 4) where we used long-range interactions by which a stem cell in the head can communicate with a stem cell in the tail, in this extended framework, stem cell communications are contained within a local region but a network of stem cells is distributed throughout the organism. This stem cell network is the driver of regeneration. Thirdly, we used an Associative memory network, a modified Hopfield neural network, as a mechanism to help the worm maintain the bioelectric gradient at equilibrium state in the body. We found a minimal network configuration for AMN (with the tail to head inhibition and head to tail activation) that efficiently stores and retrieves the equilibrium bioelectric pattern in 13 nodes each consisting of about 300 somatic cells and about 12 stem cells. This structure helps the body maintain a bioelectricity gradient from head to tail. From the AMN network configuration, we also established the connections between stem cells (directional activation and inhibition similar to the AMN).

Finally, we used three perceptron motifs which are trained for the stem cells so that the stem cell network can determine missing neighbour stem cells. Similar motifs were applied to the somatic cell network so that they can identify missing somatic neighbours. Weights of these motifs represent the bioelectrical conductance of Gap Junctions between somatic cells in living multicellular structures. These three motifs use cell voltage as inputs to communicate an output from a cell located anywhere in the organism. The two networks collaborate seamlessly to complete repair upon which the AMN restores the bioelectric gradient. For identification of various types of stem cell damage ranging from one or few stem cell damage to completely missing whole tissues, we proposed 3 simple rules applied to the pattern of the stem cell damage border consisting of stem cells that identify that their neighbours are missing.

In (Bessonov et al., 2015), all cells send and receive signals from each other and also compute the total received signals from other cells. The model has limited effectiveness due to the requirement of a lot of communications and computations from cells. In our model, somatic cells only communicate with adjacent cells through GJs and operate a simple motif. Stem cells sense the voltage in somatic cells in the neighbourhood and only interact with neighbour stem cells in a local region and do limited computation (applying the motif) when needed. The AMN with a simple structure of nodes and minimal interactions also does limited computations when it trains or retrains the network, which is quick due to the minimal network topology. Importantly, the AMN provides fault tolerance and flexibility without excessive micromanagement in bioelectric homeostasis.

In (Tosenberger et al., 2015), the authors proposed a global submodel which restores the original position of stem cells after some perturbations and a local submodel in which a stem cell maintains a circular tissue of cells. In our model, we do not arbitrarily alter the position of stem cells and restore their initial position. Instead, stem cells in our model move to the damage site and produce new cells (both stem cells and somatic cells) to repair the damage. The stem cells also regenerate the required size and shape of the worm from any damage correctly without culling cells unnecessarily (as in (Tosenberger et al., 2015)). Stem cell network repairs most damages using pattern information left in the intact tissue after damage; only in extreme cases where the whole perimeter of tissues are gone that they tap into the information field to extract minimum pattern information for the tissues.

An agent-based model (Ferreira et al., 2016) proposed by Ferreira et al. had a series of further improvements (Ferreira, Levin, & Scheutz, 2017; Ferreira, Scheutz, et al., 2017b; Ferreira et al., 2018; Lobo & Levin, 2015). Their model requires not only too much information to be remembered by stem cells but also a lot of communication between stem cells and somatic cells in the form of information packets needed for recognition of the form and regeneration. Regeneration accuracy of their model depends on the information in the packets. It is a trade-off between pattern information in the packets and regeneration rates. It can regenerate a whole organism correctly when a packet goes too far, which requires too much information to be remembered. Our framework relies on minimal communication and information burden with few neighbour rules for communication for the border, interior and corner cells; and three simple rules for identifying the extent of stem cell damage. It keeps minimal pattern information and the bioelectric gradient (attractor of the AMN) in the field so that they can be accessed by stem cells and AMN when damage is extreme.

The model in (De et al., 2017) has two elements: a neural network (representing the nervous system) and non-neuronal cells that communicate with each other. However, the model does not have the ability to sense and detect damage, while these features are important in autonomous regeneration in a living organism like the planarian. While this is one of the more bio-realistic models relying on physiological variables for regeneration, it fails to achieve complete regeneration. It produces and destroys too many cells before reaching a final form with some success. The accuracy of repair also depends on the form of damage. In our model, somatic cell and stem cell networks sense and repair damage immediately through GJs and bioelectric signals. Our model allows proper regeneration from any amputations and restoration of the equilibrium bioelectric pattern of the worm.

Although our model has efficiently emulated some important aspects of planarian regeneration, it has a number of limitations that can be improved. First, the form of the organism in this model is simple that needs to extend to a more complex worm with eyes, pharynx and a more realistic shape like the real planarian. Second, the voltage of somatic cells in each AMN node is fixed at a constant value that

approximated the known body-wide bioelectric gradient of planaria. These values can be made to vary within a node to achieve a more refined bioelectric gradient maintained by the AMN. The current-voltage values represent those that can be produced by partial differential equation-based models of voltage patterns produced by bioelectric communications between adjacent cells through Gap junctions. Our framework can combine these models and the models of the diffusion of important signalling molecules to represent bioelectric communication with molecular signalling pathways to activate biological processes such as cell division. This will further enhance the collective intelligence and decision-making capacity with the capture of all the processes involved in regeneration that will make the system more resemble living planaria.

5.9 Conclusions

In this research, we developed a conceptual framework explaining mechanisms and algorithms in autonomous regeneration or self-repair that mimicked some important features of planarian regeneration, including body-wide immortality and maintenance of bioelectric homeostasis. This computational model is very robust, plastic and fault-tolerant in simulating regeneration in an artificial organism. The communication between cells is minimised, where cells only communicate with neighbours or in a local region. The combination of the three networks enhances collective intelligence and decision making that helps the system efficiently maintain the normal state and repair when necessary. In future, we will extend the model into a self-recognising and self-organising system that is increasingly analogous to living organisms. As much knowledge of mechanisms of regeneration, cell to cell communication (between somatic cells and stem cells and among themselves) and the mechanisms of bioelectric homeostasis, is yet to be uncovered, our proposed conceptual framework allows advances to be made in generating hypotheses about these mechanisms and algorithms of regeneration. This could lead to future biological experimentation and discovery. Further, our comprehensive regeneration framework can inspire self-repair models in other domains, design of new biobots and artificial robots and promote bio-inspired computing for self-repair in other complex systems

Chapter 6

Summary, Conclusions and Future Directions

The overall goal of this thesis was to develop a conceptual framework for biological regeneration that allows regeneration from any damage similar to body-wide immortality of planaria and facilitates the generation of hypotheses for the potential mechanisms and algorithms of regeneration that allow such extreme form of recovery. The main objective of this research was to propose a conceptual framework based on intelligent systems for organism regeneration that have the capacity of detection and regeneration itself from any damage by integrating the observed features of regenerative biology, especially on planaria. The results from our framework that was developed and extended in stages through chapters 3-5 indicated that our conceptual and computational modelling of regenerative processes was successful in enhancing the capacity for complete regeneration of the organism with the minimal informational burden and computational requirements. The proposed approach, capturing the regeneration processes of the living organisms has displayed greater efficiency, plasticity, fault-tolerance, and robustness in maintaining the tissue and organism from any damage and perturbations and reproduced the important experimentally observed features of planarian regeneration. This study proposed new methods to simulate the regenerative processes in living worms that would be helpful in biology for biomedicine and in engineering for building self-repair robots and biobots. In the next section, we present a summary of what we have achieved from the whole study following the order of the three objectives. This is followed by a presentation of conclusions and contributions from this research. The last section presents suggestions for potential future research directions to extend the current framework.

6.1 Summary and contributions

In this research, the main conceptual framework contains three sub-frameworks corresponding to three main objectives that were presented in each chapter from chapter 3 to chapter 5. They were the result of asking, and incrementally addressing, some of the most fundamental questions of regeneration.

In chapter 3, **the first sub-framework, inspired by regeneration in living organisms, modelled an autonomous tissue regeneration system that is aware of its pattern and reacts to any damage to regenerate it efficiently.** This sub-framework developed the conceptual basis for a computational regeneration model for a simple tissue consisting of a stem cell surrounded by thousands of somatic cells. The tissue is in the form of an auto-associative memory network. Two types of communication exist in this model, where tissue (somatic) cells communicate only with adjacent cells (local bi-

directional communication) while the stem cell communicates with all tissue cells (long-range). This model consists of two sub-models: global sensing and local sensing that cooperate to sense, detect and repair the damage when needed.

The contributions of the computational model in this chapter are as follows. The framework allows a system to recognise itself in terms of both the system state and geometric pattern through a form of distributed collective intelligence. In the framework, global (GS) and local (LS) sensing models collaborate to detect where the damage is and how large it is and repair correctly in almost all cases. It proposes simple yet effective methods that accomplish regeneration with minimal information - the stem cell senses the damage through a change in a global property of entropy (GS), which is only activated when damage happens, and then it minimally activates the tissue network (LS) in the regeneration process. The system involves limited communication and information since only the stem cells communicate with all tissue cells while each tissue cell communicates only with adjacent cells and only global entropy and two neighbour rules are the system properties in the framework.

In chapter 4, **the second sub-framework proposed a conceptual basis and algorithms of regeneration for computational modelling of a multiple-tissue system (organism) with multiple tissue shapes that detects and regenerates a complex organism resembling a living planarian**. This is an extension of the first sub-framework to multiple tissues and an organism with some new concepts and algorithms. The organism consists of three tissues: head, body, and tail where the head and tail tissues are of triangular shape while body tissue is of rectangular shape. Each tissue model is similar to the circular model in chapter 3 that has a stem cell and surrounding tissue cells. Similar to the circular tissue, these new tissue cells form an Auto-Associative Neural Network with local interactions and the respective stem cells maintain tissue entropy. The tissues operate with Global and Local Sensing as before for tissue damage identification and recovery. Further, the three stem cells form a network with long-range interactions represented by a linear neural network that allows detecting and recovering any missing stem cells and large-scale damage to the organism. Additionally, each stem cell extracts minimum pattern information from its communication with the tissue cells and stores it in an information field shared with other stem cells. Regeneration in this model is accomplished by collaboration between the tissue cell networks and the stem cell network to recover the tissues and stem cells from any form of damage anywhere in the system resembling body-wide immortality of planaria.

This framework proposed a number of conceptual and algorithmic contributions to advancing the capability of regenerative models. From a conceptual perspective, our framework presented a new concept of tissue and stem cell cooperation to mimic the observed planarian regeneration. We also proposed a new concept of intelligent computational tissues. From an algorithmic perspective, we

introduced new but simple algorithms with the minimal computational burden. Moreover, we introduced an information field to stem cells for keeping minimum pattern information collected from the tissue and sharing with the other stem cells in the case of extreme damage. Specifically, it extended our previous circular tissue self-repair model with enhanced stem cell capability to a multiple tissue organism. Another novelty is the stem cell network which monitors the stem cell state and produces new stem cells when necessary. These tissue and stem cell network formed a 2-Level regeneration framework for efficient pattern restoration after any damage. These advancements have made the capability of our framework far exceed that of past biological regeneration models.

In chapter 5, **the third sub-framework, which brings the proposed regeneration framework to completion, provides the conceptual basis and algorithms for autonomous computational self-repair of a complex multicellular organism (*in silico*) that displays body-wide immortality and restoration of bioelectric homeostasis, much like the planarian.** This model addressed the issue of both form and function restoration during regeneration. It accomplishes this in the context of a virtual organism that more closely resembles planarian than our previous model worm. This framework significantly extended the previous one (chapter 4) with stem cells that now constitute over 4% of planarian tissues and communication between cells through bioelectricity. The model combines three levels: somatic cell networks and stem cell network for damage identification and full and accurate recovery from diverse forms of injuries, and a high-level Associative Memory neural Network (AMN) for the restoration of body-wide bioelectric homeostasis. The three networks work collectively as an integrated whole to operate the system to maintain the normal anatomical pattern and bioelectric homeostasis under any perturbations, by correctly recovering both pattern and equilibrium bioelectric state after facing any damage. Further, the AMN autonomously restores the bioelectric state under normal perturbations during regular functioning. This is the first approach that shows how an organism can maintain and restore the bioelectric pattern similar to planaria.

This model contributed several concepts and algorithms to advance computational models of planarian regeneration and to advance the knowledge of regeneration through a framework for generating hypotheses about the mechanisms of regeneration. Firstly, we proposed a greater number of stem cells that collaborate with somatic cells to make complete and accurate regeneration in a more realistic tissue system. Although a large number of stem cells exist in the organism, the communication of stem cells is reduced significantly where stem cells communicate only with their neighbour stem cells and only with somatic cells in a local range (in a small square tissue). Secondly, we introduced bioelectricity that allows cells communicate with each other, and also triggers and drives regeneration processes of the stem cell network. Thirdly, we developed a modified form of the Hopfield neural

network to maintain bioelectric homeostasis at the equilibrium state and it enabled the restoration of the bioelectric pattern after regeneration. This is the first application of an associative memory network to preserve the internal bioelectric pattern in planarian regeneration. Finally, we used the Perceptron motifs with learning ability for stem cell and somatic cell networks to determine whether neighbours are missing. Learning can be considered as the process by which cells find their neighbours, establish the connections to neighbours, and strengthen or weaken the connections that reflect the electrical conductance of Gap Junctions between somatic cells in the living tissue. Thus, this framework, with many biological features, is more realistic and far exceeds the capability of past regeneration models.

6.2 Conclusions

This study developed a conceptual framework for biological regeneration, incorporating what is known and what is required, that regenerates from any damage to accurately maintain original pattern homeostasis as well as bioelectric homeostasis, resembling the body-wide immortality in planaria. The proposed framework was developed in stages beginning with a simple computational tissue, then extending to an organism of multiple tissues, and finally extending to a complex organism with a great number of stem cells and bioelectric communication. The goal of this study was accomplished by mimicking some observed important features of planarian regeneration, including body-wide immortality and maintenance of bioelectric homeostasis. The results showed that our framework is very robust, plastic and fault-tolerant in simulating regeneration in the artificial organism with minimal communication between cells and computation in each cell. The proposed framework that outperforms the capacity of previous regeneration systems in literature creates better hypothetical explanations of the biological regenerative process, as discussed in this thesis. The promising results of these frameworks suggest the possibility for biological systems to autonomous sense and regenerate completely. Although much needs to be done to make the frameworks more resemble regeneration in living systems, they could readily be the guide for self-repair systems in synthetic biology such as bioengineered and artificial robots and pave the way for the next-generation computational frameworks for advancing and addressing some of the pressing issues in regenerative medicine in the area of birth defects, limb regeneration and many more.

6.3 Future research

The outcomes of this research suggest several directions for further research in future.

- Stem cells still account for a small fraction (approximately 4%) of the total cells that seem unrealistic for a planarian. Therefore, we plan to increase the number of cells to about 20% of the total cells.

- The form of the organism in our models is simple that needs to extend to a more complex worm with eyes, pharynx and a more realistic shape like the real planarian.

- The voltage in each AMN node is fixed at a constant value that approximated the known body-wide bioelectric gradient of planaria. These values should be changed to vary to achieve a more refined bioelectric gradient. AMN can be extended to store more than one attractor as planaria can regenerate into double-headed forms and a number of other forms with perturbations to the bioelectric state.

- We will extend the model into a self-recognising and self-organising system that is increasingly analogous to living organisms.

- The voltage pattern used in the study represents values that can be produced by partial differential equation-based models of voltage patterns. Our framework can combine these models and the models of the diffusion of important signalling molecules to represent bioelectric communication with molecular signalling pathways to activate biological processes such as cell division. This will further enhance the collective intelligence and decision-making capacity that will make the system more resemble living planaria.

- Finally, our comprehensive regeneration framework can inspire self-repair models in other domains, design of new biobots and artificial robots and promote bio-inspired computing for self-organisation in other complex systems.

Appendix A

Table A1: Weights of perceptron motifs

	Bias	Input 1	Input 2	Input 3	Input 4
Motif 1	-0.01	0.01	0.01		
Motif 2	-0.2	0.1	0.1	0.1	
Motif 3	-0.05	0.01	0.01	0.03	0.01

Table A2: Weights of Associative Memory Network (AMN)

Node	1	2	3	4	5	6	7	8	9	10	11	12	13
1	0.0	0.01	0.0	0. 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	-0.86	0.0	0.01	0.01	0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	-1.0	0.0	0.01	0.01	0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	-1.0	0.01	0.0	0.0	0.0	0.01	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	-1.0	0.01	0.0	0.0	0.0	0.0	0.01	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	-1.0	0.0	0.0	0.0	0.01	0.01	0.01	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	-1.0	0.0	0.01	0.0	0.0	0.0	0.01	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	-1.0	0.01	0.0	0.0	0.0	0.0	0.01	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	-1.0	0.0	0.0	0.0	0.01	0.01	0.01	0.0
10	0.0	0.0	0.0	0.0	0.0	0.0	-1.0	0.0	0.01	0.0	0.01	0.0	0.0
11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-1.0	0.01	0.0	0.0	0.01	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-1.0	-1.0	-1.0	0.0	0.01
13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-1.0	0.0

Node	1	2	3	4	5	6	7	8	9	10	11	12	13
Bias weight	-0.243	-0.5	-0.634	-0.688	-0.688	-0.732	-0.837	-0.837	-0.83	-0.936	-0.936	-1.99	-1.145

Further examples of implementation of the final framework – Recovery from more complex damage cases

This section presents how the same three simple pattern primitives applied to the stem cell border as explained in Chapter is able to detect even more complex damage cases and how the framework successfully and completely regenerates the whole worm. Specifically, if all three primitives apply, damage is local, and otherwise, it is whole tissue damage.

A.1 Case 1: Separation of whole tissues along with interior damage

Figure A.1 depicts an injury severing head and tail from the body and further incurring damage to the interior of the body of the worm (Figure A.1A). This is a combination of three damages: head, tail, and a group of interior body cells (AMN node in this case) missing. Figure A.1B shows the group of cells removed from the body of the worm.

For the damage in Figure A.1A, the three combined damages mean that it requires a combination of the three corresponding recovery processes. Using the three primitives, stem cells identifies two 'whole tissue' damages and the 'local damage' to the tissue. The worm achieves head and tail regeneration as in the Case E – Body regenerates Head and Tail and the case D – An AMN node damage in Chapter 5. Figure A.2 shows a set of stages in the repair of the worm starting from the remaining body tissue as in Figure A.2A and the final step of restoration of the voltage pattern. This regeneration is achieved by using the steps described for the example cases E presented in Chapter 5 of this thesis.

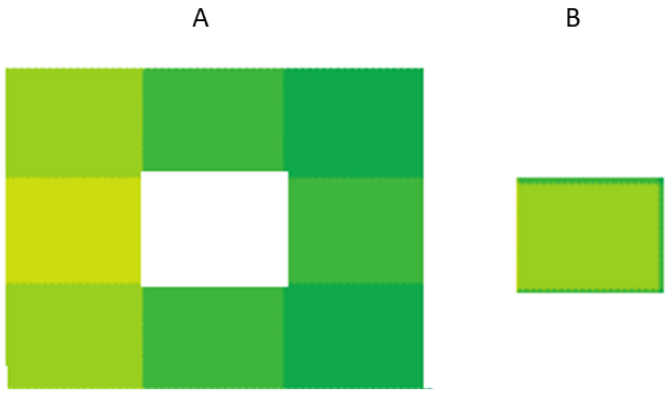


Figure A.1. Complex damage: A) Separation of parts (head and tail) and a portion of the body interior; B) portion separated from the body interior (A block represents an AMN node)

Figure A.3 shows the regeneration of the full worm from the small interior part separated from the body, as in Figure A.1B. The worm achieves this by applying the same procedures as in the case of body regeneration (Case E – body regenerates head and tail, Chapter 5). The recovery steps are illustrated in Figure A.3. After determining the border and missing tissues (State 2, 3) (Figure A.3A), the stem cells

proceed to restore the head and tail (state 4). Head and tail regenerate concurrently starting from small tissues as a small worm (Figure A.3B-D) (state 4e). The worm will then grow into the exact original form following shape information taken from the Information field (Figure A.3E). After regeneration, the AMN is re-established, and the bioelectric pattern is restored (Figure A.3F) (state 5).

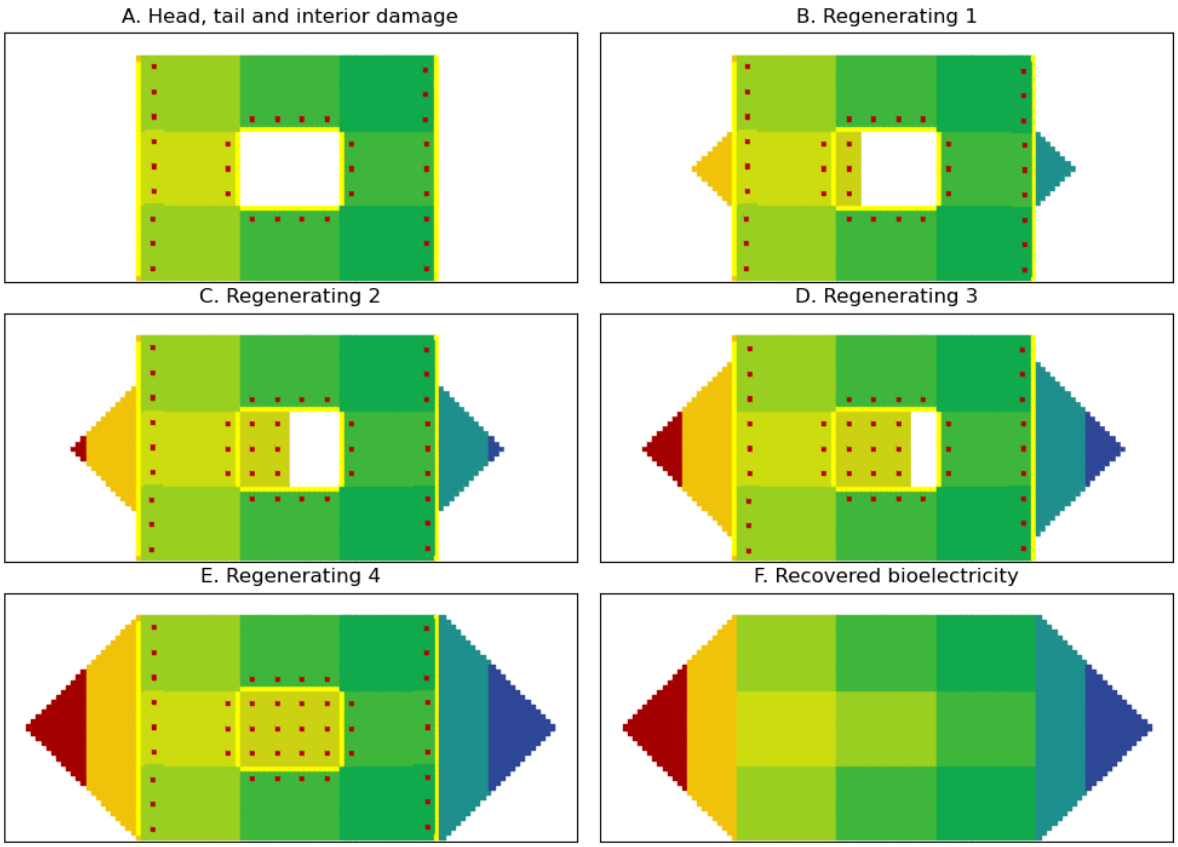


Figure A.2. Regeneration steps of damage in Figure A.1A

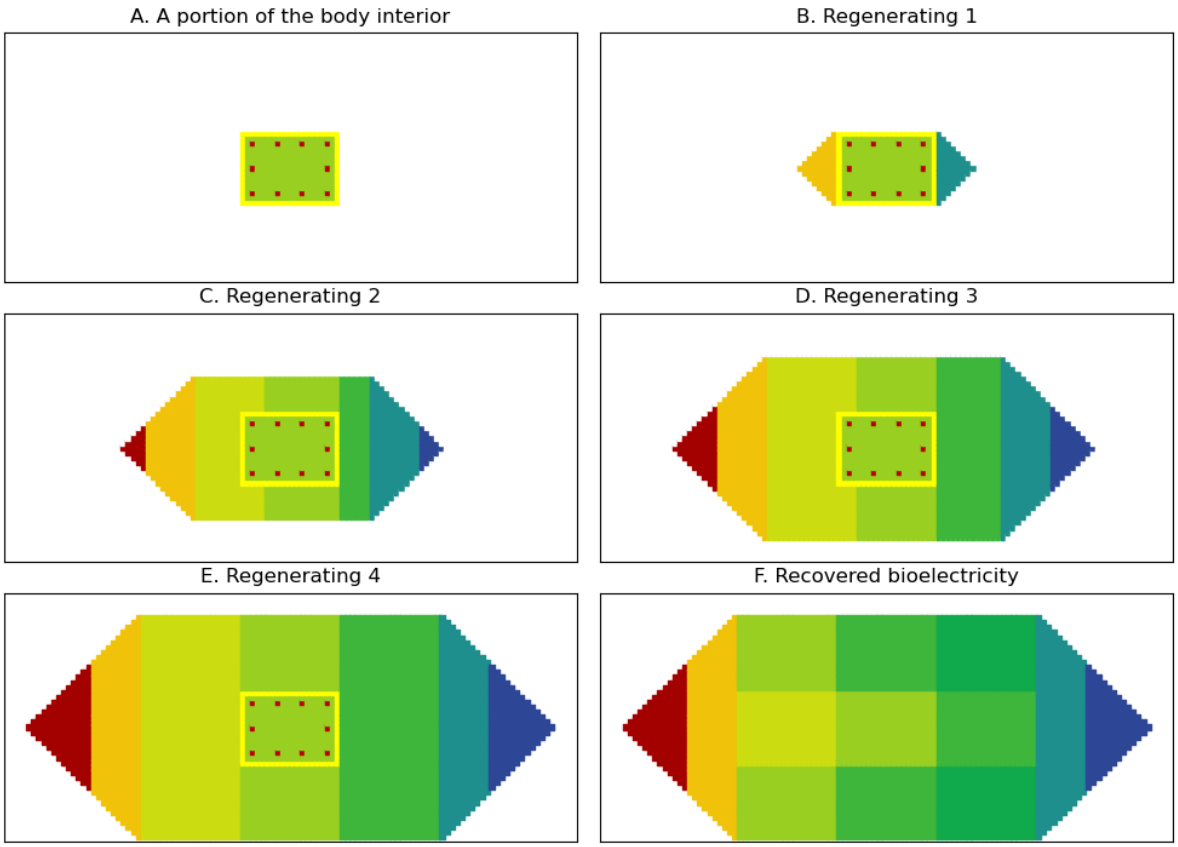


Figure A.3. Regeneration of the full form of the worm from the small fragmented part in Figure A.1B

A.2 Case 2

Figure A.4 describes another complex damage case where the worm is split into two with a vertical cut part-way through the body and then a horizontal cut through the body and tail: one part consists of complete head tissue and parts of body and tail tissues and the other part consists of the rest of the body and tail parts.

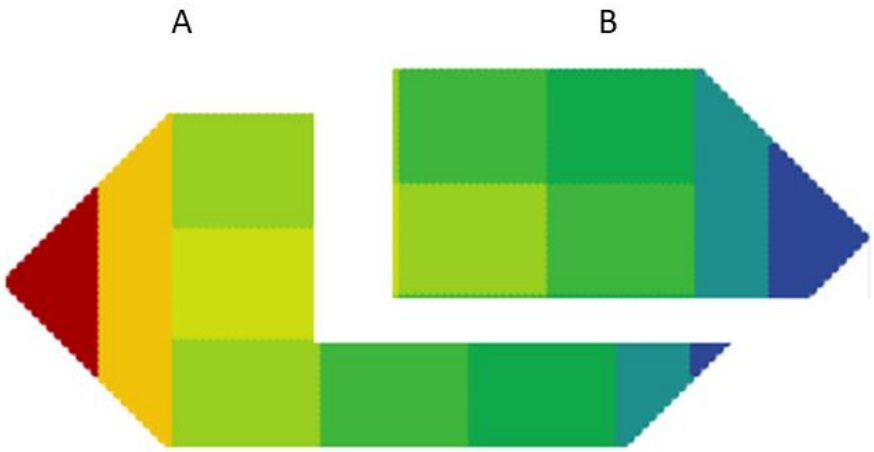


Figure A.4. Complex damage splitting the worm into two through a number of issues. (A) Part 1: Intact head and body and tail damage. (B) No head and body and tail damage.

Part 1: As shown in Figure A.4A, after damage, there is still the complete head, about half the body, and a portion of the tail tissue left. Stem cells identify and repair damage as the case of 'many stem cells missing'. The shapes of the body and tail tissues are determined from the current borders and length of sides (d) accessed from the Information field. Regeneration steps for this case are shown in Figure A.5.

Part 2: There is still about half the body and a large portion of tail tissue left after damage (Figure A.4B). The process of damage detection and identification is as in the above cases, and specifically, stem cells identify the head missing (whole tissue loss) and damage to the body and tail parts (local damage). Then, it regenerates a new head. Stem cells get pattern information for the new head from the information field (length (d), aspect ratio AR, corners (n)) and regenerates a head. The repair of the body and tail relies on the pattern information retained in the damaged tissue and extracting the length and width information from the field. Regeneration steps of this damage are shown in Figure A.6.

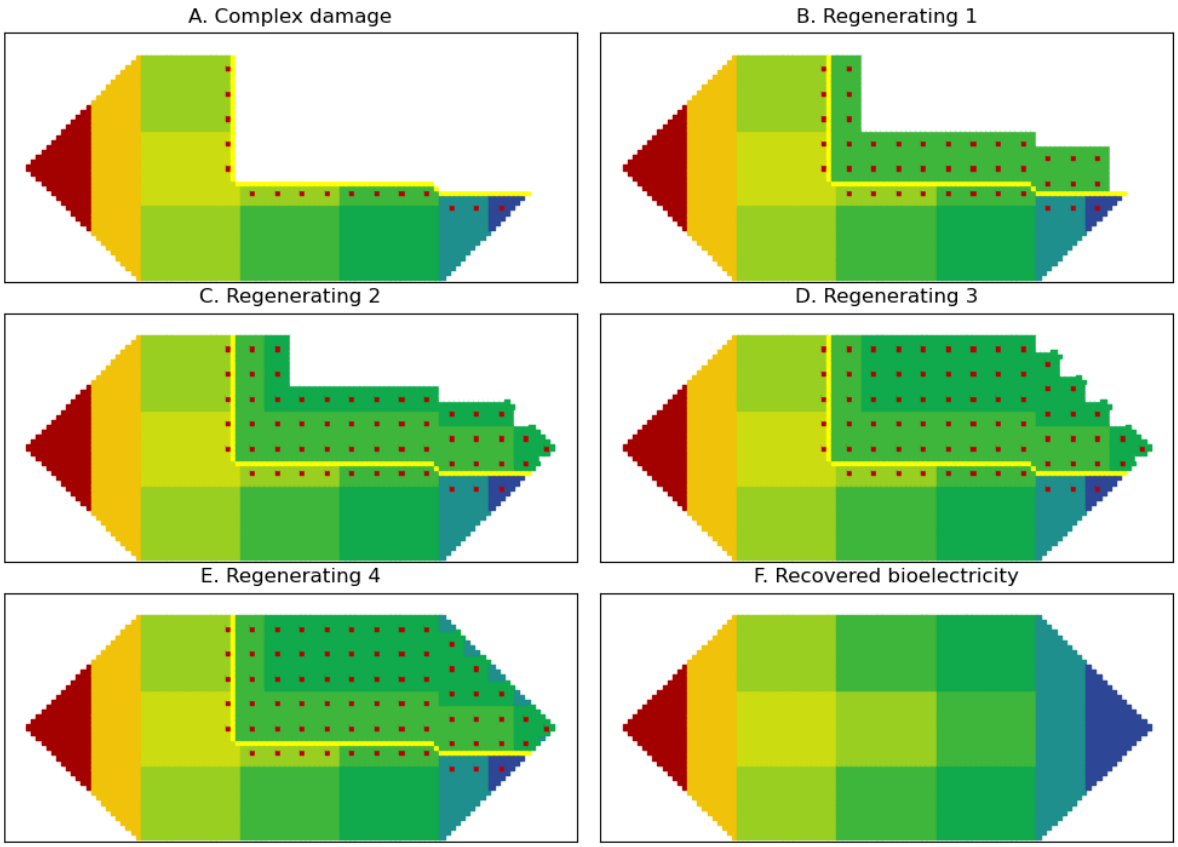


Figure A.5. Regeneration steps for the damage in Figure A.4A

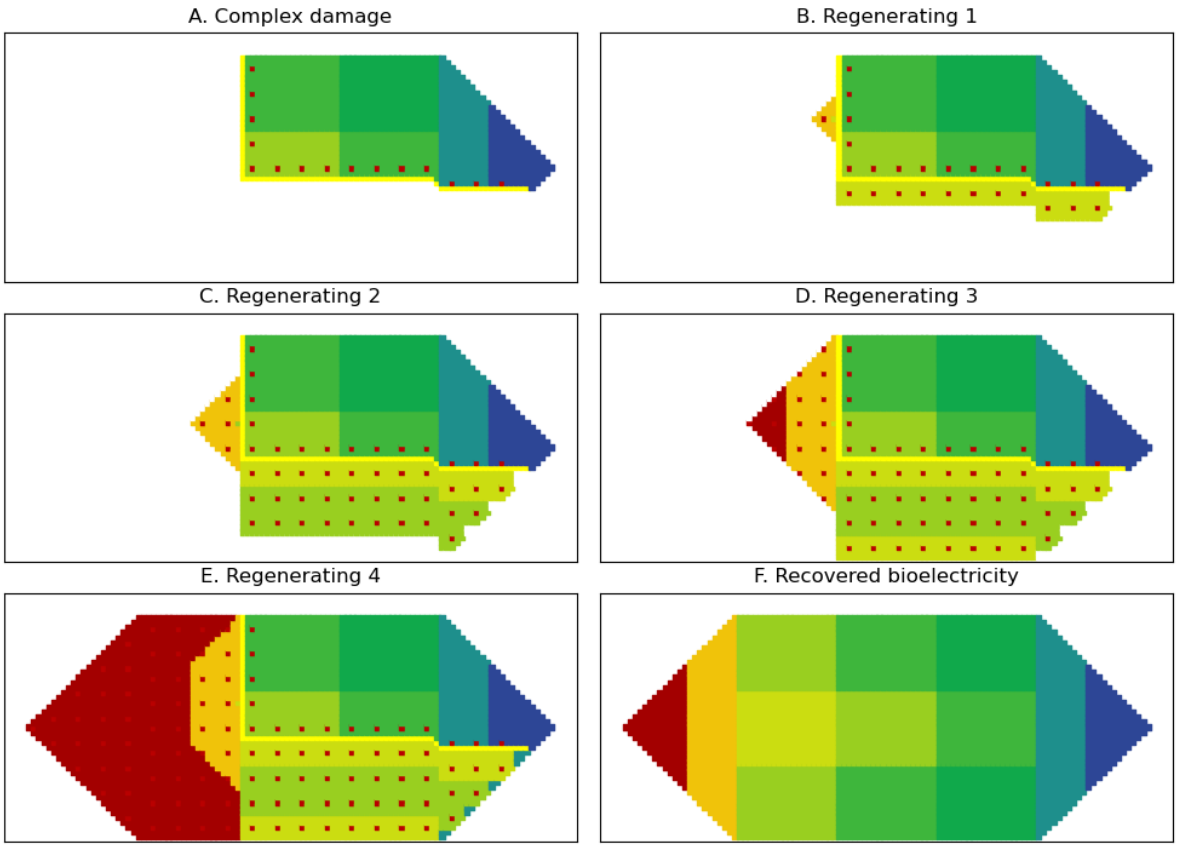


Figure A.6. Regeneration steps for the damage in Figure A.4B

A.3 Case 3

Another damage case is shown in Figure A.7A, where a large tissue from the body to tail is missing. This damage can be repaired as the damage in Figure A4.A. However, stem cells do not need to access pattern information because there remains a good portion of the head and tail from which pattern cues can be gained. New cells produced at the border are guided by existing cells in the border. Regeneration steps are shown in Figure A.7.

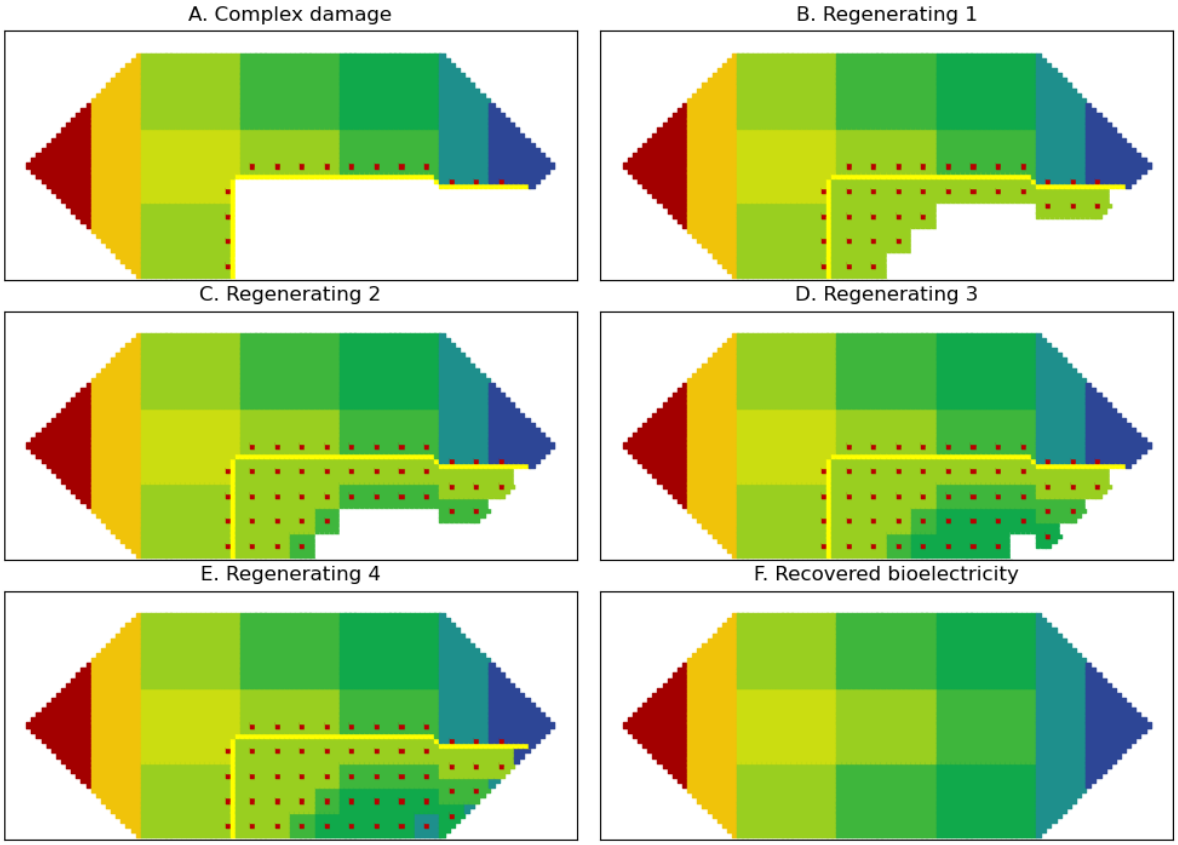


Figure A.7. A large tissue from the body to tail missing

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