

Modelling the Role of Tissue Inhibitors of Matrix Metalloproteinases as Regulators of Wound Healing

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Dedicated to Mum and Dad

Abstract

Understanding the biochemistry and pharmacodynamics of chronic wounds is of key importance, due to the millions of people in the UK affected and the significant cost to the NHS. Chronic wounds are characterised by elevated concentrations of matrix metalloproteinases (MMPs) that destroy the surrounding extracellular matrix (ECM). However, fibroblasts can produce tissue inhibitors of MMPs (TIMPs) in order to regulate wound healing. The role of MMPs and TIMPs in both acute and chronic wounds has been largely under explored in the mathematical modelling literature.

This thesis develops mathematical models to investigate the interplay between MMPs and TIMPs in wound healing. We demonstrate that the models support travelling wave solutions under parameter regimes representing both acute and chronic wounds. Through bifurcation analysis, the models replicate key qualitative behaviours observed in experimental studies and provide insights with potential clinical relevance. In particular, we show that increased TIMP production enhances regulation of chronic wounds, whereas lower levels of TIMPs hinder reversal to a healed state. One of our models is extended to a more realistic two-dimensional spatial domain to explore the application of an external TIMP source as a therapeutic strategy. Asymptotic analysis is used to elucidate how key parameters influence TIMP delivery, regulatory effectiveness, and wound healing progression. Finally, we turn to experimental data on TIMP-containing hydrogels currently being developed as a treatment for chronic wounds. We formulate a mathematical model and conduct parameter estimation, revealing significant parameter non-identifiability in the current experimental setup. To address this, we design a synthetic data study to determine the experimental design required for accurate parameter inference, thereby laying the groundwork for future studies assessing the efficacy of such hydrogels in wound treatment.

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Throughout my PhD, I've had a lot of practice trying to perfect academic writing, but here I'm going to keep this short, sweet and simple. This PhD has probably been the most difficult yet biggest accomplishment of my life, and I'm so grateful to many people, without whom it simply would not have been possible. Firstly, I would like to wholeheartedly thank my supervisors, Nabil and Reuben, for all their guidance, support and encouragement, especially when things felt overwhelming. Their wisdom and attention to detail led to two publications which is a feat I didn't think I would achieve but did, thanks to their making me a better researcher. Big love to all my wonderful friends, thank you for the constant support, the fun times, and for always making me laugh when I needed it most. A special shoutout to the gyls from Brum and my other besties who've always been there for me — you know who you are! I'm also grateful for the friends I've made throughout my PhD journey, from my Charlie Aves fam to the C32 lot, and all the other PhD pals I've met along the way. I'm also very grateful for the love and support of my family, so a shout out to them goes without saying! To Leela Massi, my second mother, Ranjit Chacha, Manisha, Vaishali and Simran, lots of love. To Sanjay and Bhushan, thank you for all your guidance — the best big brothers. Big love. To my amazing partner Lauren, thank you for putting up with me throughout this and keeping me sane! Finally to Mum and Dad, thank you for all the love, support and hard work you've done throughout your lives to give me mine. This wouldn't have been possible without you.

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Chapter 1

Introduction and Literature Review

Wound healing is a complex physiological process that involves the coordinated action of various cell types and biochemical agents to repair tissue damage [1, 2]. In the UK, approximately 2.2 million people suffer from so-called ‘chronic wounds’ [3]. These wounds usually last 12–13 months and recur in 60%–70% of cases [4], potentially leading to serious complications, including chronic infection, functional impairment, and significant reductions in quality of life [5]. They also place a significant strain on the NHS, costing over £5 billion annually [3]. Therefore, the study of wound healing and potential therapeutic treatments of chronic wounds is an area of high importance. Chronic wounds are characterised by elevated levels of matrix metalloproteinases (MMPs) which although essential for wound repair, can excessively degrade the extracellular matrix (ECM) when overexpressed [6] thereby impairing healing. In response, tissue inhibitors of metalloproteinases (TIMPs) are produced to inhibit MMP activity [7] and thus play a regulatory role in the development of chronic wounds. Although extensive research into wound healing has been conducted, the modelling literature currently lacks a thorough exploration of TIMPs as regulators

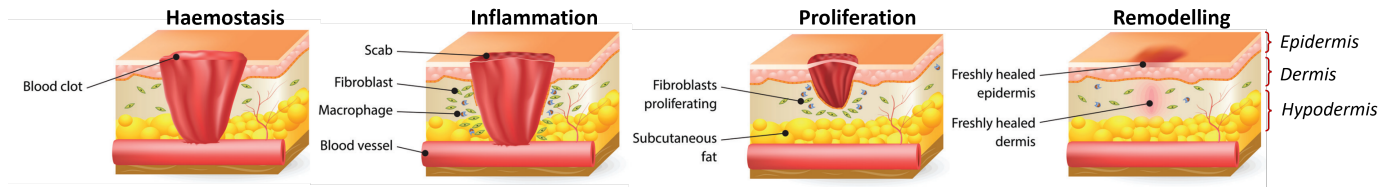


Figure 1.1: Schematic illustrating the four key stages of wound healing: haemostasis, inflammation, proliferation and remodelling. Adapted from Shield Healthcare [8].

in both acute and chronic wounds, and their potential as therapeutic treatments for chronic wounds. Therefore, in this thesis, we propose mathematical models of wound healing, focusing on the emergence of chronic wounds as a result of elevated concentrations MMPs, and the treatment of these using TIMPs as a therapeutic agent. The remainder of this chapter provides an overview of wound physiology, and presents a literature review of existing mathematical models of wound healing and associated treatments.

1.1 Biological Background

1.1.1 Stages of Wound Healing

The skin consists of three primary layers: the epidermis, dermis, and hypodermis. The outermost layer, the epidermis, acts as a protective barrier against infections and environmental factors such as sunlight and water [9]. Beneath it, the dermis accounts for approximately 90% of the skin's thickness [10] and provides tensile strength through the extracellular matrix (ECM)—a complex, three-dimensional network composed of key proteins such as collagen, elastin, and gelatin. This layer also houses blood vessels that deliver essential nutrients and oxygen to the skin. Lastly, the hypodermis, the bottom layer, is primarily made up of fatty tissue, serving as insulation to help regulate body temperature [9].

Damage to the skin tissue – whether limited to the epidermis and dermis or

extending into the hypodermis – results in a wound, initiating the four stages of the wound healing process: haemostasis, inflammation, proliferation and remodelling [1]. A wound that successfully progresses through these four stages in a well-coordinated manner is defined as an acute wound, whereas a wound that persists for a prolonged time in any of these four stages [6] and has not healed in 4 to 6 weeks is classified as a chronic wound [11]. Chronic wounds are particularly prevalent among individuals with diabetes, who are more susceptible to impaired healing and often require medical intervention to aid recovery [12]. We now outline the four stages of healing and we present a visual schematic of the stages of wound healing in Figure 1.1.

The haemostasis phase initiates wound healing by restricting bleeding from damaged blood vessels. This is achieved by first constricting the affected vessels, followed by the formation of a “platelet plug”—a cluster of platelets that temporarily seals the injury site [13]. Next, the wound undergoes the “coagulation cascade,” also known as secondary haemostasis [14], which stabilises the platelet plug to prevent further blood loss. This process activates clotting factors, enzymes that accelerate clot formation, leading to the development of the final clot, or “fibrin plug.” This fibrin plug securely seals the wound, preventing further blood loss and creating a foundation for tissue repair. As healing progresses, the clot gradually dissolves [15, 16].

With bleeding under control, the clot and surrounding tissue trigger an influx of inflammatory cells to the wound site via chemotaxis. This initiates the inflammatory phase of wound healing by activating the body’s immune response [1]. Neutrophils, the first immune cells to arrive to the wound site, play a crucial role in clearing bacteria, debris, and microbes. They also release proteases and reactive oxygen species (ROS) to further disinfect the area, ensuring a clean environment for tissue regeneration [17]. Neutrophils also stimulate the production of matrix metalloproteinases (MMPs), a family of 24 matrix-degrading proteases essential for wound healing [18], which we discuss in greater detail later.

As the inflammatory phase progresses, macrophages are recruited to the wound site, playing a pivotal role in transitioning to the proliferation phase. They achieve this by promoting the production of fibroblasts, keratinocytes, and endothelial cells, which form the single-layer lining of blood vessels [19, 20]. Fibroblasts, in turn, synthesise ECM components and release additional MMPs [6].

The proliferation phase is characterised by re-epithelialisation, during which new epithelial cells repopulate the wound site [21]. This process is driven by a complex interplay of signalling molecules, including nitric oxide (produced by macrophages), cytokines and growth factors, all originating from the inflammatory phase. Growth factors are signalling molecules that regulate cell growth, differentiation, and metabolism. Fibroblasts are responsible for creating a temporary fibrin mesh and further contribute to healing along with endothelial cells by restoring the vascular network and promoting capillary growth [20]. Capillary growth relies on two key mechanisms: angiogenesis and vasculogenesis. Angiogenesis involves the sprouting of new blood vessels from existing ones, whereas vasculogenesis refers to the formation of blood vessels from endothelial progenitor cells (EPCs) [21]. Both processes are critical, as wound healing is highly energy-intensive and depends on a continuous supply of oxygen and nutrients [22].

Following the formation of new blood vessels in the wound area, the temporary fibrin mesh produced by fibroblasts is gradually replaced by granulation tissue which is a transitional matrix primarily composed of collagen [23]. During this stage, fibroblasts also synthesise type-III collagen, which forms loosely arranged bundles that serve as a scaffold, facilitating fibroblast migration through the wound. The interaction between fibroblasts and collagen is critical for organising the ECM and laying the foundation for subsequent tissue remodelling [24]. Collagen types I and III are the primary structural components of the dermis, with type III initially being more prevalent in granulation tissue [25]. Type III collagen encases type I fibrils, resulting

in thinner fibres [26] and the balance between these collagen types is regulated by different isoforms of transforming growth factor-beta (TGF- β), a signalling molecule that plays a significant role in both the proliferative and early remodelling stages of healing. Studies have shown that modifying its concentration can reduce scarring - specifically, TGF- β 1 and TGF- β 2 promote fibrosis and scar formation, while TGF- β 3 is associated with reduced scarring and more regenerative healing [23].

The final stage of wound healing is remodelling, during which collagen is reorganised and the wound fully closes. As this process unfolds, the wound experiences contraction, and granulation tissue is gradually replaced through continuous collagen synthesis and degradation. The type III collagen in the granulation tissue is replaced by a denser arrangement of type I collagen. As this transition occurs, the tissue undergoes structural and mechanical changes, creating cellular stresses [23].

Eventually, granulation tissue matures into a scar, which differs structurally and functionally from the original tissue. The repaired area gradually gains tensile strength [1]. As angiogenesis ceases, the newly formed tissue transforms into an avascular scar, which can regain up to 80% of its original strength [24]. This process may continue for over a year as the tissue strengthens and stabilises [27]. Dysregulation of this remodelling process can lead to pathological scarring [24].

1.1.2 The role of MMPs and TIMPs in wound healing

MMPs play a crucial role in wound healing through several mechanisms. Each MMP exists in three forms, but only the active form participates in tissue repair. Key MMPs involved in this process include collagenases (MMP-1 and MMP-8), which break down collagen, and gelatinases (MMP-2 and MMP-9), which degrade gelatin. By breaking down ECM proteins, MMPs facilitate fibroblast migration, enabling tissue remodelling and proliferation [6]. Certain MMPs, such as MMP-7, are produced by cells at the wound edge and are essential for re-epithelialisation [18]. Experimen-

tal data supports this, showing that MMP expression remains elevated at the wound edge throughout all phases of acute wound healing [28]. Additionally, MMPs promote the activation of growth factors such as TGF- β by releasing them from their latent complexes in the ECM [29]. TGF- β then promotes ECM production and contributes to angiogenesis [29, 30]. Regulating MMP concentrations both spatially and temporally is crucial during wound healing. Insufficient MMP activity can lead to excessive ECM accumulation, increasing the risk of hypertrophic scarring or dermal fibrosis [31]. Conversely, chronic wounds are characterised by elevated MMP levels, leading to both prolonged ECM degradation and exacerbated inflammation and excessive TGF- β activity [30, 32]. One such cause for these elevated MMP levels is deregulated apoptosis of dermal tissue, which may be caused by uncontrolled blood sugar levels in diabetes patients [33, 34], which we elaborate upon in Section 1.1.3.

In response to elevated MMP concentrations, tissue inhibitors of metalloproteinases (TIMPs) are produced, primarily by fibroblasts. While much of MMP regulation occurs at the transcriptional level through signalling pathways [18], it is also controlled post-translationally through inhibition. TIMPs regulate MMP activity by binding to them, preventing excessive ECM degradation, and thereby maintaining balance in ECM remodelling and cellular behaviour. There are four types of TIMPs (TIMP-1, TIMP-2, TIMP-3, and TIMP-4), which function by binding tightly to the catalytic zinc atom of MMPs in a one-to-one complex, effectively controlling their enzymatic activity [35]. Some TIMPs exhibit greater affinity for specific MMPs; for instance, TIMP-1 has a strong affinity for MMP-1 [36]. Tuning TIMP activity to counteract elevated MMP levels has been shown to influence wound healing outcomes, highlighting their potential as therapeutic agents [37, 38]. One promising approach for TIMP delivery is through hydrogel-based therapies, a strategy currently being investigated in experimental research [39, 40]. In this thesis, we build on these studies by developing mathematical models to further explore this therapeutic approach.

1.1.3 Chronic Wounds

As mentioned previously, a chronic wound is one that experiences dysregulated healing – they persist for a prolonged time in any of the four stages of healing [6]. There are many causes and risk factors of pathological healing of wounds, with diabetes being one of the most significant, particularly impacting the feet and legs [5]. Understanding the biological mechanisms underlying chronic wounds is essential for developing mathematical models that capture wound behaviour during healing which we aim to do in this thesis, therefore, we outline key pathological features of chronic wounds that motivate and inform our modelling approach.

Diabetes is characterised by uncontrolled blood sugar levels which can impact healing in many ways [41]. Excessive blood sugars can cause damage to the vessels and nerves that supply wounds. This poor circulation reduces the delivery of oxygen (hypoxia) and nutrients to the wound which are essential for healing [5].

Hyperglycemia leads to excessive mitochondrial ROS production, activation of alternative glucose metabolic pathways, and impaired antioxidant defences, resulting in oxidative stress [42, 43]. In addition, ROS can directly damage ECM proteins by oxidising amino acids and disrupting cross-links [44]. Elevated ROS and oxidative stress also trigger apoptotic signalling pathways, causing dysregulated apoptosis of dermal cells [45]. This loss of normal cellular regulation sustains inflammation, and pro-inflammatory cytokines upregulate MMP expression, resulting in excessive MMP levels [34]. These elevated MMPs not only accelerate ECM degradation but also feed back to amplify inflammatory signalling [6].

Importantly, MMPs also interact with growth factor signalling: at moderate concentrations, MMP activity promotes the release and activation of latent TGF- β from the ECM [29, 30]. TGF- β plays a crucial role in stimulating fibroblast proliferation and ECM synthesis, thereby facilitating normal tissue repair [29]. However, when glucose levels are persistently elevated, oxidative stress and inflammatory sig-

nalling drive pathological MMP overexpression. In this high-MMP environment, the beneficial TGF- β -mediated healing response is overwhelmed, and ECM breakdown predominates [42]. Thus, blood glucose levels indirectly modulate the balance between constructive (TGF- β activation) and destructive (proteolysis, inflammation) roles of MMPs, tipping the system toward chronic non-healing when hyperglycemia is sustained.

Moreover, inflammation itself can damage the ECM through other mechanisms, including ROS [44] and reactive nitrogen species released by immune cells [46] and alternative proteases such as elastase and cathepsins. Together [47], these processes create a self-sustaining cycle that ultimately impairs wound healing [42, 44]. We remark that hyperglycemia may also directly promote a pro-inflammatory state through AGE-RAGE signalling and ROS production [48]. Pro-inflammatory cytokines promote deregulated apoptosis by activating death receptor signalling and inducing mitochondrial dysfunction, leading to excessive or poorly regulated cell death [49]. These interactions are summarised in the schematic in Figure 1.2.

Beyond diabetes, impaired blood circulation significantly increases the risk of chronic wounds. Proper blood flow is essential for healing, as it supplies oxygen and nutrients to damaged tissues. Conditions such as peripheral artery disease (PAD) and venous insufficiency can greatly slow the repair process. PAD narrows arteries, restricting blood supply and delaying tissue regeneration, while venous insufficiency impairs blood return to the heart, leading to fluid retention, swelling, and increased local pressure. This weakens the skin, making minor injuries more prone to becoming chronic wounds [5].

Other risk factors include weakened immune function, which slows healing or prevents wounds from closing entirely. Severe injuries that create large wounds may also overwhelm the body's healing capacity. Additionally, mechanical pressure, such as prolonged immobility in bedridden or wheelchair-bound individuals, can lead to

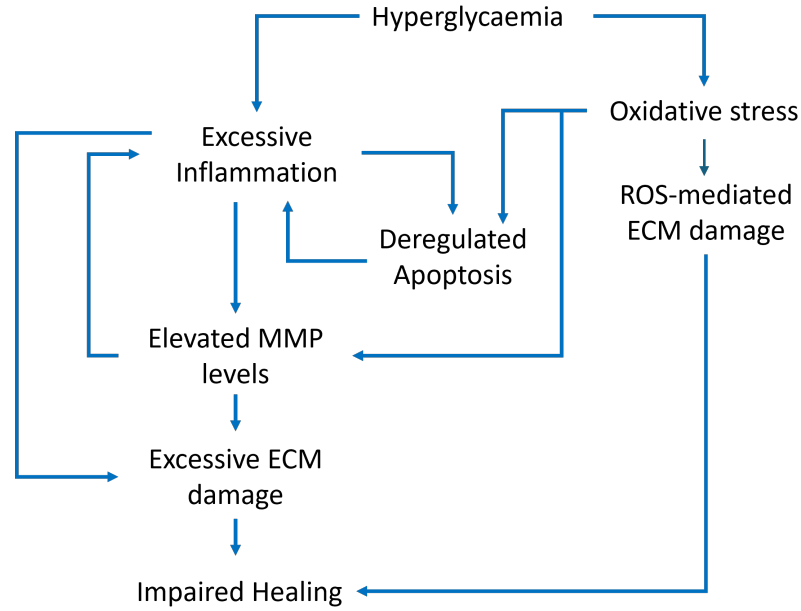


Figure 1.2: Schematic illustrating the biological processes by which hyperglycaemia impairs wound healing. Hyperglycaemia promotes oxidative stress, inflammation, dysregulated apoptosis, and excessive MMP activity. These processes interact to drive excessive ECM degradation and impaired tissue repair. This figure summarises the underlying biology motivating the modelling framework presented in subsequent sections.

chronic wounds due to sustained pressure on specific body areas [5].

Chronic wounds often require external intervention to promote healing. In the following section, we explore available treatment options.

1.1.4 Treatments of Chronic Wounds

The prevention of chronic wounds is regarded as a more efficient and sustainable strategy than treatment. Preventative measures include lifestyle adjustments that improve blood circulation, such as regular exercise and stopping smoking, as well as blood sugar control in diabetic patients. Proper footwear and good hygiene also reduce the risk of wound formation [5, 11].

However, when chronic wounds do occur, treatment options are often complex and resource-intensive. Infections may require antibiotics [5], but growing con-

cerns around antibiotic resistance limit their long-term efficacy [12]. Other approaches—such as negative pressure wound therapy [50], hyperbaric oxygen therapy (HBOT) [51], and skin grafting [5] can be effective but typically require clinical settings and specialised equipment, making them less accessible or suitable for long-term use.

An alternative treatment is the use of hydrogels, which can deliver therapeutic agents directly to the wound site in a controlled and sustained manner [52]. These offer the potential for cost-effective, localised, and non-invasive treatment [53]. Since this thesis focuses primarily on hydrogel-based treatments, we now explore them in greater detail.

1.1.5 Hydrogels

Hydrogels are a promising platform for delivering therapeutic agents to chronic wounds, such as for the sustained and localised delivery of TIMPs, which can counteract elevated MMP activity [40]. These materials are hydrated mesh networks composed of natural or synthetic polymers, engineered as an alternative to conventional drug delivery systems. They offer targeted and localised drug release while enabling controlled delivery, reducing both side effects and dosing frequency [53].

Structurally, hydrogels are three-dimensional, hydrophilic polymer networks with diverse applications in soft tissue engineering, including blood vessels, muscles, and skin. They consist of either physically or chemically cross-linked bonds, designed to replicate the skin’s microenvironment through their porous and hydrated structure [52, 53]. Hydrogels contain bioactive molecules which, once placed on the a tissue surface, may deliver these bioactive substances with precise spatial and temporal control [53]. Due to their versatility, hydrogels have been employed in the treatment of infections, cancer, and wound healing, offering a dynamic platform for drug delivery through multiple mechanisms [54]. One of the most common modes of drug release is

diffusion-controlled delivery, wherein the release is driven by concentration gradients, allowing for a diffusion-dominated transport of the drug molecule [55]. The efficiency and rate of diffusion are heavily influenced by the mesh size of the hydrogel network. When the mesh size is significantly larger than the drug molecule, the release is primarily dictated by passive diffusion. Conversely, if the mesh size is smaller than the drug, the molecule remains physically entrapped within the hydrogel matrix [52] – an essential feature for achieving controlled and sustained delivery [53].

In swelling-controlled release, drug delivery is regulated by the rate at which the hydrogel absorbs water and expands. As the hydrogel swells, the polymer network undergoes volume expansion, increasing the mesh size and gradually allowing the entrapped drug to diffuse out [55]. The release rate is thus directly linked to the swelling kinetics of the hydrogel in a physiological environment. Several factors influence this process, including pH, temperature [55], ionic strength, and the intrinsic properties of the polymer network [52]. A faster swelling rate leads to more rapid drug release, while a slower swelling rate results in prolonged and sustained delivery [52]. Alternatively, uncrosslinked polymer chains can undergo disentanglement and dissolution when exposed to a compatible solvent. While the initial swelling behaviour resembles that of swellable systems, a key difference is that the hydrogel eventually recedes inward as dissolution overtakes swelling, leading to complete polymer dissolution and thus dissolution controlled delivery.

When a drug is entrapped within the hydrogel such as through covalent bonding, its release is governed by chemically controlled mechanisms. This process relies on the cleavage of cross-linked chemical bonds within the hydrogel matrix, triggered by specific biochemical or environmental stimuli. Chemically controlled drug release systems are typically classified into two main categories: erodible and pendant chain systems. In pendant chain systems, drug release is triggered by the cleavage of a chemical bond between the drug and the polymer by hydrolysis or enzymatic activity

whereas in erodible systems drug release is governed by the degradation of the polymer matrix itself, allowing release of the drug [56, 57].

In the context of wound healing, hydrogels may be used as wound dressings, containing bioactive agents such as antibacterial agents and growth factors, which can then be delivered to the wound to accelerate its healing. We must have the correct concentration of bioactive substances in these hydrogels for optimum healing of a chronic wound [12]. An application of hydrogel-based drug delivery is the controlled release of TIMPs to counteract the elevated levels of MMPs that are characteristic of chronic wounds, offering a potential strategy for enhancing wound healing [39, 40]. In Chapter 5, we develop a mathematical model describing the behaviour of MMP-responsive hydrogels introduced in [40]. These hydrogels incorporate cross-linked bonds that are cleaved in the presence of MMPs, initiating the release of TIMPs. Following cleavage, TIMPs are released via diffusion, resulting in a system that couples chemically triggered release with diffusion-driven delivery which forms the foundation of our modelling approach in Chapter 5.

1.2 Mathematical Models of Wound Healing

Wound healing is a highly intricate process involving the coordinated actions of numerous cell types, signalling molecules, and mechanical interactions. Given this complexity, mathematical models typically seek to represent key components of healing rather than capturing the entire process. These models can be broadly categorised into discrete and continuous frameworks.

1.2.1 Discrete Models

Discrete models of wound healing often employ agent-based or cellular automata methods. Agent based models (ABMs), in particular simulate individual cellular or

molecular components and effectively capture the individual-level stochastic nature of biological processes, making them valuable for modelling systems where emergent behaviour arises from complex cell–cell or cell–matrix interactions. A notable example of an ABM is provided by Sun et al. [58] who developed a two-dimensional ABM to simulate keratinocyte colony formation. They derived rules to simulate keratinocyte proliferation, migration, and differentiation and this rule-based structure enabled an accurate representation of epidermal dynamics. Building on this work, Sun et al. [59] extended the model to a three-dimensional framework in the context of epidermal wound healing. This model incorporated interactions between keratinocytes, ECM, and TGF- β 1 signalling. In doing this, the authors were able to identify a plausible sequence of TGF- β 1 actions necessary for effective wound healing. TGF- β 1 also acts as a key regulatory factor in a study applying an agent-based model to diabetic foot ulcers. By simulating reduced TGF- β 1, Mi et al [60] reproduced delayed healing marked by chronic inflammation and impaired collagen deposition. Notably, therapeutic strategies aimed at restoring TGF- β 1 activity improved outcomes regardless of the underlying cause, highlighting its central role in wound repair and its potential as a therapeutic target. Alternatively, Walker et al. [61] developed an ABM to consider keratinocyte behaviour in wound healing but in response to varying calcium conditions. Both the *in silico* model and *in vitro* experiments showed that low calcium accelerated wound closure primarily through cell migration. While the model captured core healing dynamics, deviations from experimental data underscore the limitations of simple rule-based models. Moreover, scaling ABMs to model wound healing dynamics at the tissue level can be computationally impractical [62].

Continuous models, by contrast, describe population-level behaviours through fields such as cell density, chemical concentration, or ECM density, typically governed by systems of partial differential equations (PDEs). These models offer a range of analytical and numerical tools for further investigation and as such, they are well-

suited for exploring tissue-scale healing phenomena. In the remainder of this chapter, we focus on continuous models, which can be further classified based on their underlying techniques, including mechanochemical models, reaction-diffusion models, and multi-scale models. We place emphasis on reaction-diffusion systems in particular, which form the foundation of the modelling work in this thesis. We adopt a reaction–diffusion framework because our focus is on the chemical interactions governing ECM regulation, particularly the balance between MMPs and their inhibitors. Reaction–diffusion naturally represents both the local biochemical reactions and the spatial transport. While mechanics (e.g. fibroblast contraction, matrix stiffness) do influence wound healing, they are not the central interest here, as the influence of MMPs and TIMPs is primarily chemical rather than mechanical.

We review models representing both epidermal and dermal wound healing. The former typically involves epithelial cell migration and re-epithelialisation of the wound surface, while the latter encompasses deeper tissue repair, including ECM remodelling, fibroblast and macrophage activity, and angiogenesis.

1.2.2 Models of Epidermal Wound Healing

Mathematical modelling has been instrumental in advancing our understanding of the complex interactions between cellular behaviours and biochemical signalling that govern the process of reepithelialisation during epidermal wound healing. Many foundational models build upon the seminal work of Sherratt and Murray [63], who formulated a reaction-diffusion model to describe the interaction between epidermal cells and a generic mitosis-regulating chemical signal over time t . The system consisted of two PDEs under the assumption that a shallow wound may be modelled using a two-dimensional spatial domain, wherein epidermal cells, n undergo random motion, mitosis, and natural death, while the chemical, c , is produced by the cells, diffuses spatially, and decays over time:

$$\frac{\partial n}{\partial t} = \underbrace{D\nabla^2 n}_{\text{random cell motion}} + \underbrace{s(c)n\left(2 - \frac{n}{n_0}\right)}_{\text{mitotic generation}} - \underbrace{kn}_{\text{natural death}} \quad (1.1)$$

$$\frac{\partial c}{\partial t} = \underbrace{D_c \nabla^2 c}_{\text{diffusion}} + \underbrace{f(n)}_{\text{production by cells}} - \underbrace{\lambda c}_{\text{decay of chemical}} \quad (1.2)$$

Despite the simplicity of this model, it was able to qualitatively capture key features of wound closure dynamics, such as the temporal evolution of the wound radius. The model also highlighted the importance of chemical signalling in regulating mitotic activity during healing and the modelling framework introduced in this study was highly influential on many subsequent wound healing models [64]. One such model developed by Dale et al. [65] considered corneal wound healing, incorporating the effects of epidermal growth factor (EGF), a signalling molecule known to stimulate epithelial proliferation. Their one-dimensional model described the interaction between EGF and epithelial cells and was validated against experimental data on the speed of wound healing. Notably, the results revealed that while EGF had limited influence on cell migration, it played a crucial role in enhancing mitosis. The study further proposed that an additional EGF source, likely from wounded tissue, was required to support effective reepithelialisation. The therapeutic implications of EGF-mediated healing were explored further by Sheardown and Cheng [66], who investigated why EGF treatments in humans are less effective than in animals. Using a similar reaction-diffusion framework, they compared various EGF delivery strategies and found that continuous delivery achieved significantly better outcomes than intermittent application, such as with standard eye drops. This work emphasised the need to tailor treatment regimens to match the pharmacokinetic and biological demands of human tissue.

While the model by Dale et al. [65] accurately predicted the rate of corneal wound closure, it did not replicate observed spatial patterns of cell proliferation, er-

roniously suggesting higher mitotic activity near the wound centre. To address this discrepancy, a refined model introduced two distinct cell populations: proliferative and quiescent cells, with mitosis restricted to the proliferative cells [67]. The two cell types were assigned different migration rates and the regulatory effects of juxtacrine signalling on mitosis were included – a mechanism known to be important to healing [68]. This revised framework showed strong agreement with experimental data, successfully reproducing mitotic rates across space and time. A notably interesting feature of corneal wound healing observed experimentally is that cell migration at the wound edge responds to endogenous electric fields generated by transcorneal potential difference [69]. To account for this, Gaffney et al. [70] incorporated an electrotaxis term into their model, revealing a linear relationship between wound healing speed and electric field strength, highlighting a potential avenue for therapeutic intervention in corneal wounds.

Beyond corneal wound healing, wound geometry has been shown to play a crucial role in epidermal wound healing. In 1992, Sherratt and Murray [71] revisited their mathematical model (1.1)–(1.2) to investigate how healing time varies with wound shape. They found that for epidermal wounds with cusped shapes, healing time follows a similar pattern regardless of whether mitosis is controlled by activation or inhibition. For ovate wounds however, the two mechanisms led to different behaviours with ovate wounds flattening and healing faster under the inhibition mechanism. The authors suggested that this result is linked to the way wounds change shape during healing - pointed wounds round out over time in both cases, while rounded wounds flatten more significantly under the inhibition mechanism. This flattening expands the wound perimeter which speeds up healing, highlighting the impact of wound morphology on healing outcomes. Savakis and Maggelakis [72] approached the modelling of epidermal wound geometry from a different angle by adapting a diffusion-limited aggregation model. Their findings aligned with empirical evidence, confirming

that areas of high curvature heal first. Additionally, their model demonstrated that the healing rate is approximately proportional to the mean radius of epidermal cell clusters, reinforcing the connection between curvature and closure dynamics.

These models, building upon a foundational reaction-diffusion modelling framework have incorporated additional complexities involved in epidermal and corneal wound healing, such as growth factor signalling, juxtacrine regulation and geometric influences. However, epidermal healing is typically confined to reepithelialisation and represents only part of the wound repair process. In contrast, dermal wound healing encompasses a more intricate interplay of biochemical and cellular mechanisms, including inflammation, fibroblast activity, and angiogenesis. The increased biological complexity requires more intricate modelling approaches, thus accordingly, we now turn our attention to mathematical models of dermal wound healing.

1.2.3 Models of Dermal Wound Healing

Much of the mathematical modelling work in wound healing has focused on dermal wounds, where the biological processes are more complex and multifaceted than in epidermal healing. Broadly, these models fall into two categories: chemical models, which focus on the dynamics of growth factors, fibroblasts, and angiogenesis; and mechanochemical models, which additionally incorporate tissue mechanics, particularly the role of contraction during healing.

Angiogenesis

A central component in dermal wound healing is angiogenesis, the formation of new blood vessels from pre-existing ones, which is essential for restoring oxygen and nutrient supply to the wounded tissue [22]. One of the earliest and most influential mathematical frameworks for modelling angiogenesis was the “snail-trail model”, introduced by Balding and McElwain to describe tumour-induced angiogenesis [73],

which was adapted from Edelstein’s earlier work on fungal growth [74]. This model describes the interactions between blood vessel density, capillary tip density, and tumour angiogenesis factors within a one-dimensional spatial domain. Building on this foundation, Chaplain and Byrne [75], like Balding and McElwain, employed a one-dimensional reaction-diffusion model incorporating the same three variables, however, they extended the earlier model to include greater biological realism by introducing random sprout motility alongside chemotaxis, thereby capturing observed endothelial tip movement along ECM fibres. They also distinguished between two modes of tip proliferation—one from existing vessels and another activated by high concentrations of angiogenic factors—allowing for a more detailed representation of vascular growth. While Balding and McElwain aimed to establish a conceptual framework, Chaplain and Byrne advanced toward a quantitative one, capable of predicting vascularisation outcomes based on known parameters.

The work by Chaplain and Byrne paved the way for its application to wound healing contexts. Pettet et al [76, 77]. were among the first to do so, adapting the snail-trail framework into a wound healing context by formulating both a six-variable and a simplified three-variable system of PDEs. Their findings indicated that low oxygen levels promote healing, while excess oxygen inhibits further angiogenesis, emulating experimental observations. Further refinement came from Byrne et al. [78], who extended the model to a circular domain and fitted the results to experimental data tracking the evolution of acute and chronic wounds over time. Their analysis suggested that impaired healing may stem from factors such as capillary response, the rate of cell proliferation at capillary tips, and the production of angiogenic factors.

While the snail-trail based models primarily track blood vessel density and capillary tips, other researchers have explored alternative perspectives. Olsen et al. [79], for instance, developed a model centred on endothelial cell dynamics rather than capillary structures. Their model demonstrated that the ECM regulates the random

motion of endothelial cells (haptokinesis) and directs their movement toward areas of higher ECM density (haptotaxis). Gaffney et al. [80], on the other hand, modelled two distinct species: capillary tips and capillary sprouts. Similar to Pettet's model, capillary tips in this framework exhibit both random and directed motion. However, the directed motion term was refined under the assumption that tips migrate down the gradient of blood vessel density. Through travelling wave and asymptotic analysis, this model provided valuable insights into potential strategies for enhancing the angiogenic response.

The role of oxygen in angiogenesis has been a focus of later models. Schugart et al. [81] incorporated the effects of supplemental oxygen into their angiogenesis model. Their findings highlighted several key roles of oxygen in angiogenesis, including the existence of an optimal oxygen level, beyond which its benefits diminish. Building on biological observations, Flegg et al. [82] modelled capillary tips as migrating down the oxygen gradient and similarly discovered that excessive oxygen can impair healing. These insights provide valuable guidance for developing treatments for chronic wounds – which we explore further in Section 1.2.4.

Beyond natural wound healing, some models focus on angiogenesis within tissue-engineered scaffolds designed to support vascular growth. For example, Lemon et al. [83] developed a system of ODEs describing vascular ingrowth into porous scaffolds implanted in vivo. Unlike the models discussed earlier, this approach emphasizes vascularisation in engineered tissues aimed at promoting regeneration, providing insights that are directly relevant to scaffold design for medical applications. Additionally, a notable aspect of this model is its consideration of MMP-mediated ECM degradation which a feature often overlooked in both angiogenesis and wound healing models.

The Role of Growth Factors

A key focus in modelling dermal wound healing lies in capturing the interplay between biochemical signalling and fibroblast behaviour, particularly how growth factors regulate ECM restoration. These models often follow a structure similar to that of Sherratt and Murray (1.1)–(1.2). One notable example is the work of Olsen et al. [84], which focuses on the process of forming fibrous tissue (fibroplasia) in dermal wound healing. They developed a system of PDEs to describe fibroblast dynamics and inflammatory growth factor activity in this process. A simplified “caricature” of their model is as follows:

$$\frac{\partial n}{\partial t} = \underbrace{\frac{\partial}{\partial x} \left[D_n \frac{\partial n}{\partial x} - \frac{\alpha}{(\beta + c)^2} n \frac{\partial c}{\partial x} \right]}_{\text{cell movement}} + \underbrace{\sigma \left(1 + \frac{Ac}{B + c} \right) n(1 - \gamma n)}_{\text{mitosis}} - \underbrace{\delta n}_{\text{apoptosis}}, \quad (1.3)$$

$$\frac{\partial c}{\partial t} = \underbrace{D_c \frac{\partial^2 c}{\partial x^2}}_{\text{diffusion}} + \underbrace{\frac{\kappa_c n c}{\gamma_c + c}}_{\text{production}} - \underbrace{\delta_c c}_{\text{decay}}, \quad (1.4)$$

where $n(x, t)$ denotes the cell density, encompassing both fibroblasts and myofibroblasts, and $c(x, t)$ represents the concentration of a generic signalling chemical, such as a growth factor. Cells move randomly or in response to chemical signals (chemotaxis). Cell growth occurs via mitosis and cells undergo natural death. Chemicals spread only by diffusion and is both produced by cells and undergoes natural decay. Chemical signals control cell movement, growth, and production of the chemical in accordance with Michaelis-Menten kinetics [85]. The model highlights the critical role of chemical production rates in disease persistence and regression, linking these to the bistability of normal and pathological states. Stability and bifurcation analyses suggest novel, testable mechanisms for disease progression and control, offering insights for improved therapeutic strategies [84]. While this caricature provides a useful base-

line for subsequent models, it considers only a generic signalling chemical and omits direct interactions with the ECM. Later models have built upon this framework by incorporating specific growth factors and examining their targeted effects on ECM restoration.

Specific growth factors such as platelet-derived growth factor (PDGF) and keratinocyte growth factor (KGF) have been modelled in greater biochemical detail. Haugh et al. [86] investigated the influence of PDGF on fibroblasts during dermal wound healing. PDGF, released from the fibrin clot, acts as a chemoattractant for fibroblasts. Model simulations revealed that fibroblast chemotaxis is primarily driven by PDGF consumption, with fibroblasts sustaining a stable PDGF gradient at their leading edge. Moreover, the steepness of this gradient had little effect on the rate of chemotaxis. In contrast to PDGF, which primarily influences fibroblast behaviour, Werner et al. [87] investigated the role of KGF, a fibroblast-derived signal that stimulates keratinocyte proliferation during wound healing. This study highlighted the crucial role of KGF in wound healing, showing that its expression peaks within 24 hours of injury. Based on this, Wearing and Sherratt [88] developed a reaction-diffusion model to account for the distinct KGF concentrations in the epidermis and dermis. Their model also incorporated free and bound receptor dynamics per basal epidermal cell—cells responsible for generating new skin cells. Their findings indicated that post-wound KGF concentrations exceeded the levels required for optimal healing, suggesting that KGF plays additional roles in wound repair.

Beyond KGF, another growth factor that has received considerable attention in the context of wound healing is TGF- β , particularly due to its role in scar formation and tissue remodelling as discussed in Section 1.1.1. Two key aspects of scar tissue have been incorporated into such models: the composition of collagen and the orientation of collagen fibres, particularly how cell movement through the ECM influences fibre alignment [64]. The first approach, focusing on collagen composition is based

on the fact that balance between collagen types is regulated by different isoforms of TGF- β . This relationship was investigated by Dale et al. [89] through mathematical modelling. They developed a system of PDEs to describe the interactions between fibroblasts, TGF- β isoforms, and collagen within a wound. Their model successfully replicated the observed collagen ratios and suggested that the distinctions between normal dermis and scar tissue arise from the specific interactions of TGF- β isoforms. In a subsequent study, they further predicted that this collagen composition would only emerge if the majority of fibroblasts entered the wound from the underlying tissue rather than from the sides [90] – a hypothesis later confirmed through experimental observations [91].

A second line of modelling work instead addressed how TGF- β contributes to fibre alignment during healing. One of the earliest models in this area was developed by Olsen et al. [92] who constructed a two-species system of PDEs representing fibroblast and ECM densities. In the model, ECM synthesis, degradation, and orientation are regulated by fibroblasts, while the ECM, in turn, influences fibroblast behaviour through haptotaxis, haptokinesis, and proliferation. Simulations in a one-dimensional spatial domain demonstrated how key parameters affect fibre alignment, with results suggesting that faster healing leads to greater alignment and thus more severe scarring. Building on this work, Dallon and Sherratt [93] developed a model in a more realistic wound geometry that allowed fibroblasts and collagen fibres to align at arbitrary angles in two dimensions, rather than being restricted to perpendicular directions. Their model incorporated both random and fibre-guided reorientation of collagen but did not account for fibroblast migration or changes in collagen density. Using integrodifferential equations, they explored various patterns of fibroblast and collagen alignment through both analytical and numerical methods. They found that the key factors that influence alignment include initial collagen deposition and fibroblast-collagen interactions.

While insightful, the previous models offered a simplified view of collagen fibre orientation. To address this, Dallon et al. [94] extended earlier approaches by incorporating both collagen fibre alignment and fibroblast migration. Unlike previous models that treated fibroblasts as a continuous density, this framework represented fibroblasts as discrete entities moving across the wound bed, guided by contact guidance – the tendency of cells to migrate along aligned fibres. The model included both fibrin and collagen, with fibroblasts degrading fibrin clots and replacing them with a new collagen matrix. Simulations revealed that increased fibroblast speed promoted greater fibre alignment, while reduced contact guidance led to more disorganised fibre patterns. Additionally, fibroblast ECM production had minimal influence on alignment. The model also demonstrated that collagen fibres tend to align perpendicular to the skin due to the directional entry of fibroblasts, a pattern reinforced over time. In a subsequent refinement, the model was extended to include the effects of TGF- β , modelled as a time-dependent concentration influencing cell speed [95]. While TGF- β nearly doubled fibroblast proliferation, it had little effect on fibre alignment. However, when the model allowed cells to change direction more frequently, reflecting TGF- β 's role in regulating filopodia dynamics, simulations showed fibre alignment patterns that matched experimental observations [96]. Most recently, McDougall et al. [97] introduced fibroblast chemotaxis in response to a wound-generated chemoattractant diffusing into adjacent tissue. Their work demonstrated how gradient-driven fibroblast migration could further influence collagen fibre orientation and proposed that the spread of such signals may affect cross-linking between scar tissue and surrounding ECM.

The frameworks reviewed above offer a robust basis for modelling chemical signalling in the dermis. By incorporating key processes such as chemotaxis, mitosis, apoptosis, and ECM interactions with mechanistic depth, they provide a versatile platform for extension. These modelling principles are readily adaptable to other

crucial chemical mediators in wound healing, such as MMPs, whose roles remain underexplored in the current modelling literature. In this thesis, we build on these established approaches to develop new models that capture MMP dynamics within the wound environment.

Wound Contraction

During healing wounds experience contraction, particularly in the remodelling stage of healing as discussed in Section 1.1.1. Dysregulation of this process can lead to pathological scarring and hence several mathematical models have explored the interplay between mechanical forces and chemical signalling [24]. In this thesis, however, we focus primarily on chemical models, and therefore provide only a brief overview of the relevant mechanical modelling literature. One of the earliest models to address wound contraction during healing was developed by Murray et al. [98], who demonstrated that a general tissue biomechanics framework, incorporating both viscous and elastic effects through stress-strain relationships, could be adapted to simulate wound dynamics. Building on this foundation, Tranquillo and Murray [99] introduced a mechanochemical model that integrated cellular behaviours such as proliferation and migration with the mechanical properties of the ECM. Their model incorporated mass conservation for both fibroblasts and ECM, treating the tissue as an isotropic viscoelastic material that contracts passively. Notably, the model closely aligned with experimental data on wound contraction in rats, predicting that both the contraction rate and final wound size were largely independent of the wound's initial shape or area.

This modelling approach laid the groundwork for later studies. For example, a subsequent model extended the framework to simulate interactions between fibroblasts, myofibroblasts, and growth factors, again representing the ECM as a viscoelastic medium [100]. Its predictions corresponded well with experimental observations of

PDGF clearance from the wound centre with contraction [101]. Another model incorporated collagen strain and concentration both inside and outside of the wound, offering flexibility by adapting to both no plasticity and elastoplastic cases through a single adjustable parameter, achieving strong agreement with experimental data [102].

Travelling Waves in Wound Healing Models

Many of the models discussed above exhibit travelling wave solutions. These models are typically formulated as reaction-diffusion PDEs, where cell movement may be influenced by factors such as chemical gradients or mechanochemical cues, as previously described. In a notable early study, Maini et al. [103, 104] were among the first to incorporate an experimental wound healing assay into their mathematical modelling framework and observed that, following an initial transient phase, cells at the leading edge advanced at an approximately constant speed. This behaviour was accurately captured by the classical Fisher equation, both in terms of the wave speed and the asymptotic convergence to this value over time. While the model employed in their analysis is a highly simplified representation of the wound healing process, it nevertheless highlights the utility of travelling waves in wound healing modelling, which features significantly in the work in this thesis.

1.2.4 Dysregulated Wound Healing and Treatment

The literature discussed above has shown how mathematical modelling can effectively model wound behaviour under normal physiological conditions. However, to inform therapeutic intervention, it is equally important to understand how these processes become disrupted in chronic wounds. In this section, we therefore focus on models that explore pathological healing of wounds and how these insights have been used to evaluate and guide treatment strategies.

Oxygen plays a critical role in wound healing, particularly in chronic wounds where hypoxia is a defining feature as discussed in Section 1.1.3. Schugart et al. [81] first examined this relationship using a mathematical model considering tissue oxygen tension. Their model consisted of a system of nonlinear partial differential equations to capture the spatial and temporal dynamics of key biological factors involved in wound angiogenesis. The framework includes variables representing endothelial cells, fibroblasts, inflammatory cells, ECM, oxygen, and angiogenic growth factors such as VEGF. By simulating the interactions between these components, including the chemotactic response of fibroblasts and the oxygen-dependent proliferation of endothelial cells, they found that supplemental oxygen can promote healing by enhancing angiogenesis. Building on this, Xue et al. [105] developed a model of ischemic wounds: wounds with restricted blood and oxygen supply, treating the ECM as a viscoelastic material. Their findings showed that oxygen deprivation impairs healing by limiting macrophage proliferation. Additional studies have further highlighted the importance of fibroblast dynamics in chronic wound healing. For instance, Menke et al. [106] identified fibroblast death and recruitment rates as potential therapeutic targets. Similarly, Segal et al. [107] developed an ODE model to examine how oxygen levels influence collagen accumulation, concluding that stimulating fibroblast production may be more effective than antibiotics in promoting healing.

Flegg and collaborators expanded this line of inquiry by mathematically modelling a range of chronic wound treatments, with a particular focus on hyperbaric oxygen therapy (HBOT). In this treatment, the patient enters a chamber and breathes in pure oxygen at higher-than-normal pressure levels which enhances oxygen delivery to the wound. Their work demonstrated that HBOT can effectively promote the healing of chronic wounds [108], and further extensions of this work suggested that treatment sessions should continue until healing is fully complete [109]. In additional studies, asymptotic analysis on a simplified version of the model identified critical parameter

thresholds such as the oxygen supply rate needed to stimulate angiogenesis [82]. In related work, they developed a model comparing two types of bandages designed to enhance oxygen delivery from surrounding healthy tissue: short-stretch bandages and three-layer bandages. By fitting a moving boundary in the model to experimental data, they found that three-layer bandages promote faster healing due to their ability to facilitate greater oxygen flow [110].

Beyond oxygen, other physiological imbalances contribute to dysregulated healing. Inspired by the work of Zykora [111], who showed that a balance between repair and inflammatory macrophages is essential for effective healing, Waugh and Sherratt [112] developed an ODE model that explicitly separated the actions of different macrophage phenotypes. Their results predicted that the distribution of these phenotypes is different in chronic wounds compared to acute wounds. This model was later extended to explore the use of engineered skin tissue substitutes, with findings indicating that such treatments increase hyaluron levels in the wound which promotes healing [113]. While many mathematical models focus on angiogenesis as a key process in wound healing, Bianchi et al. [114] proposed that chronic wounds may also be linked to impaired lymphangiogenesis: the formation of lymphatic capillaries. To explore this, they developed a system of five ODEs to model lymphangiogenesis in response to wounding. Their findings revealed that the balance between TGF- β and VEGF, rather than their absolute concentrations, plays a pivotal role in regulating lymphatic vessel growth. This suggests therapeutic strategies aimed at reducing macrophage-driven TGF- β stimulation while enhancing lymphatic endothelial cell proliferation could be effective in promoting healing, pointing to new therapeutic avenues. Similarly, Friedman and Siewe [115] broadened the scope by considering four different systemic risk factors — including elevated glycation and insulin resistance. Their model considered how these conditions restrict oxygen delivery to the wound site and impair the transition between macrophage phenotypes, thereby encompass-

ing many of the previously discussed mechanisms. In agreement with Waugh and Sherratt [112], their results emphasised the necessity of maintaining a delicate balance between inflammatory and repair macrophage populations. Additionally, similar to Bianchi's [114] findings, they identified the balance between TGF- β and VEGF as a critical determinant in wound healing.

Another significant contribution to chronic wound modelling came from Orazov et al [116], who challenged the assumption that wound hypoxia is solely due to impaired circulation. They proposed that microbial activity within the wound actively depletes oxygen, thereby sustaining a chronic state. Gas plasmas, known for their bactericidal properties, have been shown to effectively eliminate bacteria within wounds and promote healing. In response to this, Orazov and collaborators developed a six-species reaction–diffusion model to evaluate the efficacy of gas plasma therapy as a treatment for chronic wounds. The results demonstrated that while reducing bacterial levels is crucial, preventing bacterial regrowth must be limited to avoid pathological healing. Wang et al. [117] extended this work to include nitric oxide identifying that an effective therapeutic approach would involve both reducing bacterial load and enhancing nitric oxide levels.

More recently, innovative approaches have targeted the treatment of bacterial biofilms that are resistant to healing in chronic wounds. A novel approach involves the use of silver-doped glass fibres as an antimicrobial treatment. Mathematical modelling of this strategy has underscored several important considerations, including the dissolution stability of the fibres, which is essential for ensuring sustained therapeutic efficacy [118].

These studies demonstrate the important role that mathematical modelling can play in evaluating treatment strategies for chronic wounds. More broadly, there is growing recognition of the potential for mathematical and computational modelling to support the entire regenerative medicine pipeline, from early experimental design to

clinical translation as highlighted in a recent review by Waters et al [119]. Embedding modelling in regenerative medicine research offers a cost-effective, predictive complement to traditional experimental approaches, with the potential to accelerate the development and optimisation of therapies. Despite these advances, a key pathological feature of chronic wounds remains largely overlooked in the modelling literature: the role of MMPs. To date, mathematical models have scarcely addressed how dysregulated MMP activity drives chronic pathology, nor have they systematically evaluated treatment strategies targeting MMP imbalance such as the use of TIMPs. This thesis aims to fill this critical gap by developing a comprehensive modelling framework that captures the consequences of dysregulated MMP activity and explores potential therapeutic interventions involving TIMPs to regulate elevated concentrations of MMPs. In doing so, it contributes a novel perspective to the mathematical understanding of chronic wound healing.

1.2.5 Hydrogel Models

In this thesis, we focus on the development of chronic wounds driven by dysregulated MMP activity, and explore treatment strategies based on TIMP-mediated interventions, specifically through the use of hydrogels. To inform our modelling approach, we evaluate existing mathematical models of hydrogels with various release mechanisms.

Diffusion Controlled Release

As discussed in Section 1.1.5, there are multiple mechanisms by which drugs can be released from hydrogels. The simplest of these is diffusion-controlled release. Although drug release from most hydrogels is not governed solely by diffusion, diffusion often plays a significant role in the overall release dynamics. As a result, many mathematical models describe drug transport at the hydrogel interface using Fick's law of diffusion [120].

One of the earliest and most widely adopted models for drug release from matrix systems was introduced by Higuchi [121]. Originally developed to fit experimental data, the Higuchi model has since become a foundational approach for describing drug diffusion in porous matrices. The classical Higuchi equation, derived under pseudo-steady-state assumptions, expresses drug release as proportional to the square root of time. However, its applicability relies on several assumptions: a high initial drug concentration, one-dimensional diffusion, negligible polymer swelling, a constant diffusion coefficient, and ideal sink conditions, many of which may not hold in real hydrogel systems, particularly those designed for biologically complex environments such as wound healing.

To account for more complex release behaviours, a more general semi-empirical power law, known as the Peppas equation, was introduced [57]. This model extends the Higuchi framework by incorporating additional mechanisms such as polymer relaxation and convective transport. The equation characterises drug release kinetics using an exponent that varies depending on the system geometry [122]. While alternative approaches such as the Weibull function, an exponential-based statistical distribution that captures the time-dependent behaviour of heterogeneous systems, and Monte Carlo simulations, which model the random movement and release of individual drug particles, offer refined representations of drug transport [123–125], the simplicity and interpretability of the Peppas model makes it widely used in pharmaceutical research [57].

1.2.6 Swelling Controlled Release

We now turn to swelling-controlled drug release from hydrogels, which undergo complex macromolecular transformations during the release process. In these systems, water-soluble drugs are embedded within glassy polymers. When exposed to a solvent, the polymer swells as the solvent diffuses into the polymer. This swelling is

characterised by two moving fronts: the swelling interface, marking the transition from glassy to rubbery states as the polymer swells inwards, and the polymer interface where the polymer meets the solvent and moves outward [57].

As the polymer absorbs water, chain relaxation occurs at the swelling front, directly influencing drug diffusion. The resulting transport behaviour can be either Fickian or non-Fickian, depending on the balance between relaxation and diffusion. These systems are typically modelled as Stefan-type moving boundary problems, using either concentration-dependent or constant diffusion coefficients, with both interfaces treated as moving boundaries [120].

Adding macromolecular relaxation to the model yields a more biologically realistic representation of drug release [126]. A key parameter in determining transport behaviour is the Deborah number ($De = \text{relaxation time}/\text{diffusion time}$). High or low De values indicate that Fickian transport is dominated by relaxation or diffusion, respectively, while intermediate values lead to anomalous diffusion [127]. Two additional metrics help describe release dynamics are the swelling interface number, which compares water movement to drug diffusion [128], and the swelling area number, which captures how changes in surface area during swelling influence release [129].

These models have been extended to account for additional complexities such as front synchronisation [130], non-uniform drug distribution [131], time-dependent diffusion [132], and environmental factors like temperature, pH, and ionic strength [133–135]. These models have helped establish the conditions necessary for achieving zero-order release which is a controlled, constant drug release rate over time that maintains a stable therapeutic concentration in the bloodstream or target tissue.

In addition to swelling and relaxation, uncrosslinked polymer chains can undergo disentanglement and dissolution when exposed to a compatible solvent as discussed in Section 1.1.5. While the initial swelling behaviour resembles that of swellable sys-

tems, a key difference is that the polymer interface, after initially expanding outward, eventually recedes inward as dissolution overtakes swelling, leading to complete polymer dissolution [136]. Harland et al. demonstrated that zero-order release can be achieved when the swelling and dissolution fronts move are synchronised [137]. Expanding on this concept, Ju et al. developed models incorporating both swelling and dissolution in radial systems [138]. Further refinement was provided by Narasimhan and Peppas, who introduced a molecular-level model incorporating chain disentanglement dynamics and polymer chain mobility, capturing the transition between Fickian and non-Fickian release and identifying the conditions required to achieve zero-order release [139].

Further developments have focused on multi-dimensional and mechanistic modelling to predict drug release more precisely across various hydrogel formulations. Siepmann and co-workers accounted for axial and radial drug transport, non-uniform swelling, various geometries, and drug loading levels — successfully predicting drug release from hydroxypropyl methylcellulose systems [140] and similar approaches were adapted for other polymers such as polyethylene oxide [141]. Focusing on a mechanistic approach, Borgquist and colleagues introduced a framework validated by experimental data, successfully predicting drug and polymer release profiles [142]. Collectively, these models have significantly advanced our understanding of drug delivery from dissolving polymer systems [57].

1.2.7 Chemically Controlled Release

Chemically controlled drug release systems are typically classified into two main categories: erodible and pendant chain systems as discussed in Section 1.1.5. We focus on the modelling literature of erodible systems, where drug release is governed by the degradation of the polymer matrix itself. Here, the release mechanism is either predominately diffusion-controlled, if matrix erosion is slow, or predominately

erosion-controlled, if drug diffusion is minimal. Erosion may occur through bulk erosion, where degradation happens uniformly throughout the polymer, or surface erosion, where degradation is limited to the surface of the polymer [57].

To model drug release from erodible polymers, researchers use three main approaches. Phenomenological models have adopted a reaction-diffusion structure [143, 144] and have accounted for autocatalytic effects [145]. These models often incorporate the evolving physical and chemical properties of the polymer matrix, including crystallinity [146], surface structure [147], and moving boundaries [148]. Probabilistic models adopt a stochastic perspective, using Monte Carlo simulations to assign lifetimes to polymer elements and calculate erosion probabilities, though they often overlook drug diffusion [149, 150]. In contrast, empirical models rely on experimental data to describe drug release [151, 152]; however, they offer limited generalisability and provide minimal insight into the underlying release mechanisms [57].

Some reaction-diffusion models, which incorporate both surface and bulk erosion mechanisms, provide a more comprehensive and realistic framework for predicting drug release behaviour [153, 154]. One such model was developed by Rothstein et al. [155], who formulated a system of reaction-diffusion PDEs to describe a range of degradation scenarios, including purely surface-eroding, purely bulk-eroding, and transitional systems that evolve from surface to bulk erosion as degradation progresses. Their model explicitly accounts for key physical processes such as hydration, dissolution, and pore formation, all of which significantly influence the transport dynamics within the polymer matrix.

Of particular interest to this work is the treatment of pore formation and its effect on molecular diffusivity. In Rothstein et al.'s framework, the diffusivity of agents within the polymer is not constant but is instead modulated by the evolving porosity of the matrix. This is captured through an effective diffusion coefficient, D_{eff} , which depends on the polymer's molecular weight and is modelled as:

$$\frac{\partial C}{\partial t} = \nabla(D_{\text{eff}}\nabla C) + f(C). \quad (1.5)$$

Here, D_{eff} is defined as a function of the matrix porosity ϵ , such that $D_{\text{eff}} = D \cdot \epsilon$. The porosity ϵ , which varies over time and space as the polymer degrades, is modelled using a cumulative normal distribution based on the local molecular weight M :

$$\epsilon = 1 - \frac{1}{2} \left[\text{erf} \left(\frac{M - M_r}{\sqrt{2\sigma^2}} \right) + 1 \right]. \quad (1.6)$$

In this expression, M_r represents the critical molecular weight at which drug release becomes significant, and σ^2 denotes the variance, which is determined by the crystallinity of the polymer and the associated distribution of degradation rates. This formulation allows the model to link spatiotemporal changes in the polymer matrix structure with transport properties. The validity of Rothstein et al.'s model was demonstrated through comparisons with experimental release data from various polyanhydride and poly(ortho ester) drug delivery systems [155]. This model forms the basis of the approach we adopt and build upon in Chapter 5, particularly in relation to the porosity-dependent diffusion framework for erodible polymer systems.

1.3 Motivation and Thesis Structure

While extensive research has been conducted on modelling various processes involved in wound healing and treatment approaches for pathological healing, there is a notable gap in the literature regarding the role of MMPs in chronic wound development and the role of TIMPs as regulators of these processes.

Modelling studies have been conducted on the role of MMPs and TIMPs in tumour development, since many MMPs are overexpressed during tumour progression, enhancing the tumour's ability to invade surrounding tissue [156–159]. However, to

our knowledge, this has not been extensively applied to wound healing contexts. One such model, developed by Krishna et al. [160], used a system of ODEs to describe the behaviour of MMPs and TIMPs in a wound. While insightful and offering a valuable foundational framework, the model does not account for cellular migration or the influence of chemical gradients, and its analysis is limited to a parameter sensitivity analysis, without investigating the dynamic interrelationships between these components or identifying potential therapeutic targets.

In this thesis, we present a comprehensive analysis of the role of MMPs in chronic wound development, alongside the regulatory function of TIMPs. The overarching objective is to explore their potential as therapeutic targets for chronic wounds. Our approach involves the use of PDE models, along with analytical techniques and extensive numerical simulations performed in MATLAB.

In Chapter 2, we introduce a simplified two-component reaction-diffusion model that describes the interaction between MMPs and dermal tissue during wound healing, focusing on how the restoration of the ECM is influenced by MMP concentration levels. To streamline the model, we combine the effects of the ECM and fibroblasts into a single variable, “dermal tissue.” We demonstrate that this model gives rise to travelling wave solutions in both cases of acute and chronic wounds, modelled using different parameter sets; thus motivating a travelling wave analysis. We also explore the impact of deregulated cell apoptosis on wound healing, as motivated by the biological literature discussed in Section 1.1.2, through bifurcation and phase plane analyses. We also examine the effects of elevated MMP production levels on wound healing, also motivated by the literature discussed in 1.1.2.

Chapter 3 builds on this preliminary model by extending the model framework to separately account for the actions of fibroblasts and the ECM, while also incorporating TIMPs and their regulatory effects on MMPs. In this chapter, we present numerical simulations of our extended model, which similarly reveals the existence of travelling

wave solutions in both acute and chronic wound contexts. This motivates an analysis of steady states and bifurcations, with a particular focus on the role of TIMPs in the regulation of chronic wound formation, giving insights that were not provided by Chapter 2. We then conduct a parameter sensitivity analysis, which allows us to derive a reduced model which we demonstrate supports the same qualitative dynamics as the original model.

Chapter 4 further extends the reduced Chapter 3 model to two dimensions, where we demonstrate that elevated MMP production levels can lead to the formation of chronic wounds. Additionally, we show that treatment of such wounds with a TIMP-containing hydrogel, modelled with a Robin boundary condition, can reverse chronic wound formation. We perform an asymptotic analysis of the system, deriving analytic solutions for the four variables involved. This analysis shows that the influx of TIMPs into a chronic wound can facilitate full healing, and provides a profile for TIMP activity.

While Chapter 4 offers a simplified view of TIMP influx via an external source, Chapter 5 presents a more comprehensive model of the TIMP-delivering hydrogel. Currently in development for chronic wound treatment, these hydrogels are designed so that MMPs cleave the cross-linked bonds within the material, triggering the release of TIMPs to the wound site through both diffusion and erosion-driven mechanisms enabled by the hydrogel's porosity. We develop a mathematical model to capture the system dynamics of this hydrogel-mediated delivery. Using experimental data, we attempt to estimate key parameters of the model, but encounter significant parameter non-identifiability. This insight motivates the construction of a synthetic parameter set, which in turn informs the experimental design needed to accurately estimate the unknown parameters and gain deeper insight into the hydrogel's delivery dynamics.

Finally, in Chapter 6, we summarise the main conclusions of the thesis and outline potential extensions of this work, particularly with a view to combine the chronic

wound model from Chapter 4 and the hydrogel model from Chapter 5 to gain further insight into hydrogel dynamics and ultimately inform treatment recommendations.

1.4 Statement of Originality

At the time of submission, two papers [161, 162] have been published from this thesis, containing the work in Chapters 2, 3 and Chapter 4 (Sections 4.2 and 4.3). These papers were co-authored by S. Dari, N. T. Fadaei and R.D O'Dea (supervisors).

Chapter 2

Modelling the impact of matrix metalloproteinases in wound healing

2.1 Introduction

In this Chapter, we consider the role that MMPs play in wound healing, with particular consideration given to chronic wounds. We propose a two-component reaction-diffusion model on a one-dimensional spatial domain that describes the interaction of MMPs with dermal tissue, in which the restoration of the ECM is affected by MMP concentration levels. Using parameter values representing both acute and chronic wound healing, this model gives rise to travelling waves. From the arising travelling wave analysis, we observe that deregulated apoptosis results in the emergence of chronic wounds, characterised by elevated MMP concentrations, aligning with the biological literature discussed in Chapter 1. We also observe hysteresis effects when both the apoptotic rate and MMP production rate are varied, providing further insight into the management (and potential reversal) of chronic wounds.

We develop our mathematical model in Section 2.2. Direct numerical simulations indicate the existence of travelling wave solutions, motivating a travelling wave analysis in Section 2.3. We then consider the effect of deregulated apoptosis on the healing of a wound via bifurcation and phase plane analysis in Section 2.4. Finally, we consider the effects of elevated MMP production levels on the healing of a wound in Section 2.7, before discussing our results in Section 2.8. This chapter contains the results shown in [161].

2.2 Model Development

We begin by presenting a mathematical formulation to describe MMP concentration dynamics in the presence of a wound. Elevated MMP levels are a key contributor to chronic wound persistence, yet their role has been largely overlooked in existing models. To address this, we develop a reaction-diffusion model that captures interactions between MMPs and dermal cells during wound healing, aiming to better understand how MMP dynamics contribute to pathological healing and inform potential treatment strategies. For simplicity, we consider a wound with a one-dimensional Cartesian geometry, where x represents the longitudinal distance across a fixed domain, which evolves over time t . This model consists of two populations within a wound: the dermal cell density, denoted as $n(x, t)$, and the MMP concentration, denoted as $m(x, t)$. We take $n(x, t)$ to incorporate the actions of both fibroblasts and ECM for the sake of simplicity, since fibroblasts are responsible for creating the components of the ECM.

We assume that dermal cells undergo mitosis with intrinsic growth rate σ and carrying capacity K_n , noting that $n = 0$ corresponds to the complete absence of cell tissue. We model this mitosis term using logistic-type growth, following a similar approach to Olsen et al. [84], as outlined in Section 1.2.3. As discussed previously, MMPs play a dual role in wound healing: at moderate levels, they promote tissue

repair by releasing latent TGF- β from the ECM, which stimulates fibroblast proliferation and ECM synthesis. However, when MMP concentrations exceed a threshold, denoted m_{thresh} , their activity shifts from constructive to destructive. Excessive MMPs accelerate ECM degradation and amplify inflammation, leading to impaired healing.

These complex regulatory effects are not represented mechanistically in our framework — we do not model TGF- β release or inflammatory mediators as explicit variables. Instead, their net influence is captured phenomenologically by modifying the logistic growth with a biphasic function $f(m)$.

Furthermore, dermal cells undergo apoptosis with rate δ_n and dermal cell motion within the wound is represented by linear diffusion with effective diffusivity D_n . This apoptotic process is also highlighted in our biological schematic and is represented mechanistically in the model. When apoptosis is excessive, as occurs in the hyperglycaemic environment, the resulting loss of reparative cells contributes to impaired healing (see Figure 1.2).

MMP production by fibroblasts, denoted by a production function $g(n)$, occurs in response to inflammatory chemicals which are found in abundance in a wound. MMP production is therefore assumed here to be proportional to the absence of healthy tissue. MMPs undergo natural decay with rate δ_m and diffuse through the wound with diffusivity D_m . These aforementioned processes are shown in Figure 2.1. Combining these modelling elements provides the following system of PDEs:

$$\frac{\partial n}{\partial t} = \underbrace{D_n \frac{\partial^2 n}{\partial x^2}}_{\text{Cell motion}} + \underbrace{\sigma(1 + f(m))n \left(1 - \frac{n}{K_n}\right)}_{\text{Mitosis}} - \underbrace{\delta_n n}_{\text{Apoptosis}}, \quad (2.1)$$

$$\frac{\partial m}{\partial t} = \underbrace{D_m \frac{\partial^2 m}{\partial x^2}}_{\text{Diffusion}} + \underbrace{g(n)}_{\text{Production of } m} - \underbrace{\delta_m m}_{\text{Decay}}. \quad (2.2)$$

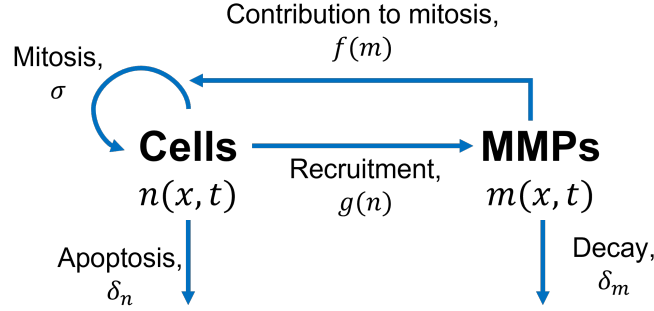


Figure 2.1: Schematic of the interactions of dermal cells with MMPs. Labelled rates are defined as in the system (2.1)–(2.2).

We now discuss the functional forms of $f(m)$ and $g(n)$ appearing in the system (2.1)–(2.2). The function $f(m)$ in (2.1) represents the contribution to wound healing by MMPs and is defined to be an increasing function of m until $m = m_{\text{thresh}}$, at which $f(m) = f_{\text{max}}$, the maximum factor by which mitosis can be enhanced due to the presence of MMPs. Larger concentrations of MMPs hinder the wound healing process and thus, for m larger than m_{thresh} , $f(m)$ is a decreasing function of m . A suitable choice of $f(m)$ is therefore

$$f(m) = \frac{f_{\text{max}}}{m_{\text{thresh}}} m \exp\left(1 - \frac{m}{m_{\text{thresh}}}\right); \quad (2.3)$$

a schematic of $f(m)$ is shown in Figure 2.2(a). We note that $f(m)$ and hence the parameter f_{max} are dimensionless. For elevated levels of MMPs, $f(m)$ decreases to zero and the mitosis term reduces to logistic growth with zero healing contribution due to MMPs. One could also allow $f(m)$ to take negative values for large m , representing a negative healing contribution to the ECM. One such function that captures this behaviour is the following:

$$\tilde{f}(m) = \frac{\tilde{f}_{\text{max}}}{m_{\text{thresh}}} m \exp\left(1 - \frac{m}{m_{\text{thresh}}}\right) - \frac{\kappa m}{1 + m}, \quad (2.4)$$

for some values of $\tilde{f}_{\text{max}}$ and κ . A schematic of $\tilde{f}(m)$ is shown in Figure 2.3. We demonstrate in Section 2.2.3 that the choice (2.4) yields largely similar qualitative

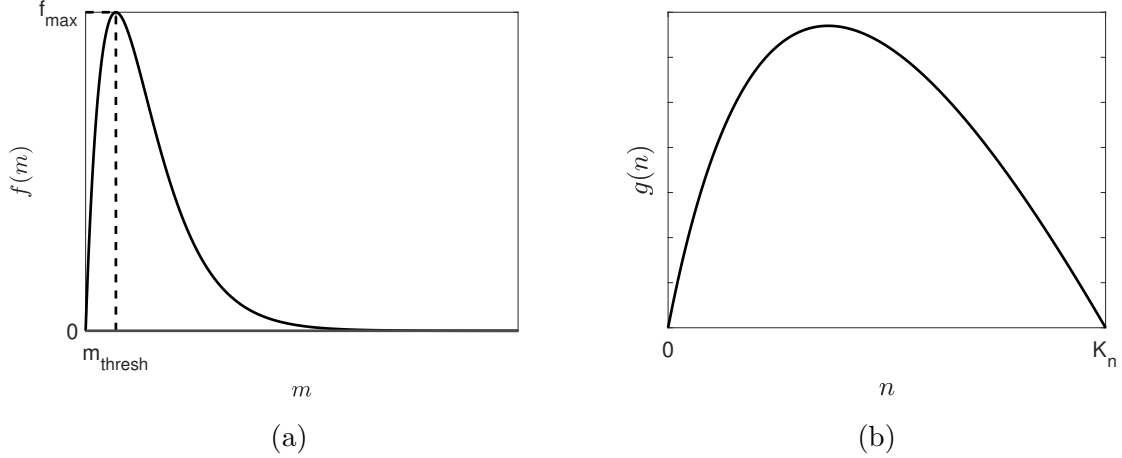


Figure 2.2: Schematic functional forms of (a) the healing contribution by MMPs function, $f(m)$ in (2.3), and (b) the MMP production function, $g(n)$ in (2.5).

features to the functional form (2.3). Given these similarities, we restrict attention to the choice of $f(m)$ as in (2.3) in all subsequent analysis as this choice results in simpler analytic computations.

The production function $g(n)$ in (2.2) represents the production of MMPs by the cells in proportion to the absence of healthy tissue. Consequently, we can represent $g(n)$ as

$$g(n) = \left(1 - \frac{n}{K_n}\right) \frac{k_m n}{\gamma_m + n}, \quad (2.5)$$

where k_m is the maximum reaction rate and γ_m is a Michaelis–Menten constant [85]. A schematic of $g(n)$ is shown in Figure 2.2(b).

2.2.1 Initial Conditions and Boundary Conditions

Assuming the wound is symmetric about its centre and that the tissue is fully healed far from the wound, we define the initial conditions of the system, (2.1)–(2.2) to represent a wound starting at $x = \lambda$. The wound spans the region $[\lambda, L]$ and the tissue surrounding the wound occupies $[0, \lambda)$. A small concentration of MMPs, η , is

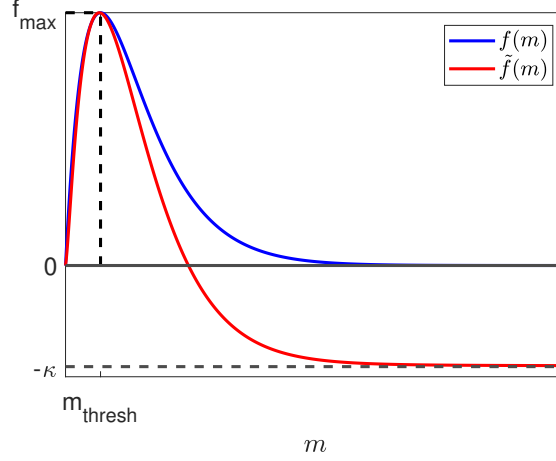


Figure 2.3: Schematic functional forms of the healing contribution by MMPs functions $f(m)$ as in Figure 2.2(a) and $\tilde{f}(m)$ as in (2.4).

initially present at the wound edge λ :

$$n(x, 0) = K_n H(\lambda - x), \quad (2.6)$$

$$m(x, 0) = \eta \exp\left(-\left(\frac{x - \lambda}{\nu}\right)^2\right), \quad (2.7)$$

where ν represents the initial spread of MMPs. In (2.6), H is the Heaviside function while $n = K_n$ corresponds to the cells being at carrying capacity. We adopt zero-flux boundary conditions on both endpoints of the finite domain of length L , i.e:

$$\frac{\partial}{\partial x} n(x, t) = \frac{\partial}{\partial x} m(x, t) = 0 \quad \text{at} \quad x = 0, L. \quad (2.8)$$

2.2.2 Nondimensionalisation

In this section, we non-dimensionalise the system (2.1)–(2.3), (2.5)–(2.8) in order to reduce the number of parameters and simplify the system. We are interested in the dynamics of the system on the timescale of mitotic rate of dermal cells and therefore introduce the following dimensionless variables:

$$N = \frac{n}{K_n}, \quad M = \frac{m}{m_{\text{thresh}}}, \quad X = \sqrt{\frac{\sigma}{D_n}}x, \quad T = \sigma t. \quad (2.9)$$

With these scalings, we obtain the following dimensionless equations:

$$\frac{\partial N}{\partial T} = \frac{\partial^2 N}{\partial X^2} + [1 + f_{\max} M \exp(1 - M)] N(1 - N) - \delta_N N, \quad (2.10)$$

$$\frac{\partial M}{\partial T} = D_M \frac{\partial^2 M}{\partial X^2} + \frac{\alpha N(1 - N)}{\gamma_M + N} - \delta_M M, \quad (2.11)$$

where

$$D_M = \frac{D_m}{D_n}, \quad \alpha = \frac{k_m}{m_{\text{thresh}} \sigma}, \quad \gamma_M = \frac{\gamma_m}{K_n}, \quad \delta_N = \frac{\delta_n}{\sigma}, \quad \delta_M = \frac{\delta_m}{\sigma}. \quad (2.12)$$

We note that $0 \leq N(X, T) \leq 1$, since we do not expect the cell density to exceed its carrying capacity once healing is completed. The dimensionless initial and boundary conditions are:

$$N(X, 0) = H(\bar{\lambda} - X), \quad (2.13)$$

$$M(X, 0) = \bar{\eta} \exp\left(-\left(\frac{X - \bar{\lambda}}{\bar{\nu}}\right)^2\right), \quad (2.14)$$

and

$$\frac{\partial}{\partial X} N(X, T) = \frac{\partial}{\partial X} M(X, T) = 0 \quad \text{at} \quad X = 0, \bar{L}, \quad (2.15)$$

where $\bar{\eta} = \frac{\eta}{m_{\text{thresh}}}$, $\bar{\lambda} = \lambda \sqrt{\frac{\sigma}{D_n}}$, $\bar{\nu} = \nu \sqrt{\frac{\sigma}{D_n}}$ and $\bar{L} = L \sqrt{\frac{\sigma}{D_n}}$. We note that the dimensionless values $\bar{\lambda}, \bar{\nu}, \bar{\eta}$ and $\bar{L}$ are chosen for illustrative purposes and are given in Table 2.2. Unless otherwise specified, the initial conditions (2.13)–(2.14) are used for all subsequent numerical simulations, with the corresponding parameter values

listed in Table 2.2.

We define the dimensionless system (2.10)–(2.15) as the ‘wound healing model’. Suitable values for the dimensional parameters appearing in (2.1)–(2.3) and (2.5) are given in Table 2.1; we remark that these parameters correspond to ‘normal’, or healthy, wound healing and, unless otherwise stated, are employed in all simulations. These parameter values are used to calculate the dimensionless parameter values in (2.12)–(2.15) and are given in Table 2.2. We remark that where parameter values were unable to be obtained from literature, these were estimated heuristically based on exploratory simulations to ensure healthy wound healing behaviour.

2.2.3 Model Simulations

In order to obtain numerical solutions of the wound healing model, we use the method of lines and discretise on a one-dimensional spatial domain, using second order finite differences to approximate spatial derivatives. We then integrate the resulting system of time-dependent ODEs using MATLAB’s `ODE15s` solver.

To ensure reliability of the PDE simulations, we performed several standard checks. For 1D simulations for this Chapter and Chapter 3, we verified that spatially homogeneous steady states of the discretised system agreed with the analytically predicted equilibria of the continuum equations. Numerical convergence was confirmed by mesh refinement: increasing the number of spatial nodes from $N_x = 200$ to $N_x = 10000$ produced no appreciable changes in wave speeds, steady-state values, or qualitative behaviour. Time integration was performed with MATLAB’s stiff solver `ODE15s`, and results were checked for robustness to solver tolerances. For 2D simulations in the following Chapters (MATLAB PDE Toolbox), mesh refinement was tested by halving the maximum element size; x-averaged quantities and wave propagation speeds were consistent with those obtained in 1D simulations, indicating good agreement across dimensions. These checks confirm that the qualitative and

Parameter	Description	Value	Reference
D_n	Diffusion coefficient of cells	$6.12 \times 10^{-5} \text{ mm}^2 \text{ h}^{-1}$	[81]
D_m	Diffusion coefficient of MMPs	$9 \times 10^{-4} \text{ mm}^2 \text{ h}^{-1}$	[163]
σ	Mitotic rate of cells	0.0385 h^{-1}	[164]
K_n	Carrying capacity of cells	$1250 \text{ cells mm}^{-2}$	[164]
k_m	Maximal rate of MMP production by cells	$4 \text{ pg}/\mu\text{g h}^{-1}$	Estimate
γ_m	Density of cells at which half of k_e is attained	625 cell mm^{-2}	Estimate
$f_{\max}$	Maximum factor with which mitosis can be enhanced due to the presence of MMPs	50	Estimate
δ_m	MMP decay rate	0.009 h^{-1}	[165]
δ_n	Cell apoptosis rate	0.005 h^{-1}	[164]
m_{thresh}	Threshold concentration of MMPs at which maximum healing contribution $f_{\max}$ is attained	$7 \text{ pg}/\mu\text{g}$	[166]

Table 2.1: Values for the dimensional parameters appearing in (2.1)–(2.5).

quantitative behaviours reported are accurate.

The model produces travelling wave solutions that capture the progression of wound healing. In this framework, we distinguish between acute and chronic wounds based on the long-time behaviour of the cell density N . In a successful healing outcome, N approaches a value close to unity, representing restoration of healthy tissue, with small deviations reflecting the normal background of cell turnover. In contrast, a chronic wound corresponds to convergence to an abnormal steady state where N remains significantly below unity, indicating that regeneration has stalled and the tissue fails to recover to healthy levels.

An example numerical simulation of the wound healing model is shown in Figure 2.4. As seen in Figure 2.4(a), the cell density $N(X, T)$ evolves into a travelling wave where a front of tissue of density $N \approx 1$ is ‘invading’ the wound ($N = 0$). This state of $N \approx 1$ is close enough to unity to be considered as a healed state; we elaborate on

Parameter	Value
D_M	14.706
α	14.842
$f_{\max}$	50
δ_N	0.130
δ_M	0.234
γ_M	0.5
$\bar{\lambda}$	3
$\bar{\nu}$	1
$\bar{\eta}$	0.1
$\bar{L}$	200

Table 2.2: Values of the dimensionless parameter values appearing in the wound healing model in (2.10)–(2.15).

the classification of a healed state further in Section 2.3. Since we observe a travelling wave where a healed front of tissue invades the wound, this scenario corresponds to a wound healing to completion. In Figure 2.4(b), we also observe the formation of a travelling wave for $M(X, T)$ in which a ‘spike’ in MMP concentration is found at the edge of the healing wound. These qualitative phenomena are in agreement with biological literature, which suggests the increased expression of MMPs at the edge of an acute wound at all stages in the healing process [28].

In Section 3.2 we considered an alternative functional form of $f(m)$ which describes the healing contribution of dermal cells by MMPs. We denoted this $\tilde{f}(m)$ in (2.4). In non-dimensionalising the PDE system (2.1)–(2.2) subject to $\tilde{f}(m)$ given in (2.4), using the scaling given in (2.9), we obtain the simulations given in Figure 2.5. Initial and boundary conditions are chosen as in (2.13)–(2.15) and we take $\tilde{f}_{\max} = 67.46$ and $\kappa = 20$; all other parameter values are as given in Table 2.2. In Figure 2.5, we observe largely similar qualitative features as those seen in Figure 2.4: the invasion of a wound by a healed front of cells with an increased expression of MMP concentration at the wound edge.

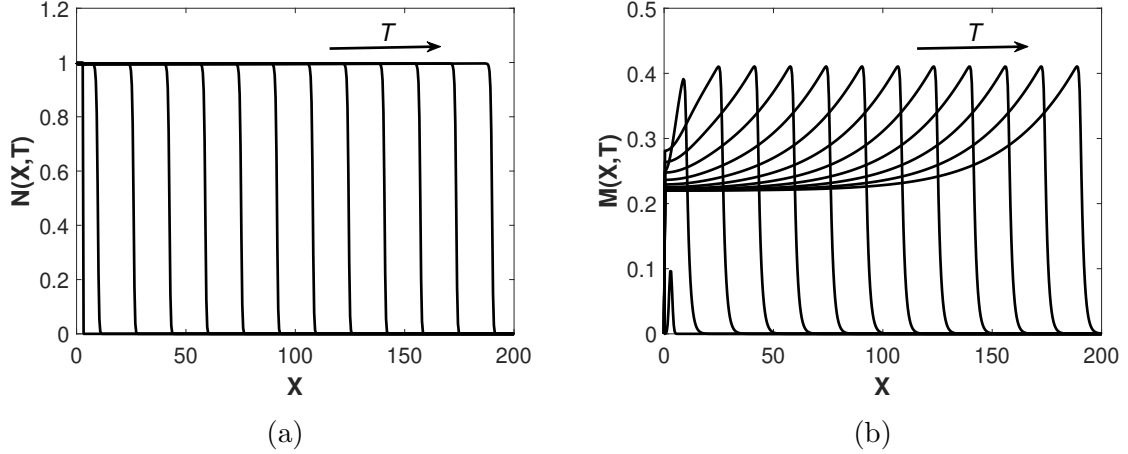


Figure 2.4: Simulation of the wound healing model (2.10)–(2.15) at regular time intervals $T = 2$ with parameter values as in Table 2.2 (a) Evolution of cell density $N(X, T)$ across the spatial domain, (b) Evolution of MMP concentration $M(X, T)$ across the spatial domain.

2.3 Steady State and Travelling Wave Analysis

2.3.1 Steady State Analysis

From the previous section, we observe that the wound healing model gives rise to travelling wave solutions. Therefore, in this section we conduct a travelling wave analysis of the model. To determine the far-field states of the travelling waves, we first examine the uniform steady-states of (2.10)–(2.11). In particular, the invading far-field state for $N(X, T)$ will indicate whether or not a wound is healing to completion. The uniform steady states $(\bar{N}, \bar{M})$ of (2.10)–(2.11) satisfy the following:

$$\bar{M} = \frac{\alpha}{\delta_M} \frac{\bar{N}(1 - \bar{N})}{\gamma_M + \bar{N}}, \quad (2.16)$$

$$\delta_N \bar{N} = (1 + f_{\max} \bar{M} \exp(1 - \bar{M}))(1 - \bar{N}) \bar{N}. \quad (2.17)$$

Combining (2.16)–(2.17), we find that all non-trivial steady states may be written in terms of the single variable $\bar{N}$:

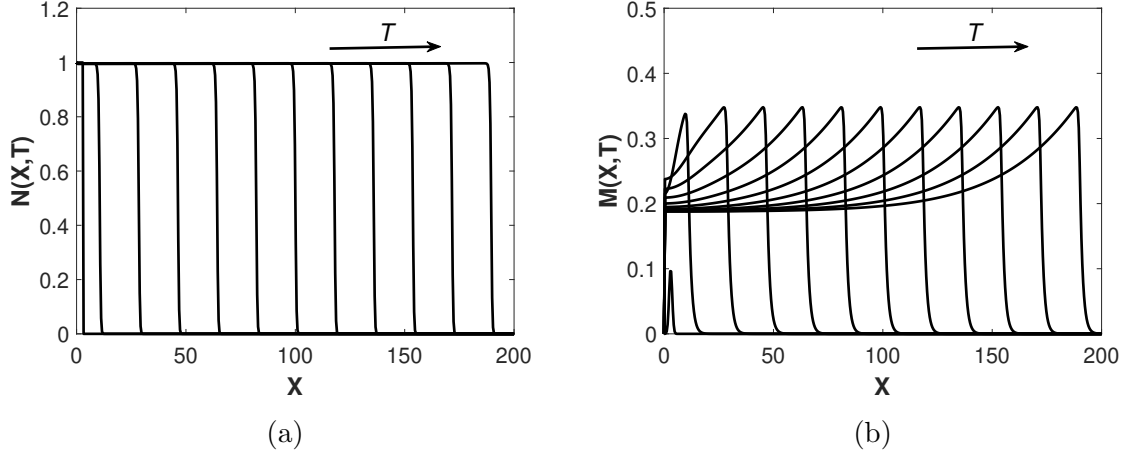


Figure 2.5: Simulation of the system (2.1)–(2.8), non-dimensionalised according to (2.9) with $f(m)$ as in (2.4) at regular time intervals with $\tilde{f}_{\max} = 67.46$, $\kappa = 20$ and other parameter values as in Table 2.2 (a) Evolution of cell density $N(X, T)$ across the spatial domain, (b) Evolution of MMP concentration $M(X, T)$ across the spatial domain.

$$\delta_N = \frac{\alpha f_{\max}(1 - \bar{N})^2 \bar{N}}{\delta_M(\gamma_M + \bar{N})} \exp\left(1 - \frac{\alpha \bar{N}(1 - \bar{N})}{\delta_M(\gamma_M + \bar{N})}\right) + 1 - \bar{N}. \quad (2.18)$$

It is clear from (2.17) that we always have the trivial steady state, i.e. $(\bar{N}, \bar{M}) = (0, 0)$ for all positive parameters. To find the remaining steady state solutions, we use the `fimplicit` function in MATLAB, to determine a numerical solution for $\bar{N}$ using (2.18) and subsequently $\bar{M}$ using equation (2.16).

As discussed in Section 2.2.3, we characterise a wound as healing to completion when a far-field state of $N \approx 1$ is achieved. This is because for $\delta_N = 0$, $\bar{N} = 1$ satisfies (2.17). For non-zero δ_N , however, $\bar{N}$ does not attain the value one, since apoptosis of cells is continually occurring. For small δ_N , i.e. values corresponding to normal healing, (2.17) provides $\bar{N}$ close to unity, and we hence consider this state to describe healed tissue. Additionally, if an unhealed or partially healed steady-state of $\bar{N}$, i.e. not qualitatively close to one, invades a state corresponding to damaged tissue, we characterise this as a chronic wound.

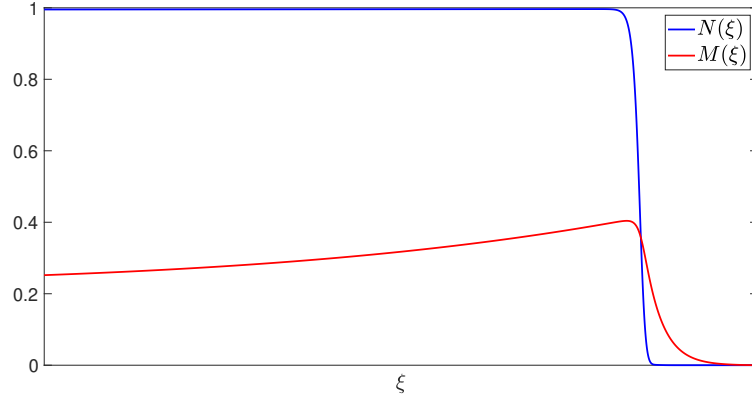


Figure 2.6: Numerical solution of the boundary value problem (2.19)–(2.21) obtained using MATLAB's BVP5c solver with parameter values as in Table 2.2

2.3.2 Travelling Wave Analysis

In order to verify the existence of travelling solutions to the wound healing model, we consider a travelling wave analysis. Employing the travelling wave coordinates $\xi = X - cT \in \mathbb{R}$, where c is the wave speed, the wound healing model (2.10)–(2.15) reduces to the boundary value problem (BVP):

$$\frac{d^2 N}{d\xi^2} + c \frac{dN}{d\xi} + (1 + f_{\max} M \exp(1 - M)) N(1 - N) - \delta_N N = 0, \quad (2.19)$$

$$D_M \frac{d^2 M}{d\xi^2} + c \frac{dM}{d\xi} + \alpha \frac{N}{\gamma_M + N} (1 - N) - \delta_M M = 0, \quad (2.20)$$

with boundary conditions

$$\lim_{\xi \rightarrow \pm\infty} N'(\xi) = \lim_{\xi \rightarrow \pm\infty} M'(\xi) = 0. \quad (2.21)$$

Using MATLAB's BVP5c solver, we present numerical simulations of the BVP (2.19)–(2.21) in Figure 2.6 with parameter values given in Table 2.2, which verifies the simulations of the wound healing model in Figure 2.4. We are also able to use BVP5c

to obtain a nondimensional wavespeed of $c = 9.6$, which translates to a dimensional wavespeed of $c_{\text{dim}} = 1.504 \times 10^{-2} \text{mm h}^{-1}$. This wavespeed is of the correct order of magnitude observed from wound healing assays from [103].

2.3.3 Stability Analysis

In this section, we analyse the stability of the uniform steady states $(\bar{N}, \bar{M})$, since in the subsequent sections, we examine the behaviour of the wound healing model under variation of key parameters using bifurcation analysis. This analysis will inform the stability of the resulting branches. We perform linear stability analysis of the analogous spatially-independent form of the wound healing model:

$$\frac{dN}{dT} = (1 + f_{\max} M \exp(1 - M))N(1 - N) - \delta_N N, \quad (2.22)$$

$$\frac{dM}{dT} = \frac{\alpha N}{\gamma_M + N}(1 - N) - \delta_M M. \quad (2.23)$$

The Jacobian of this system is given by:

$$J = \begin{pmatrix} (1 - 2N)(1 + f_{\max} M \exp(1 - M)) - \delta_N & f_{\max} N(1 - N)(1 - M)(\exp(1 - M)) \\ \frac{\alpha(\gamma_M - N^2 - 2\gamma_M N)}{(\gamma_M + N)^2} & -\delta_M \end{pmatrix}. \quad (2.24)$$

At the steady state $(\bar{N}, \bar{M}) = (0, 0)$, the Jacobian becomes

$$J|_{(0,0)} = \begin{pmatrix} 1 - \delta_N & 0 \\ \frac{\alpha}{\gamma_M} & -\delta_M \end{pmatrix},$$

which provides the following eigenvalues:

$$\begin{aligned}\lambda_1 &= -\delta_M, \\ \lambda_2 &= 1 - \delta_N.\end{aligned}\tag{2.25}$$

Since $\delta_M, \delta_N > 0$, the zero state is an unstable saddle for $\delta_N < 1$ and is a stable node for $\delta_N > 1$. For all other steady states, we use MATLAB's `fsolve` function to determine $(\bar{N}, \bar{M})$ which are then substituted into (2.24) and whose eigenvalues are computed using MATLAB's `eig` function. We note that when considering solutions in travelling wave coordinates, the stability of $\bar{N}$ and $\bar{M}$ in the bifurcation diagrams in Figure 2.7 and 2.14 are swapped, i.e. stable branches are unstable in travelling wave coordinates and vice versa, due to the change of variables $\xi = X - cT$.

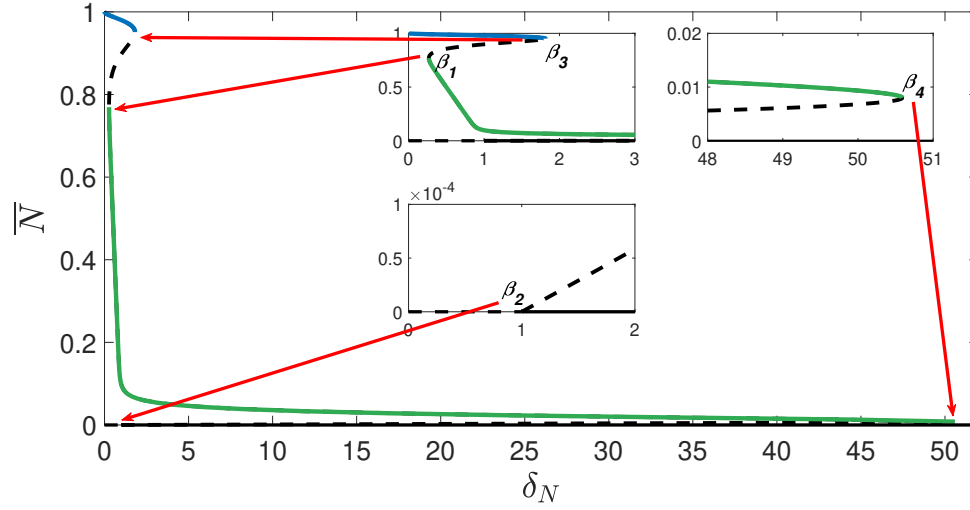
2.4 Deregulated Apoptosis

As mentioned in Section 1.1.2, a potential cause for the emergence of chronic wounds is deregulated apoptosis of cells. In this section, we examine the behaviour of the wound healing model under variation of the apoptotic rate of cells, δ_N . Figure 2.7 shows bifurcation diagrams for $N(X, T)$ and $M(X, T)$ against δ_N ; the stability of the branches is determined by linear stability analysis that is detailed in Section 2.3.1.

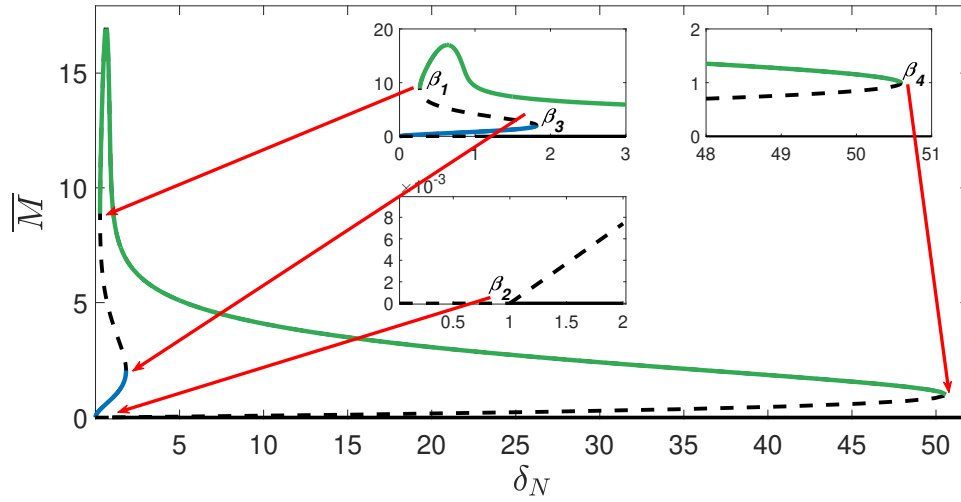
Inspection of the bifurcation diagrams in Figure 2.7 show that we have four bifurcation points that separate five parameter regimes, in which solution behaviours of the wound healing model differ qualitatively. The intervals for each regime are denoted as follows:

$$I_1 = [0, \delta_N^1); \quad I_2 = (\delta_N^1, \delta_N^2); \quad I_3 = (\delta_N^2, \delta_N^3); \quad I_4 = (\delta_N^3, \delta_N^4) \text{ and } I_5 = (\delta_N^4, \infty).\tag{2.26}$$

The positions of the interval boundaries $\delta_N^1 < \delta_N^2 < \delta_N^3 < \delta_N^4$ are given in Table 2.3.



(a)



(b)

Figure 2.7: Bifurcation diagrams of (2.16)–(2.18) showing the steady-states (a) $\bar{N}$ and (b) $\bar{M}$ under variation of δ_N . Other parameter values are given in Table 2.2. The bifurcation diagrams show four bifurcation points $\delta_N^1, \delta_N^2, \delta_N^3$ and δ_N^4 separating five regimes I_1, I_2, I_3, I_4 and I_5 . Dashed lines represent unstable branches and solid lines represent stable branches. The stable branches are colour-coded such that the green branch of steady states of $\bar{N}$ in (a) corresponds to the green branch of steady states of $\bar{M}$ in (b), and similarly for blue branches.

Bifurcation Point i	δ_N^i	$\bar{N}$	$\bar{M}$	Type
1	0.2695	0.7693	8.8787	Saddle Node
2	1	0	0	Transcritical
3	0.8164	0.9508	2.0465	Saddle Node
4	50.5904	0.007998	0.9916	Saddle Node

Table 2.3: Values of the bifurcation points δ_N^1 , δ_N^2 , δ_N^3 , and δ_N^4 shown in Figure 2.7 for varying δ_N . The remaining parameter values are listed in Table 2.2. The corresponding steady-state values of $\bar{N}$ and $\bar{M}$ are also provided.

and the method used to calculate them are given below.

From the bifurcation diagrams in Figure 2.7, we observe that there are four bifurcation points: one transcritical and 3 saddle nodes. The value of δ_N at which the transcritical bifurcation point occurs may be deduced from the argument in Section 2.3.1: from (2.25), we conclude that the zero state is an unstable saddle for $\delta_N < 1$ and is a stable node for $\delta_N > 1$. This change in stability confirms that the transcritical bifurcation point occurs at $(\delta_N, \bar{N}, \bar{M}) = (1, 0, 0)$.

The values of $\bar{N}$ at which the saddle node bifurcation points occur may be calculated by computing the derivative of (2.18) with respect to $\bar{N}$ and setting this equal to zero, i.e. by solving the equation:

$$\begin{aligned} \frac{\alpha f_{\max}}{\delta_M(\gamma_M + \bar{N})^2} & \left(\gamma_M(1 - 4\bar{N} + 3\bar{N}^2) - 2\bar{N}^2(1 - \bar{N}) \right. \\ & \left. + \frac{\alpha \bar{N}(1 - \bar{N})^2(2\gamma_M \bar{N} - \gamma_M + \bar{N}^2)}{\delta_M(\gamma_M + \bar{N})} \right) \exp\left(1 - \frac{\alpha \bar{N}(1 - \bar{N})}{\delta_M(\gamma_M + \bar{N})}\right) + 1 = 0. \end{aligned} \quad (2.27)$$

We use MATLAB's **fzero** function to find the $\bar{N}$ values of the saddle node bifurcation points, and then substitute these into (2.16) and (2.18) to find the corresponding $\bar{M}$ and δ_N values respectively.

The values of $\delta_N^1, \delta_N^2, \delta_N^3$ and δ_N^4 as in Table 2.3, subject to other parameter values as in Table 2.2, are $\delta_N^1 = 0.2965, \delta_N^2 = 1, \delta_N^3 = 1.9164$ and $\delta_N^4 = 50.5904$. These

critical values of δ_N mark the boundaries between the distinct dynamical regimes, each characterised by different qualitative behaviours. While each interval is discussed in greater detail in subsequent subsections, we begin with a brief overview of the qualitative behaviours observed in each region.

For $\delta_N \in I_1$, we have two steady-states for $\bar{N}$: a state approaching unity and the trivial state. As discussed in Section 2.2.3, this parameter regime corresponds to a wound healing to completion, with an increased expression of MMPs at the wound edge. For $\delta_N \in I_2, I_3$, it is unclear from the bifurcation diagram whether or not a wound will heal to completion, due to the existence of other non-trivial steady states. The bifurcation diagram for $\bar{N}$ indicates that we have 3 and 4 nontrivial states for I_2 and I_3 respectively: with one being a healed state ($\bar{N} \approx 1$), and the others being partially healed states. At $\delta_N = \delta_N^3$, we observe a saddle node bifurcation point at which the healed state is destroyed. For $\delta_N \in I_4$, there are two non-trivial states for $\bar{N}$, both being unhealed states which indicates that for $\delta_N \in I_4$, a wound will not heal to completion and hence a chronic wound will persist. Finally, for $\delta_N \in I_5$ only the trivial state exists.

2.4.1 $\delta_N \in I_1$

The bifurcation diagram in Figure 2.7 indicates that we have two steady-state solutions, with one being the trivial state and the other being $\bar{N} \sim 1$. As discussed in Section 2.3, this regime describes the invasion of a wound by a healed front of cells and thus the healing of an acute wound. These features are reflected in direct simulation of the full system in Figure 2.8(a), for which $\delta_N \in I_1$. We also note that travelling waves for $M(X, T)$ in Figure 2.8(b) display an increased expression of MMP concentration at the wound edge, which is in agreement with experimental studies as discussed in Section 1.1.2. From the phase plane diagram in Figure 2.8(c), we see that regardless of initial conditions, trajectories in (N, M) space always arrive at the

healed state, $(\bar{N}, \bar{M})$. Therefore, for $\delta_N \in I_1$ and other parameter values as in Table 2.2, a wound will always heal to completion.

2.4.2 $\delta_N \in I_2, I_3$

For $\delta_N \in I_2, I_3$, the bifurcation diagram in Figure 2.7 shows that we have 3 and 4 nontrivial states, respectively. The phase plane diagrams in Figure 2.8(c) indicate that trajectories of the spatially homogeneous ODE model can approach different steady states depending on the initial condition, with high initial concentrations of MMPs leading to trajectories towards an unhealed equilibrium. This, however, does not immediately transfer to the PDE model. In the representative PDE simulations shown in Figure 2.8(a), using the baseline initial conditions from (2.13)–(2.14) with parameter values from Table 2.2, the wound evolves to complete healing.

The PDE dynamics in these regimes nonetheless depend on initial conditions in a more subtle way. By modifying the initial MMP profile, it is possible to alter which state ultimately invades. For example, with $\delta_N = 1.5$ the baseline set-up produces a healing front connecting the tissue-free state to the healed state, as reproduced in Figure 2.9(a). If, however, the initial MMP amplitude and spread are increased, a second front may emerge that connects the healed state to the unhealed state. This behaviour is shown in Figure 2.9(b), where altered values of $\bar{\nu}$ and $\bar{\eta}$ lead to invasion by the unhealed state. The establishment of this second front also depends on $\bar{\lambda}$, which sets the location of the wound edge. With a larger $\bar{\lambda}$ there is a greater reservoir of healthy tissue available to resist invasion. Figures 2.9(c) and (d) illustrate this: for $\bar{\lambda} = 15$ the second front persists and the wound fails to heal, whereas for $\bar{\lambda} = 16$ the additional healthy tissue suppresses the unhealed front and the wound heals.

These examples demonstrate that while the ODE phase plane correctly signals the possibility of multiple outcomes, the PDE dynamics are governed not only by the equilibria themselves but also by the spatial distribution of initial data and the

competition between travelling fronts. From the ODE analysis, we conclude that low baseline MMP levels reduce the risk of chronic wounds. The PDE model refines this picture since chronic wound formation is promoted not only by elevated MMP levels but also by their amplitude and distribution relative to the wound edge. Large, widespread initial MMP concentrations can generate fronts that drive the system to an unhealed state, whereas sufficient surrounding healthy tissue can suppress this invasion. Thus, in the PDE context, avoiding chronic wounds requires both keeping baseline MMP concentrations low and limiting their spatial spread.

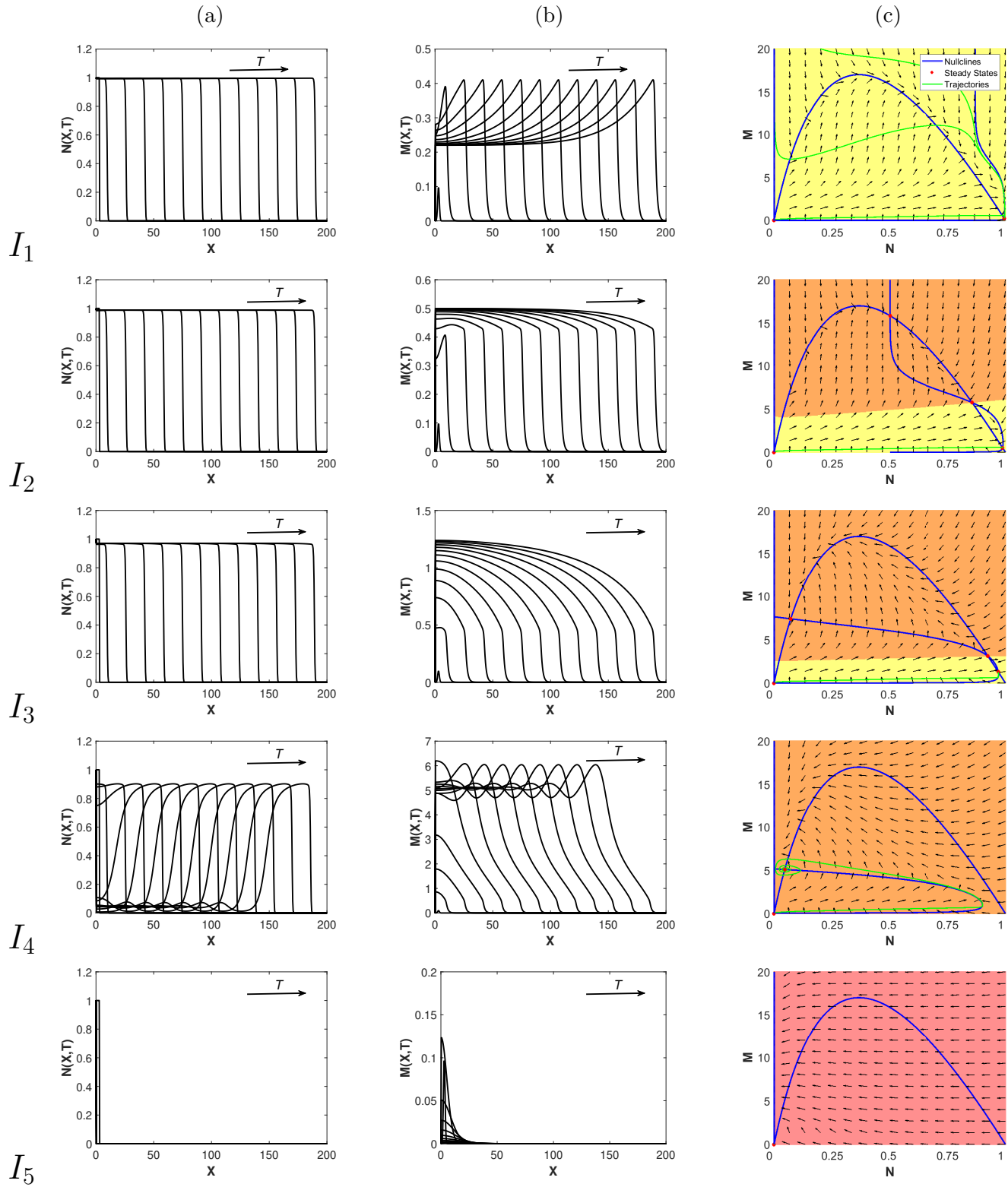
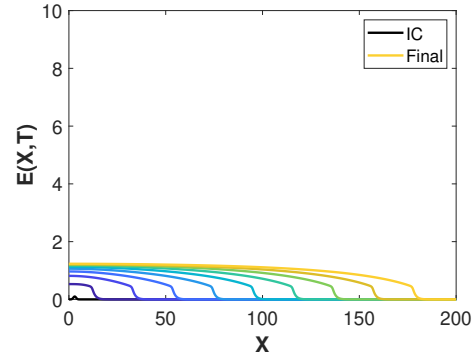
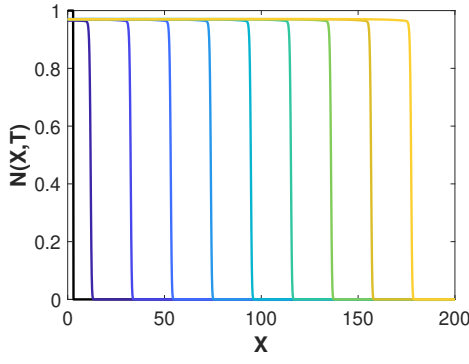
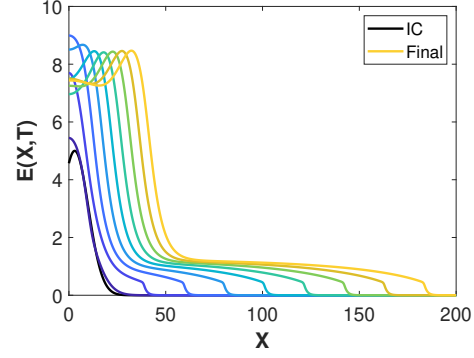
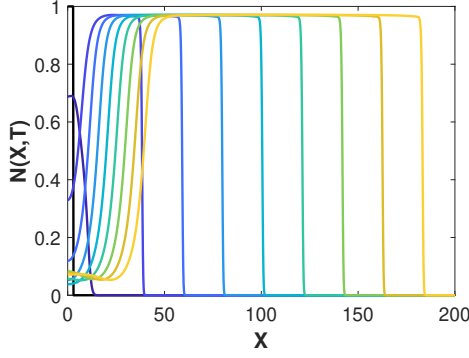


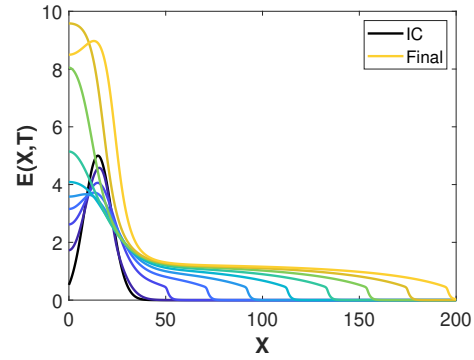
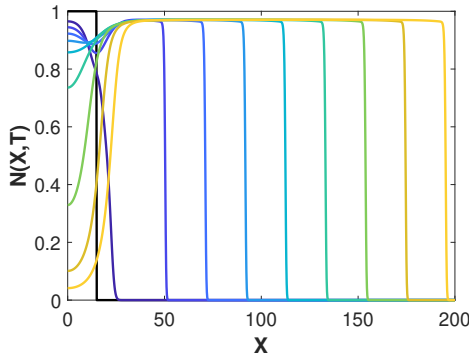
Figure 2.8: Direct simulations of the wound healing model, for δ_N values (top to bottom) 0.13, 0.5, 1.5, 5 and 51, for (a) $N(X, T)$ and (b) $M(X, T)$. The phase plane diagrams in (c) correspond to the spatially independent ODE system (2.22)–(2.23). Blue lines represent nullclines, green lines indicate representative trajectories of the ODE system and red dots represent the steady states. The yellow highlighted section of the domain represents the set of all the initial conditions (ICs) in the (N, M) phase space whose trajectories go to the healed state, the orange section represents the ICs whose trajectories go to an unhealed state and the red section represents the ICs whose trajectories go to the zero state. All other parameter values are as given in Table 2.2 We note that for I_2 and I_3 , only the fully healed travelling waves (obtained using initial conditions as in (2.13)–(2.14)) are shown in (a) and (b). We also note that the M -axis changes in each row.



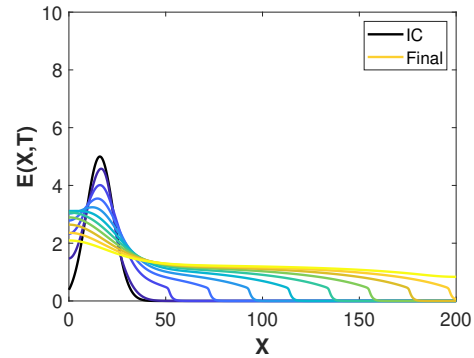
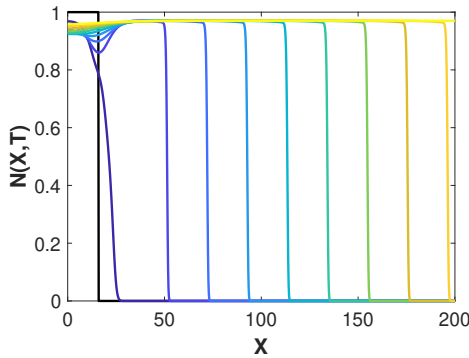
(a) $\bar{\lambda} = 3, \bar{\nu} = 1$ and $\bar{\eta} = 0.1$



(b) $\bar{\lambda} = 3, \bar{\nu} = 10$ and $\bar{\eta} = 5$



(c) $\bar{\lambda} = 15, \bar{\nu} = 10$ and $\bar{\eta} = 5$



(d) $\bar{\lambda} = 16, \bar{\nu} = 10$ and $\bar{\eta} = 5$

Figure 2.9: Numerical simulations of the wound healing model for $\delta_N = 1.5 \in I_3$, showing travelling waves of cell density $N(X, T)$ (left panels) and MMP concentration $M(X, T)$ (right panels) at the time interval $T = 2$. Initial conditions are given by (2.13)–(2.14), with parameter values $\bar{\lambda}, \bar{\nu}, \bar{\eta}$ as indicated in subfigures (a)–(d). All remaining parameters are fixed as in Table 2.2. Varying the amplitude, spread, and location of the initial MMP profile determines whether the system evolves to a healed or unhealed state.

2.4.3 $\delta_N \in I_4$

For $\delta_N \in I_4$, all trajectories arrive at one of two unhealed states for all initial conditions (Figure 2.8(c)). These states have complex eigenvalues, leading to spirals in the phase plane diagram and we therefore expect a travelling wave corresponding to a chronic wound with fluctuating MMP levels about its nonzero state. This is verified by the simulations in Figure 2.8(a) and 2.8(b), where we observe travelling waves with qualitatively different features to those observed in previously discussed intervals.

This change in the qualitative features of the travelling waves corresponds to a saddle-node bifurcation point at $\delta_N = \delta_N^3$ observed in the bifurcation diagrams in Figure 2.7. At this bifurcation point, the branch of healed states $\bar{N} \sim 1$, shown in blue (and the corresponding branch for $\bar{M}$) is destroyed. This loss of a healed state of $\bar{N}$ indicates that the invading state in the travelling wave simulations will transition to an unhealed state, which is verified by Figure 2.8(a) where we also observe increased density of dermal tissue at the edge of the wound. As discussed in Section 2.3, we characterise this as a chronic wound. The emergence of a chronic wound also corresponds to the invading state of $\bar{M}$ in the travelling wave simulations transitioning to a larger value as shown in Figure 2.8(b), suggesting that elevated MMP concentrations prevent the complete healing of the wound. These results are consistent with studies that have identified that deregulated apoptosis is a cause of the emergence of chronic wounds, which are characterised by having raised MMP concentrations.

2.4.4 $\delta_N \in I_5$

For $\delta_N \in I_5$, we find that N and M both tend to zero for all initial conditions. This collapse of the travelling wave is expected in the case where apoptosis of dermal cells is very high, as cells die faster than they can reproduce.

2.5 Varying Parameters

In this section we revisit the bifurcation analysis from the previous section and discuss how the ordering of the bifurcation points changes under variation of other parameter values in the model. Since the travelling waves of the PDE model always connect to the trivial zero state, the ordering of bifurcations determines which equilibria can act as invading states. The bistability intervals, where two stable nontrivial states coexist, play a central role, because the invading state depends on the basin of attraction of the initial conditions as demonstrated in Figure 2.9. In the original reference configuration from Figure 2.7(a), the ordering of bifurcation points is saddle node, transcritical, saddle node, saddle node. This ordering yields the behaviour described previously: for intermediate values of δ_N (in I_2 and I_3), travelling waves may originate either from the healed branch ($\bar{N} \sim 1$) or from the unhealed branch, depending on initial conditions. There are 3 other possibilities in terms of bifurcation point arrangements and we give examples of these in Figure 2.10, along with the different possible travelling wave profiles, illustrating the possible wave connections.

2.5.1 Configuration 1: Exchange of δ_N^2 and δ_N^3

In the first alternative configuration in Figure 2.10(a), the ordering becomes saddle node, saddle node, transcritical, saddle node. Here δ_N^2 and δ_N^3 swap order, so the healed branch is destroyed before the transcritical point. This reduces the parameter interval where healed states are accessible. For values of δ_N between δ_N^1 and δ_N^2 , healed and unhealed branches coexist, and the invading state in the PDE depends on the initial conditions as discussed in Section 2.4.2. Thus in this region, there are two possible travelling wave profiles: one where the healed state connects to the tissue free state and one where the unhealed state invades and connects to the healed state, similar to in Figure 2.9(a) and (b). However, once δ_N exceeds δ_N^2 , the healed state no

longer exists, so only unhealed fronts remain up until δ_N^4 . Thus, in this configuration the chronic waves dominate over a larger parameter range. Similar to before, after δ_N^4 , only the trivial state remains.

2.5.2 Configuration 2: Loss of the final saddle-node

In the second configuration, the final saddle-node δ_N^4 is absent, leaving the ordering saddle node, transcritical, saddle node. Here the unhealed branch connects directly to the zero branch at the transcritical bifurcation point. For values of δ_N between δ_N^1 and $\delta_N^2 = 1$, there is bistability between healed and unhealed states, and the invading state depends on initial conditions as previous, so either acute or chronic travelling waves may occur. Just beyond the transcritical point $\delta_N^2 = 1$, between $\delta_N^2 = 1$ and δ_N^3 , there is bistability between the healed and zero states, so one may expect only acute-type waves are supported. However, not only acute waves, but also we see waves where the invading state is zero, they jump to the healed state and return to the zero state. This phenomenon continues once δ_N exceeds δ_N^3 also, even after the healed state is destroyed, leaving only the zero state. This phenomenon can be explained by the persistence of slow dynamics in the neighbourhood of the destroyed equilibrium branch (the ‘ghost’ of the saddle-node). Thus, while no true healed state exists in this regime, the PDE solutions may transiently visit the healed state before collapse. As well as this, we also observe full collapse of the travelling wave to the trivial state once δ_N becomes large enough.

2.5.3 Configuration 3: Loss of the first two saddle-node

In the final configuration, only two bifurcation points remain: the transcritical bifurcation and a saddle-node. The earlier saddle-nodes are absent. In this scenario, it no longer makes sense to distinguish “healed” and “unhealed” branches, since the single stable branch varies continuously with δ_N . All travelling waves connect this

stable branch to the trivial zero state. Beyond the saddle-node, the stable branch disappears, leaving only the trivial zero state. This corresponds biologically to a simple dichotomy: for $\delta_N < \delta_N^2$ tissue may persist in various states of health through travelling waves connecting the nontrivial state to the trivial state, but for $\delta_N > \delta_N^2$ we settle at zero.

These alternative orderings illustrate how parameter variations reshape the bistability intervals and therefore control the qualitative features of travelling waves. In the original configuration, both acute and chronic waves may occur depending on initial conditions. In Configuration 1, the healed branch is destroyed earlier, so chronic waves dominate over a wider parameter range. In Configuration 2, a large range of waves may occur, even where the zero equilibrium connects back to itself with transients connecting to a phantom healed state. In Configuration 3, only the transcritical and a single saddle-node remain, the distinction between healed and unhealed states disappears, and waves either connect a single stable branch to zero or collapse entirely. The biological outcome is therefore determined not only by the apoptotic rate, δ_N , itself, but also by how the bifurcation points are ordered, since this dictates which equilibria persist and which wave connections are possible.

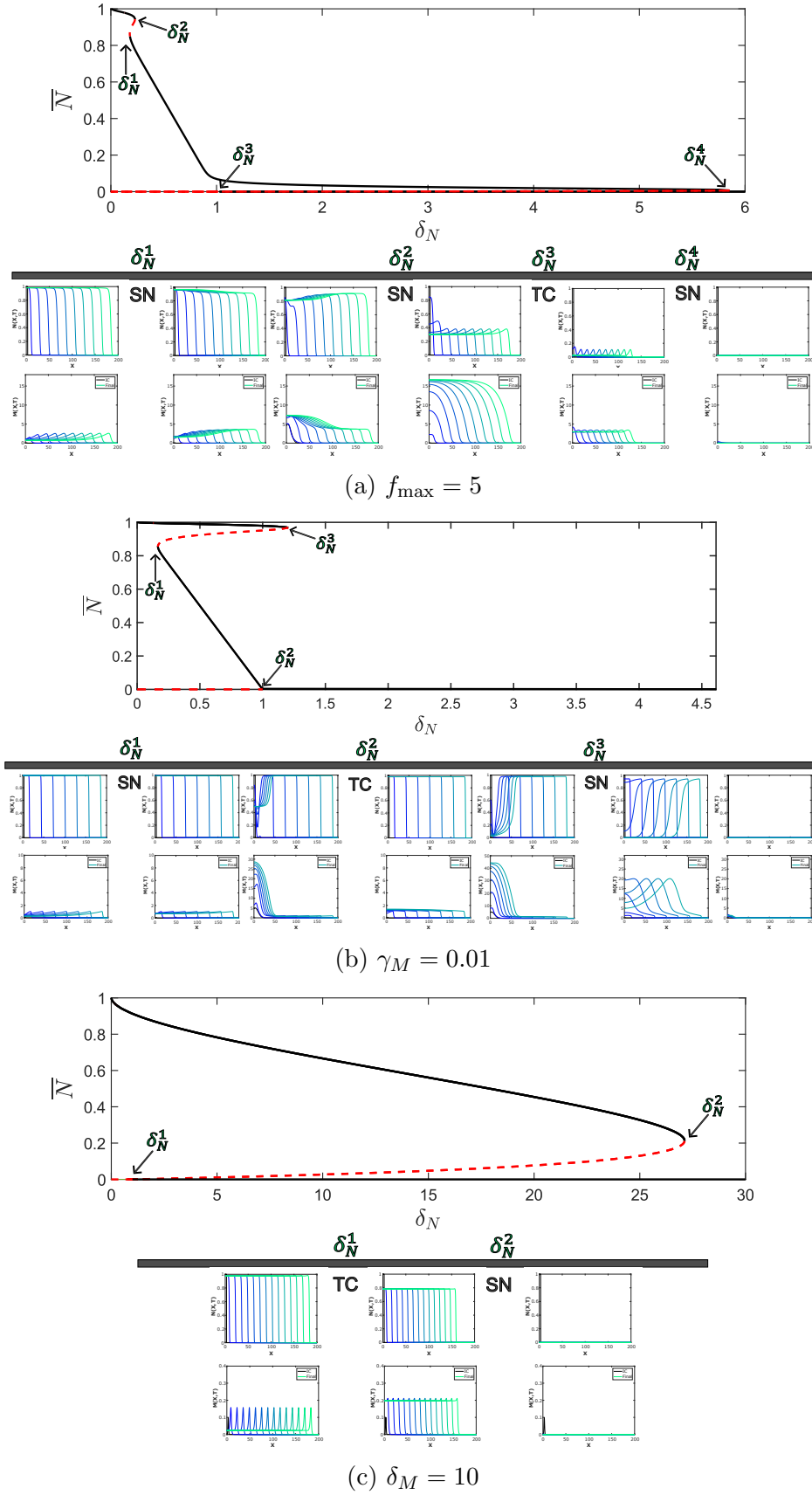


Figure 2.10: Bifurcation diagrams of the steady-state $\bar{N}$ as a function of δ_N for three parameter configurations (a)–(c). In each case, the varied parameter is indicated, while all other parameters are as listed in Table 2.2. The bifurcation points are labelled together with their types (saddle-node or transcritical). Distinct parameter regimes correspond to different sets of possible travelling wave profiles in the PDE model, as discussed in Section 2.5.

2.6 Multistability and Hysteresis

As discussed in Chapter 1, a potential cause for the emergence of chronic wounds is deregulated apoptosis, which may be caused by uncontrolled blood sugar levels in diabetes patients. In this section, we therefore examine how the apoptotic rate, δ_N controls the transition from a healthy to a chronic state. We also investigate whether or not a chronic wound may be reversed if the apoptotic rate were to be regulated, for example, by regulating blood sugar levels. As discussed in Section 2.4.3, a saddle-node bifurcation point occurs at $\delta_N = \delta_N^3$, which results in the emergence of a chronic wound where high concentrations of MMPs prevent the wound healing to completion. The destruction of the healed branch at this bifurcation has important implications for the solution behaviour, which we demonstrate in direct simulations of the wound healing model by allowing δ_N in (2.10) to slowly increase in time:

$$\delta_N(T) = \widehat{\delta}_N + \epsilon T, \quad (2.28)$$

where $\widehat{\delta}_N = 0.1 \in I_1$. Here we choose ϵ sufficiently small so that the evolution of δ_N is slow compared with the dynamics of the waves. This ensures that the fronts have time to adjust and the transition between different wave behaviours can be observed clearly, rather than being skipped over if δ_N were to change too quickly.

As we see in Figure 2.11, the wave for $N(X, T)$ transitions from a completely healed wound to a chronic wound with elevated MMP concentrations, which verifies the results outlined in Section 2.4.3.

A saddle-node bifurcation point also occurs at $\delta_N = \delta_N^1$ as discussed in Section 2.4.3. As such, we consider the case of decreasing δ_N to investigate the implications for the solution behaviour upon decreasing δ_N below δ_N^1 from a regime in which a chronic wound persists. We take a value of $\widehat{\delta}_N$ such that $\widehat{\delta}_N > \delta_N^3$ (to ensure the persistence of a chronic wound), and allow δ_N in (2.10) to slowly decrease in time via (2.28) by

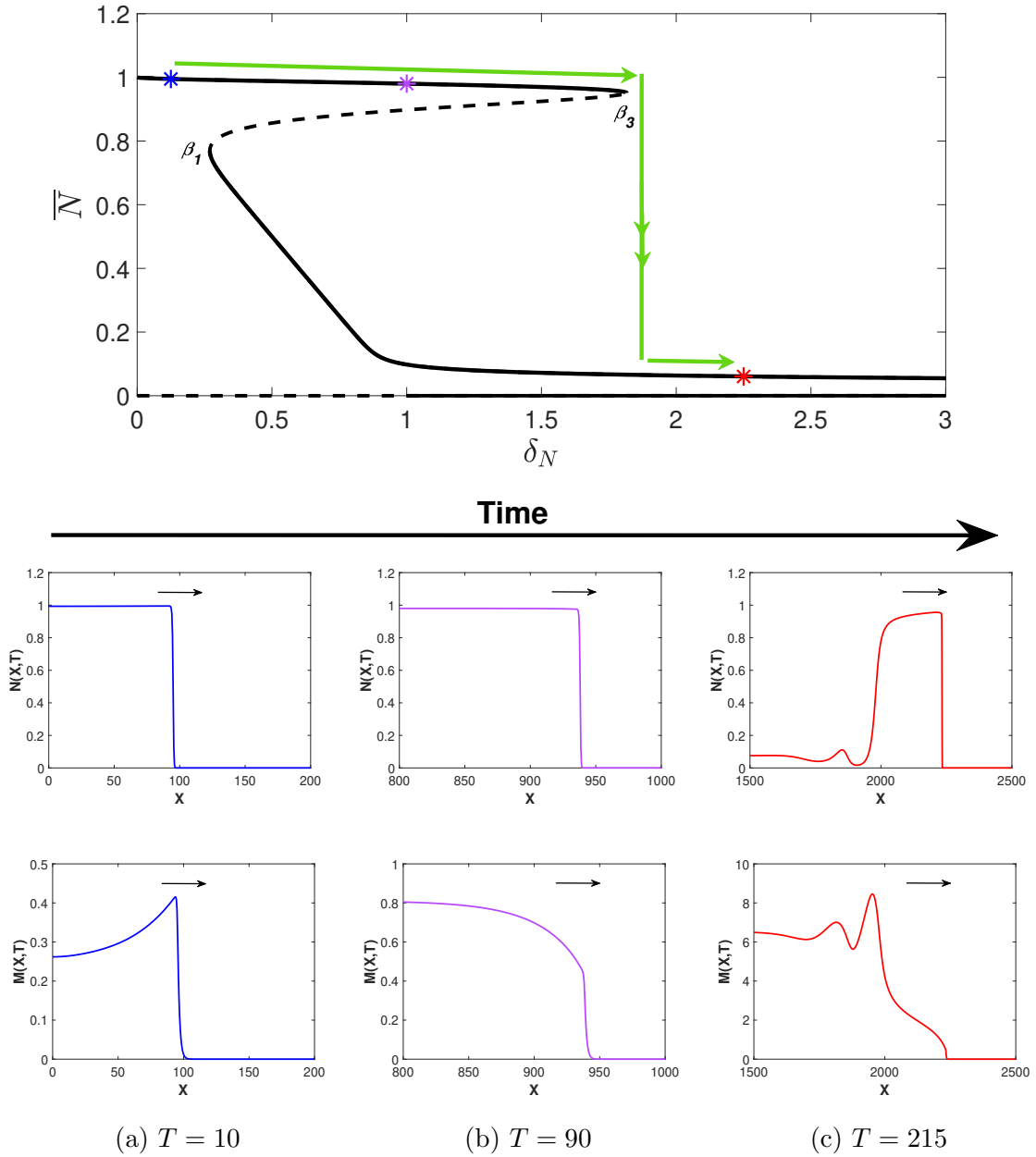


Figure 2.11: Simulations of the wound healing model with (2.28) applied to (2.10), i.e. δ_N increasing in time with $\hat{\delta}_N = 0.1$ and $\epsilon = 0.01$. Other parameter values are as given in Table 2.2. The bifurcation diagram is as given in Figure 2.7(a) for $\delta_N \in [0, 3]$ and coloured asterisks represent the $\delta_N(T)$ values at the time points given in (a), (b) and (c). The green arrows represent the evolution of the invading states of the waves as δ_N is increased. We note that all waves shown connect to the zero state and the X and M -axis changes at each time point. The X -axis in each subplot has been cropped to focus on the qualitative features of the waves. Arrows at each time point indicate that the waves are propagating forwards.

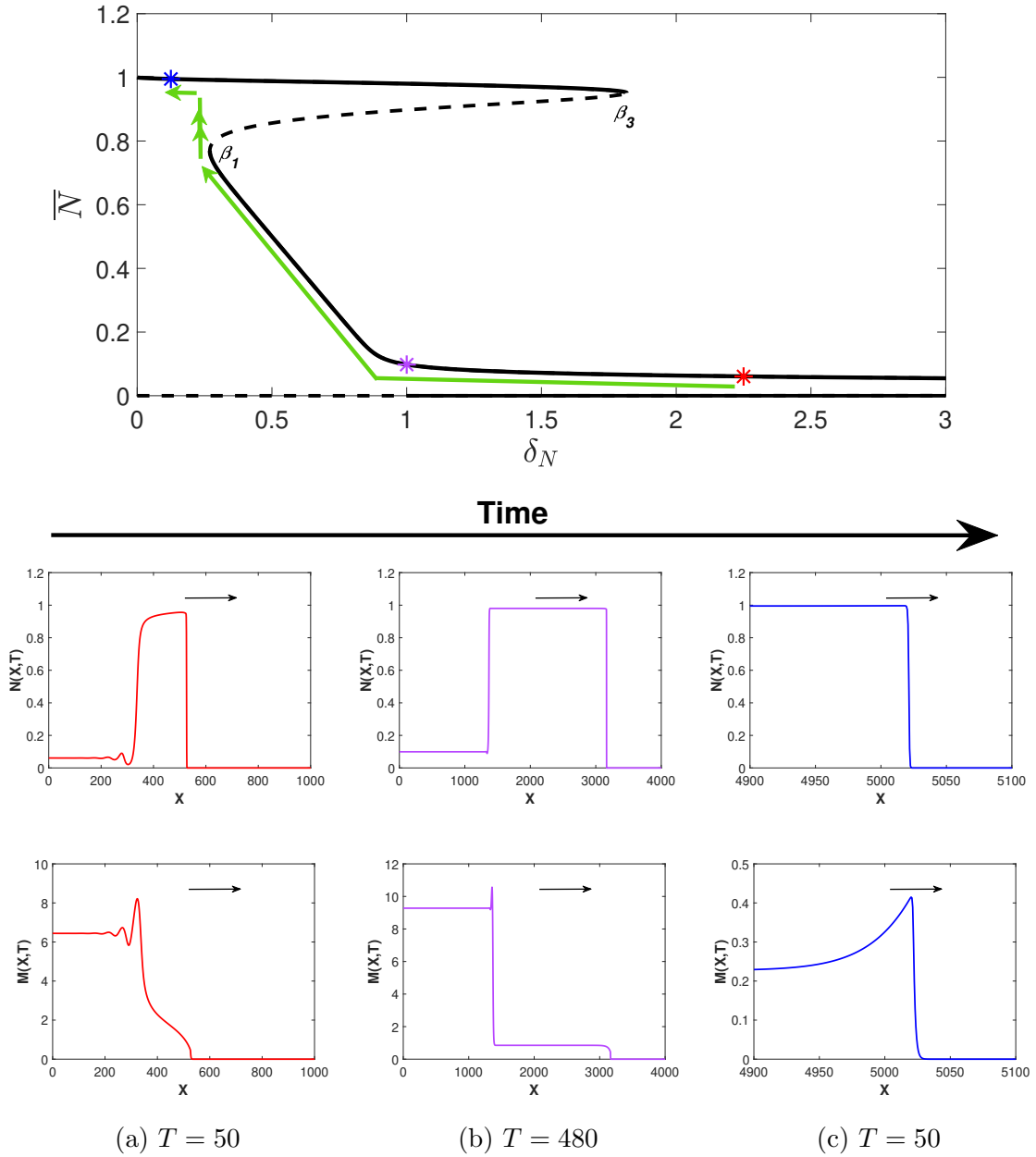


Figure 2.12: Simulations of the wound healing model with (2.28) and $\epsilon \rightarrow -\epsilon$ applied to (2.10), i.e. δ_N decreasing in time with $\hat{\delta}_N = 2.5$ and $\epsilon = 0.005$. Other parameter values are as given in Table 2.2. The bifurcation diagram is as given in Figure 2.7(a) for $\delta_N \in [0, 3]$ and coloured asterisks represent the $\delta_N(T)$ values at the time points given in (a), (b) and (c). The green arrows represent the evolution of the invading states of the waves as δ_N is increased. We note that all waves shown connect to the zero state and the M -axis changes at each time point. The X -axis in each subplot has been cropped to focus on the qualitative features of the waves. Arrows at each time point indicate that the waves are propagating forwards.

changing ϵ with $-\epsilon$. In Figure 2.12, we take $\hat{\delta}_N = 2.5 \in I_4$ and simulate the wound healing model with $\delta_N(T)$ applied to (2.10). From Figure 2.12(c), we observe the emergence of a chronic wound as we expect, displaying qualitative features similar to those in Figure 2.11(c) when considering increasing δ_N . For $\delta_N^1 < \delta_N < \delta_N^3$, i.e. $\delta_N \in I_2, I_3$, however, we observe another partially-healed wound in the wave simulations in Figure 2.12(b) in contrast to Figure 2.11(b) and demonstrates the existence of two qualitatively different wave profiles, depending on the choice of initial conditions. We observe from Figure 2.12(a) that for a chronic wound to heal, $\delta_N(t)$ must be decreased to when $\delta_N < \delta_N^1$, demonstrating the multistable nature of different wave profiles for $\delta_N^1 < \delta_N < \delta_N^3$. We remark that since δ_N is time-dependent, the dynamics are transient and vary with time; hence the fronts observed are waves, but not true travelling waves. Moreover, by multistable waves, we mean that within the bistable parameter window between $\delta_N^1 < \delta_N < \delta_N^3$, which is bounded by saddle-node bifurcations, the system supports two different stable travelling-wave profiles. One profile corresponds to the high N state invading the zero state and the other corresponds to the low N state connecting the high N state. Both are dynamically stable solutions of the PDE, but which one is realised in practice depends on the history of the system. There are multiple, qualitatively distinct wave profiles that can each persist stably. This bistability also gives rise to a hysteresis loop, meaning the system exhibits history dependence, thus the particular profile of the wave that emerges depends on the initial condition and whether the apoptotic rate is increasing or decreasing. We conclude that chronic wounds may persist if apoptotic rates reach an unregulated state, but these chronic wounds may heal to completion if δ_N were able to be regulated from a deregulated state, for example by controlling blood sugar levels. However, successful healing will require substantial reduction in δ_N so as to pass beneath the bistable region and transition back up to the healed branch, meaning that the wound is more difficult to heal than by simply reducing δ_N slightly.

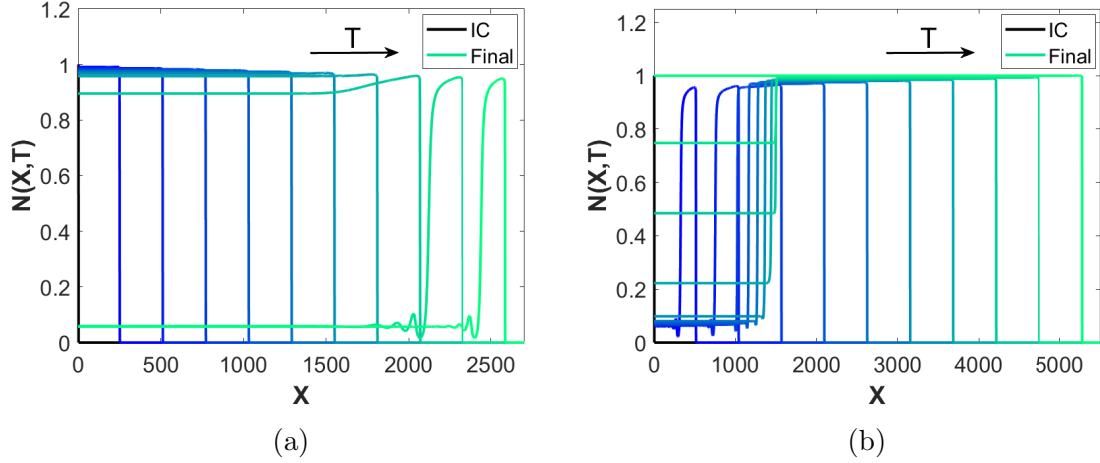


Figure 2.13: Simulations of the wound healing model. Wave progression for time-dependent δ_N . Shown are snapshots of $N(X,T)$ at regular time intervals $T = 30$, with initial (IC) and final profiles indicated. (a) (2.28) applied to (2.10), i.e. δ_N increasing in time with $\hat{\delta}_N = 0.1$ and $\epsilon = 0.01$. Other parameter values are as given in Table 2.2. For increasing δ_N , the front propagates forward and the wound evolves to a chronic state (low N). (b) (2.28) and $\epsilon \rightarrow -\epsilon$ applied to (2.10), i.e. δ_N decreasing in time with $\hat{\delta}_N = 2.5$ and $\epsilon = 0.005$. Other parameter values are as given in Table 2.2. For decreasing δ_N , the front likewise propagates forward, but the wound returns to the healed state (high N).

Since both wave profiles are stable, the fronts propagate forward in space for both increasing and decreasing δ_N . This behaviour is indicated by the arrows in Figures 2.11 and 2.12, and is illustrated more clearly in Figure 2.13, where we show the full wave progression at regular time intervals. In the case of increasing δ_N the wound evolves towards a chronic state, while for decreasing δ_N it returns to a healing state, yet in both cases the fronts advance forward.

2.7 Elevated MMP Production

As described in Section 1.1.2, chronic wounds are characterised by elevated levels of MMPs which prevent its healing. MMP production is a factor that may potentially be targeted with treatments for chronic wounds. In a similar fashion to the varying of δ_N , we now examine the qualitative changes to travelling wave profiles when we

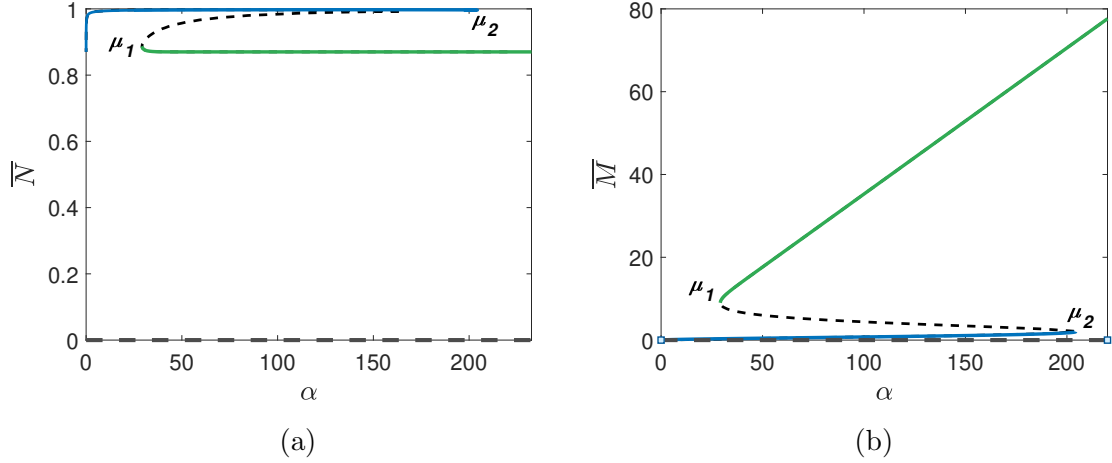


Figure 2.14: Bifurcation diagrams of α against (a) $\bar{N}$, and (b) $\bar{M}$. Other parameter values are as given in Table 2.2 Dashed lines represent unstable branches and solid lines represent stable branches. The stable branches are colour-coded such that the green branch of steady states of $\bar{N}$ in (a) correspond to the green branch of steady states of $\bar{M}$ in (b); equivalent for blue branches.

vary the production rate of MMPs, α .

We present bifurcation diagrams for $\bar{N}$ and $\bar{M}$ against α in Figure 2.14; the stability of the branches is determined by linear stability analysis detailed in Section 2.3.3. Inspection of the bifurcation diagrams reveals two saddle-node bifurcation points (α_1 and α_2) separating three parameter regimes. At α_2 (at which α is large), the branch of healed states of $\bar{N}$ is destroyed and hence, similar to in Section 2.4.3, we expect the emergence of a chronic wound. We also note that the unhealed branch in Figure 2.14 consists of states much higher than those in Figure 2.7 upon varying δ_N . This contrast is due to our choice of $f(m)$ in (2.3). If we allowed $f(m)$ to take negative values for large m , then the unhealed branch would take much lower values.

Similar to Section 2.6, we now investigate the dynamics of the wound healing model for both increasing and decreasing α . Firstly, we allow α in (2.11) to slowly increase in time:

$$\alpha(T) = \hat{\alpha} + \epsilon T, \quad (2.29)$$

where $\hat{\alpha} = 0 < \alpha_1$. As we see in Figure 2.15, the wave for $N(X, T)$ transitions from a completely healed wound to a partially healed wound with elevated MMP concentrations (see the bifurcation diagram in Figure 2.14(b)). These dynamics occur as α exceeds a value $\alpha_* \approx 103.2$, where, despite the invading state of the wave attaining a healed value, the wave visits the unhealed state, resulting in a partially healed wound. Increasing α further results in the the invading state transitioning to an unhealed value, and thus the emergence of a chronic wound at α_2 . We deduce that the threshold MMP production rate at which wounds cease to heal to completion is α_* , rather than the saddle-node bifurcation point α_2 as we may have originally expected.

We now consider decreasing α to investigate whether a chronic wound may be reversed under a treatment based on regulation of MMP production. In Figure 2.16, we take $\alpha = 300 > \alpha_2$ and allow α in (2.11) to slowly decrease in time via (2.29) under the replacement $\epsilon \rightarrow -\epsilon$. From Figure 2.16(d), we observe the emergence of a chronic wound as we expect, showing consistent qualitative features as in Figure 2.15(d) when considering increasing α . For $\alpha_1 < \alpha < \alpha_2$ however, we observe wounds that do not heal to completion in contrast to the case of increasing α , again demonstrating the existence of multistable travelling wave profiles and the profile that emerges depends on the choice of initial conditions. We also observe a change in qualitative features at $\alpha = \alpha_*$. For $\alpha < \alpha_*$, the travelling wave visits the healed state resulting in a partially healed wound. From Figure 2.16(d), we deduce that $\alpha(T)$ must be reduced such that $\alpha < \alpha_1$ for a chronic wound to heal.

The value of α_* was determined heuristically by systematically exploring simulations. Specifically, we fixed the same initial condition and then varied α incrementally (both increasing and decreasing in small steps), rerunning the simulations until we identified the approximate value of α at which the qualitative behaviour of the travelling wave changed. We also note that the change in qualitative behaviours at α_* leads

to more intricate behaviours in terms of direction of propagation than the δ_N case. For increasing α , for $\alpha < \alpha_*$, the wound heals with a single forward-propagating front. When α surpasses α_* , the wave visits the lower state despite the invading state being on the upper branch: a secondary subfront forms, the leading front continues forward, while the trailing subfront slows, stalls, and then propagates backwards. This reflects the influence of the competing basins of attraction, the lower state dominates and leads to a switch from a healing to a chronic outcome. Conversely, when α decreases from high values, the invading state is initially chronic, but as α passes back through α_* the healed state reasserts dominance: both subfronts propagate forward, and the wound transitions back to healing. Thus, unlike the δ_N case where the wave direction is preserved, the α -driven dynamics involve the coexistence of multiple subfronts with distinct directions of motion, and the outcome depends sensitively on the history of the trajectory and its basin of attraction. This behaviour is indicated by the arrows in Figures 2.15 and 2.16, and is illustrated more clearly in Figure 2.17, where we show the full wave progression at regular time intervals.

As in Section 2.6, a hysteresis loop arises, leading to history dependence in the travelling wave profiles as α is increased or decreased. Bistability in the range $\alpha_1 < \alpha < \alpha_2$ gives rise to multiple possible travelling wave profiles, with the specific profile attained depending on the initial condition due to this history dependence. However, unlike in Section 2.6, we observe an inconsistency in the qualitative features of the travelling waves within this bistable region, due to the presence of a threshold value α_* . We conclude that wounds do not fully heal beyond $\alpha = \alpha_*$. Before α reaches α_2 , a small reduction in α may be sufficient to return the system to a fully healed state. However, if α surpasses α_2 , reversing the chronic wound to a state of complete healing will require substantial reduction in α so as to pass beneath the bistable region and transition back up to the healed branch, meaning that the wound is more difficult to heal than by simply reducing α slightly. This suggests that wounds

become significantly harder to heal once the system moves too far into the deregulated regime.

2.8 Conclusions and Discussion

In this chapter, we developed a two-variable reaction-diffusion model, describing the interaction of MMPs with dermal cells in the wound healing process. In particular, we focus attention on the emergence of chronic wounds, since elevated levels of MMPs play a key role in their emergence. Our mathematical model gives rise to travelling wave solutions and in particular is able to emulate key qualitative features of the wound healing process observed in experimental and clinical studies. One such property is that under parameter regimes representing healthy healing, we observe acute wounds healing to completion with an increased expression in MMP concentration at the edge of the healing wound.

To contrast acute wounds with chronic wounds, we consider the effect of varying the apoptotic rate of dermal cells. Small apoptotic rates correspond to healthy biological functioning and thus we observe the complete healing of a wound. Provided initial concentrations of MMPs are kept low, an increase in apoptotic rate also results in the healing of a wound, which suggests that in order to avoid risk of the emergence of a chronic wound, baseline levels of MMPs must be kept minimal. Elevated apoptotic rate beyond a threshold value leads to the emergence of a chronic wound, with increased levels of MMPs invading the wound which prevents its healing. Moreover, the presence of a bistable region in the bifurcation diagrams for the apoptotic rate gives rise to multistable travelling wave profiles. The specific profile obtained depends on the initial condition, reflecting the system's history dependence. As a result, reversing a chronic wound to a fully healed state requires reducing the apoptotic rate below a critical threshold. This highlights the importance of regulating apoptosis in chronic

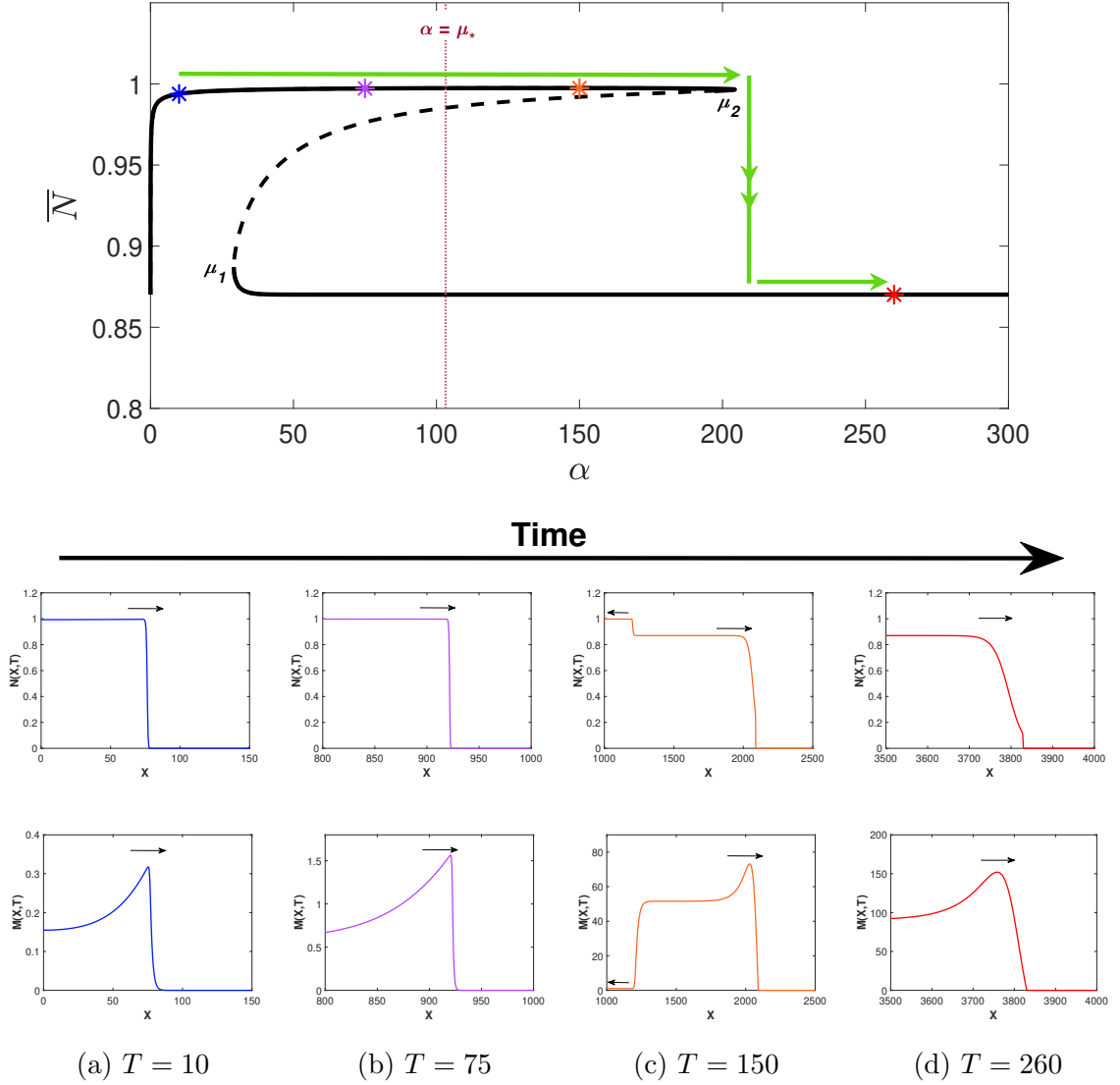


Figure 2.15: Simulations of the wound healing model with (2.29) applied to (2.11), i.e. α increasing in time with $\hat{\alpha} = 0$ and $\epsilon = 1$. Other parameter values are as given in Table 2.2. The bifurcation diagram is as given in Figure 2.14(a) for $\alpha \in [0, 300]$ and $\bar{N} \in [0.8, 1]$ and coloured asterisks represent the $\alpha(T)$ values at the time points given in (a), (b), (c) and (d). The green arrows represent the evolution of the invading states of the waves as α is increased. The maroon dotted line represents the value $\alpha = \alpha_*$. We note that all waves shown connect to the zero state and the M -axis changes at each time point. The X -axis in each subplot has been cropped (indicated by dotted lines) to focus on the qualitative features of the travelling waves; however, the physical scale of the X -axis remains unchanged and is the same as in Figure 2.8(a) and (b).

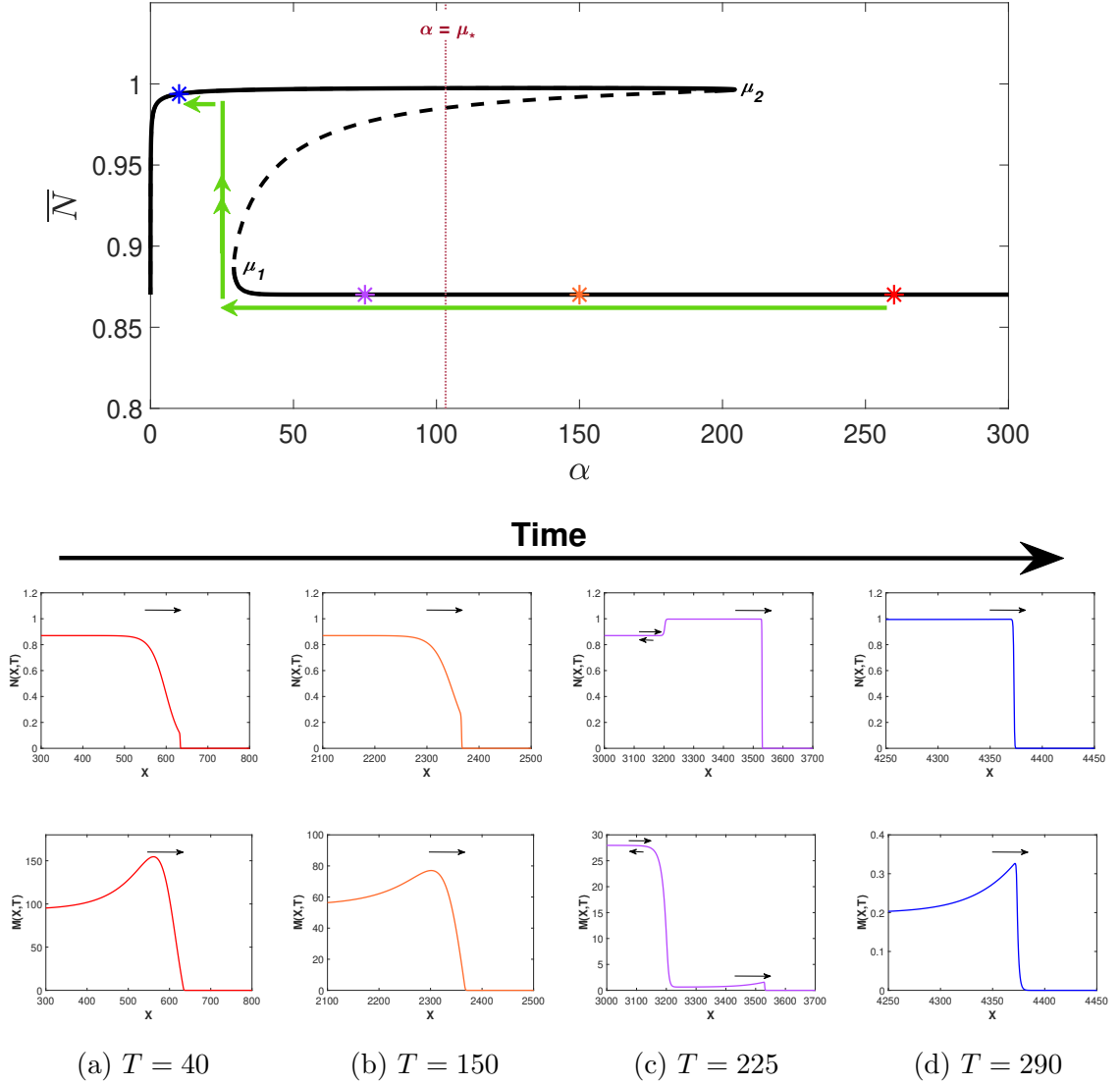


Figure 2.16: Simulations of the wound healing model with (2.29) and $\epsilon \rightarrow -\epsilon$ applied to (2.11), i.e. α decreasing in time with $\hat{\alpha} = 300$ and $\epsilon = 1$. Other parameter values are as given in Table 2.2. The bifurcation diagram is as given in Figure 2.14(a) for $\alpha \in [0, 300]$ and $\bar{N} \in [0.8, 1]$ and coloured asterisks represent the $\alpha(T)$ values at the time points given in (a), (b), (c) and (d). The green arrows represent the evolution of the invading states of the waves as α is increased. The maroon dotted line represents the value $\alpha = \alpha_*$. We note that all waves shown connect to the zero state and the M -axis changes at each time point. The X -axis in each subplot has been cropped (indicated by dotted lines) to focus on the qualitative features of the travelling waves; however, the physical scale of the X -axis remains unchanged and is the same as in Figure 2.8(a) and (b).

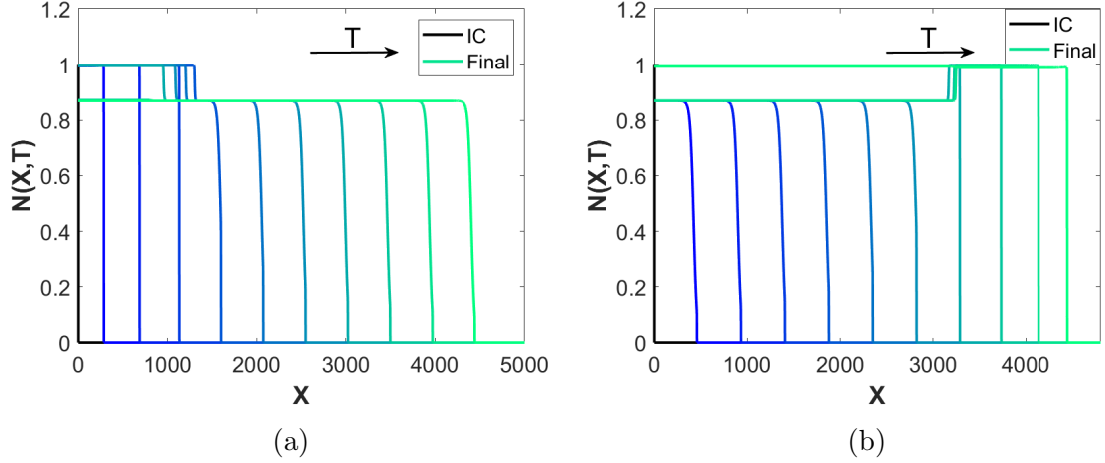


Figure 2.17: Simulations of the wound healing model. Wave progression for time-dependent α . Shown are snapshots of $N(X, T)$ at regular time intervals $T = 30$, with initial (IC) and final profiles indicated. (a) (2.29) applied to (2.10), i.e. δ_N increasing in time with $\hat{\alpha} = 0$ and $\epsilon = 1$. Other parameter values are as given in Table 2.2. (b) (2.29) and $\epsilon \rightarrow -\epsilon$ applied to (2.10), i.e. α decreasing in time with $\hat{\alpha} = 300$ and $\epsilon = 1$. Other parameter values are as given in Table 2.2.

wound treatment, potentially through interventions such as blood sugar control in diabetic patients. Crucially, successful healing demands a substantial reduction in the apoptotic rate to exit the bistable region and transition back to a healed state, making recovery significantly more challenging than by simply decreasing apoptotic rate slightly.

We also consider the effect of varying MMP production rate on the healing of a wound. Similar to the analysis for apoptotic rate, we obtain threshold values at which chronic wounds persist and may be reversed. However, contrasting from the variation of the apoptotic rate, we observe a change in the qualitative features of the travelling wave solutions at a different threshold value μ_* . At this new threshold, occurring in the middle of the multistable regime, a wound transitions from healing completely to only being partially healed, before reaching a regime where chronic wounds persist.

We remark that in the simplified model that we have developed in this chapter,

the role of TIMPs in the wound healing process has not been considered. Moreover, we have simplified the process of wound healing by combining the action of fibroblasts and the extracellular matrix. Therefore, in the next Chapter, we extend our model to address these simplifications.

Chapter 3

Modelling the regulation of chronic wounds by tissue inhibitors of matrix metalloproteinases

3.1 Introduction

Extensive research has been conducted into treatments for chronic wounds, however, the modelling literature currently lacks a thorough exploration of TIMPs as regulators in both acute and chronic wounds, and their potential as therapeutic treatments for chronic wounds. In this chapter, we extend the model framework presented in Chapter 2 to examine the role of TIMPs as regulators of MMP concentrations and hence as regulators of chronic wounds. We observe that, subject to parameter sets corresponding to both acute and chronic wound healing, this mathematical model gives rise to travelling wave solutions. Using bifurcation analysis, we demonstrate that excessive degradation of the ECM results in the emergence of chronic wounds, and the reversal of these chronic wounds is prohibited for lower TIMP production values. These results are replicated within a simplified model obtained via a parameter

sensitivity analysis.

In Section 3.2, we develop our mathematical model which we then nondimensionalise in Section 3.3. We present numerical simulations of the model in Section 3.4 which demonstrates the existence of travelling wave solutions, thus motivating a steady state and bifurcation analysis in Section 3.5, similar to that undertaken in Chapter 2. In Section 3.6, we conduct a parameter sensitivity analysis and exploit this to obtain a reduced model that we then demonstrate supports the same qualitative dynamics as the original model. Finally, we discuss our results in Section 3.7. This chapter contains the results from [162].

3.2 Model Development

We present a mathematical formulation to describe the dynamics of MMPs and TIMPs within a wound and consequently, the impact on the restoration of the ECM by fibroblasts. Elevated concentrations of MMPs contribute to the emergence of chronic wounds, and TIMPs are produced in order to regulate MMP concentrations. We construct a reaction-diffusion model to capture these dynamics in the wound healing process. For simplicity, we consider a wound in a one-dimensional Cartesian geometry, where x denotes the longitudinal distance across a fixed domain $[0, L]$, and we examine the wound's evolution within this domain over time t as in Chapter 2. However, this model consists of four populations: ECM density, denoted as $c(x, t)$; fibroblast cell population density, $f(x, t)$; MMP concentration, $m(x, t)$ and TIMP concentration, $p(x, t)$. We note that the variable $f(x, t)$ refers to the spatial-temporal density of fibroblasts and is distinct from the function $f(m)$ introduced in the previous section. Our model seeks to include all the key processes involved in the interactions between these populations in the context of a wound (see Figure 3.1), resulting in a complex system with numerous parameters. We remark that the schematic in Figure

3.1 extends that from Chapter 2 (Figure 2.1). In Section 3.6, we will use a parameter sensitivity analysis to reduce the complexity of the model.

We assume that the components of the ECM are produced by fibroblasts with production rate σ_c , which decreases to zero as the ECM concentration approaches the carrying capacity c_0 , noting that $c = 0$ corresponds to the complete absence of ECM. As mentioned in Section 1.1.2, MMPs contribute positively to healing due to the activation of TGF- β up to a certain concentration, beyond which their healing contribution diminishes rapidly due to excessive inflammation, effectively reaching negligible levels. We do not model explicitly TGF- β or the relevant inflammatory factors or processes; instead, following the same phenomenological approach described in Chapter 2, we capture their combined influence by a production rate that depends on MMP concentration m .

The degradation of the ECM by MMPs occurs with rate η and is modelled mechanistically, since MMPs are included explicitly. We also include a further ECM degradation term to represent damage independent of MMPs, with rate δ_C , such as oxidative stress, which may be elevated in chronic wounds as discussed in Section 1.1.3. This additional pathway is represented phenomenologically, as ROS is not included explicitly in the model. We note that such degradation is minimal in healthy wound healing.

Fibroblasts undergo mitosis: they are produced by local connective tissue and reproduce by logistic growth. Moreover, fibroblasts undergo apoptosis with rate δ_f , linear diffusion with effective diffusivity D_f , and migrate chemotactically towards the absence of healthy tissue, with rate χ_f , in response to various chemical signals such as growth factors and cytokines at the wound site, which arise from various cell types such as fibroblasts and keratinocytes. Similarly to above, growth factors and cytokines are not explicitly included in our model and so we represent these chemotaxictic effects using an effective term that accounts for the absence of healthy

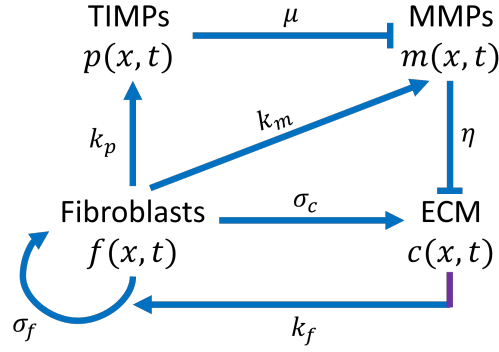


Figure 3.1: Schematic of the interactions of ECM, fibroblasts, MMPs and TIMPs

tissue. We note that the effect of MMPs on fibroblast proliferation is omitted for simplicity.

MMPs are produced by fibroblasts in response to inflammatory chemicals which are found in large quantities in a wound, whose impact on the model variables we describe in a similar way to that outlined above. MMPs diffuse through the wound with diffusivity D_m and decay with rate δ_m . MMPs are inhibited by TIMPs and thus become inactive, represented by their effective removal with rate μ . We remark that this PDE for MMPs is as in Chapter 2 with the only addition being the inhibition term. TIMPs are produced by fibroblasts in response to the presence of MMPs, decay with rate δ_p , and are also depleted in the process of MMP inhibition with rate μ .

Combining these aforementioned assumptions yields the following set of PDEs describing the spatiotemporal evolution of $c(x, t)$, $f(x, t)$, $m(x, t)$ and $p(x, t)$:

$$\frac{\partial c}{\partial t} = \sigma_c \left(1 - \frac{c}{c_0}\right) f \left[1 + \frac{f_{\max}}{m_{\text{thresh}}} m \exp\left(1 - \frac{m}{m_{\text{thresh}}}\right)\right] - \eta c m - \delta_c c, \quad (3.1)$$

$$\frac{\partial f}{\partial t} = D_f \frac{\partial^2 f}{\partial x^2} - \frac{\partial}{\partial x} \left[\chi_f f \frac{\partial}{\partial x} \left(1 - \frac{c}{c_0}\right) \right] + \sigma_f f \left(1 - \frac{f}{f_0}\right) - \delta_f f, \quad (3.2)$$

$$\frac{\partial m}{\partial t} = D_m \frac{\partial^2 m}{\partial x^2} + \frac{k_m f}{\gamma_m + f} \left(1 - \frac{c}{c_0}\right) - \mu m p - \delta_m m, \quad (3.3)$$

$$\frac{\partial p}{\partial t} = D_p \frac{\partial^2 p}{\partial x^2} + \frac{k_p f m}{\gamma_p + f} - \mu m p - \delta_p p. \quad (3.4)$$

We remark the following. The Chapter 2 system is a deliberately lumped, two-variable reaction–diffusion model in which the biphasic MMP effect acts on the net tissue field n . The Chapter 3 system separates ECM, fibroblasts, MMPs and TIMPs, moves the biphasic action to ECM production, introduces MMP–TIMP binding and ECM-guided migration, and makes MMP production depend on both fibroblasts and ECM. Because of these structural differences, i.e. number of variables, placement of the biphasic nonlinearity, additional kinetics, and transport terms, the Chapter 2 model cannot be obtained as sub-limit of Chapter 3. The models address different levels of biological resolution and are therefore complementary rather than hierarchical.

As in Section 2.2.1, the initial conditions are chosen to model a symmetric wound starting at $x = \lambda$. The wound spans the region $[\lambda, L]$ and the tissue surrounding the wound occupies $[0, \lambda)$. Specifically within healthy tissue $[0, \lambda)$, ECM and fibroblasts are initially at carrying capacity (c_0 and f_0 respectively) and absent within the wound region $(\lambda, L]$. We also assume that a small spike in MMPs, κ_1 is initially present at the wound edge, λ while TIMPs are absent throughout. These modelling assumptions yield the following initial conditions:

$$(x, 0) = c_0 H(\lambda - x), \quad (3.5)$$

$$f(x, 0) = f_0 H(\lambda - x), \quad (3.6)$$

$$m(x, 0) = \kappa_1 \exp\left(-\left(\frac{x - \lambda}{\kappa_2}\right)^2\right), \quad (3.7)$$

$$p(x, 0) = 0 \quad (3.8)$$

where ν represents the initial spread of MMPs. In (3.5)–(3.6), H is the Heaviside function, c_0 and f_0 correspond to the carrying capacity of the ECM and fibroblasts, respectively, κ_1 represents the peak value of MMPs, and κ_2 corresponds to the spike width of MMPs in (3.7). Since we assume the wound to be symmetric and the surrounding tissue to be healed far away from the wound, we adopt zero-flux boundary conditions on both ends of the finite spatial domain $[0, L]$:

$$\frac{\partial}{\partial x} f(x, t) = \frac{\partial}{\partial x} m(x, t) = \frac{\partial}{\partial x} p(x, t) = 0 \quad \text{at} \quad x = 0, L. \quad (3.9)$$

3.3 Non-dimensionalisation

We non-dimensionalise the system (3.1)–(3.9) in order to reduce the number of parameters and thus simplify the analysis of the system. Since we are interested in the behaviour of the system on the timescale of production rate of the ECM, we introduce the following dimensionless variables:

$$C = \frac{c}{c_0}, \quad F = \frac{f}{f_0}, \quad M = \frac{m}{m_{\text{thresh}}}, \quad P = \frac{p}{m_{\text{thresh}}}, \quad X = \sqrt{\frac{\sigma_c}{D_f}} x, \quad T = \sigma_c t. \quad (3.10)$$

Applying these scalings to (3.1)–(3.4) yields the following dimensionless equations:

$$\frac{\partial C}{\partial T} = \sigma_C F(1 - C)(1 + f_{\max} M \exp(1 - M)) - \bar{\eta} C M - \delta_C C, \quad (3.11)$$

$$\frac{\partial F}{\partial T} = \frac{\partial^2 F}{\partial X^2} - \frac{\partial}{\partial X} \left(\chi_F F \frac{\partial}{\partial X} (1 - C) \right) + \sigma_F F(1 - F) - \delta_F F, \quad (3.12)$$

$$\frac{\partial M}{\partial T} = D_M \frac{\partial^2 M}{\partial X^2} + \frac{k_M F}{\gamma_M + F} (1 - C) - \bar{\mu} M P - \delta_M M, \quad (3.13)$$

$$\frac{\partial P}{\partial T} = D_P \frac{\partial^2 P}{\partial X^2} + \frac{k_P F M}{\gamma_P + F} - \bar{\mu} M P - \delta_P P. \quad (3.14)$$

We take $\nu = \sqrt{\frac{\sigma_c}{D_f}}$ in (3.5)–(3.7) and $\kappa_2 = 1$ in (3.7). These choices simplify the specification of the initial conditions and reduce the number of parameters. We remark in passing that numerical investigations (that we omit for brevity) indicate that solution dynamics are unaffected (beyond initial transients) by different choices of initial data. Using the above, we obtain the following nondimensional initial and boundary conditions:

$$C(X, 0) = H(\bar{\lambda} - X), \quad (3.15)$$

$$F(X, 0) = H(\bar{\lambda} - X), \quad (3.16)$$

$$M(X, 0) = \bar{\kappa}_1 \exp\left(-\left(\frac{X - \bar{\lambda}}{\bar{\kappa}_2}\right)^2\right), \quad (3.17)$$

$$P(X, 0) = 0, \quad (3.18)$$

and

$$\frac{\partial}{\partial X} F(X, T) = \frac{\partial}{\partial X} M(X, T) = \frac{\partial}{\partial X} P(X, T) = 0 \quad \text{at} \quad X = 0, \bar{L}, \quad (3.19)$$

where $\bar{\kappa} = \frac{\kappa_1}{m_{\text{thresh}}}$, $\bar{\lambda} = \lambda \sqrt{\frac{\sigma_c}{D_f}}$, $\bar{\kappa}_2 = \kappa_2 \sqrt{\frac{\sigma_c}{D_f}}$ and $\bar{L} = L \sqrt{\frac{\sigma_c}{D_f}}$. We remark that the dimensionless values $\bar{\lambda}$, $\bar{\kappa}_1$, $\bar{\kappa}_2$ and $\bar{L}$ are chosen for illustrative purposes and the

corresponding dimensional parameter values are straightforward to calculate. We denote the dimensionless system (3.11)–(3.19) as the ‘regulated wound healing model’ (RWHM).

Dimensional parameter values appearing in (3.1)–(3.4) are listed in Table 1, together with citations to their sources. In contrast to the previous chapter, which served primarily as a proof of concept with limited attention to parameter selection, we now place greater emphasis on the biological realism of parameter values by drawing upon data from experimental studies and relevant biological literature. Where possible, these values were obtained from relevant experimental and biological literature, although we note that some of the referenced studies differ in the types of MMPs (e.g., collagenases, gelatinases) and TIMPs examined, as well as the specific skin tissue types used in the experiments. Nonetheless, these give the best available estimates, in the absence of comprehensive and tailored experimental data. The remainder were estimated heuristically to construct a parameter set representing the complete healing of an acute wound through exploratory numerical simulations. The corresponding dimensionless parameter values are given in Table 2 and we note that unless otherwise stated, these values are used in all subsequent numerical simulations.

Values for the degradation rates of MMPs and TIMPs, δ_M and δ_P were calculated based on the half-lives obtained from [167] and [168] using $\ln(2)/\text{half life}$; those for the production rates of MMPs and TIMPs, k_M and k_P , were converted to units appropriate for our model via the molecular masses also specified in the literature; and the diffusion coefficient for fibroblasts, D_F , was estimated using the fibroblast migration rate reported in [169], applying the classical relation $\langle x^2 \rangle = 4Dt$. Lastly, we estimate the chemotactic constant for fibroblasts as $\chi_f = 3D_f$. This value is supported by experimental findings indicating that directed migration can exceed random movement by up to five-fold [170], and a threefold increase lies well within this biologically plausible range. Moreover, this choice is consistent with previous

modelling studies that have similarly assumed the chemotactic rate to be three times the diffusivity [171, 172]. The rate of ECM degradation by MMPs, η , was derived from kinetic data on human collagen hydrolysis reported in [173]. The published catalytic efficiency was given in units of $M^{-1}s^{-1}$, which we converted into $M^{-1}h^{-1}$ for consistency with the model. For inhibition of MMPs by TIMPs, we used kinetic binding parameters from [174], where the association constant (k_{on}) was also reported in $M^{-1}s^{-1}$ and similarly converted to $M^{-1}h^{-1}$. The ECM carrying capacity was obtained from [175] using the reported bulk density of type I collagen. Fibroblast carrying capacity was estimated from [176], which reported fibroblast densities (cells/mm²) in histological sections of breast tissue. The maximum reported density was taken as an upper bound and converted into model units (cells/g, then g/cm²) using published values for cell numbers per gram of tissue [177]. For fibroblast apoptosis, [178] reported approximately 1% TUNEL-positive cells in human dermis after 72 hours, indicating that approximately 1% of fibroblasts undergo apoptosis over this period. Assuming apoptosis occurs at a constant fractional rate, this corresponds to a mean apoptosis rate of 0.000139 h^{-1} . Finally, for ECM production, [179] reported a 0.44 increase in collagen I mRNA expression over 24 h in arm tissue; assuming a linear increase, this yields the production rate value listed in Table 3.2.

3.4 Model Simulations

To obtain numerical simulations of the RWHM, we discretise the one-dimensional spatial domain $[0, L]$ into $N_x = 1000$ equally-spaced nodes, using second-order finite differences to approximate spatial derivatives. The resulting system of time-dependent ODEs was then integrated using MATLAB's `ODE15s` function.

Subject to the parameter values in Table 2, which represent well-regulated wound healing, we obtain the numerical solutions of the RWHM in Figure 3.2. Here, we

Parameter	Description	Value	Reference
D_f	Diffusion coefficient for Fibroblasts	$1.96 \times 10^{-6} \text{ cm}^2\text{h}^{-1}$	[169]
D_m	Diffusion coefficient for MMPs	$9 \times 10^{-6} \text{ cm}^2\text{h}^{-1}$	[163]
D_p	Diffusion coefficient for TIMPs	$9 \times 10^{-6} \text{ cm}^2\text{h}^{-1}$	[163]
χ_f	Chemotactic coefficient for Fibroblasts	$5.88 \times 10^{-6} \text{ cm}^2\text{h}^{-1}$	[169, 170], Estimate
σ_c	Production rate of ECM	0.0183h^{-1}	[179]
c_0	Carrying capacity of ECM	0.68 g cm^{-3}	[175]
$f_{\max}$	Maximum factor with which mitosis can be enhanced due to the presence of MMPs	15	Estimate
m_{thresh}	Threshold concentration of MMPs at which maximum healing contribution $f_{\max}$ is attained	$9 \times 10^{-10} \text{ M}$	Estimate
η	Rate of ECM degradation by MMPs	$9.144 \times 10^6 \text{ M}^{-1}\text{h}^{-1}$	[173]
δ_c	ECM degradation rate by factors other than MMPs	0.00001h^{-1}	Estimate
σ_f	Mitotic rate of fibroblasts	0.15h^{-1}	Estimate
f_0	Carrying capacity of fibroblasts	0.632 g cm^{-3}	[176, 177]
δ_f	Fibroblast apoptotic rate	0.000139h^{-1}	[178]
k_m	Maximal rate of MMP production by fibroblasts	$2.23 \times 10^{-10} \text{ Mh}^{-1}$	[180, 181]
γ_m	Density of fibroblasts at which half of k_m is attained	0.3g cm^{-3}	Estimate
μ	Inhibition rate of MMPs by TIMPs	$3.65 \times 10^7 \text{ M}^{-1}\text{h}^{-1}$	[174]
δ_m	MMP decay rate	0.0141h^{-1}	[167]
k_p	Maximal rate of TIMP production by fibroblasts	0.235 h^{-1}	[180, 182]
γ_p	Density of fibroblasts at which half of k_p is attained	0.35g cm^{-3}	Estimate
δ_p	TIMP decay rate	0.1733h^{-1}	[168]

Table 3.1: Values, definitions and sources of the dimensional parameters appearing in (3.1)–(3.4).

Parameter	Definition	Value
σ_C	$\frac{f_0}{c_0}$	0.9294
$\bar{\eta}$	$\frac{\eta m_{\text{thresh}}}{\sigma_c}$	0.4497
f_{max}	-	15
δ_C	$\frac{\delta_c}{\sigma_c}$	5.4645×10^{-4}
χ_F	$\frac{\chi_f}{D_f}$	3
σ_F	$\frac{\sigma_f}{\sigma_c}$	8.1967
δ_F	$\frac{\delta_f}{\sigma_c}$	0.0076
D_M	$\frac{D_m}{D_f}$	4.5918
k_M	$\frac{k_m}{m_{\text{thresh}} \sigma_c}$	15.3188
γ_M	$\frac{\gamma_m}{f_0}$	0.4747
$\bar{\mu}$	$\frac{\mu m_{\text{thresh}}}{\sigma_c}$	1.7705
δ_M	$\frac{\delta_m}{\sigma_c}$	0.7730
D_P	$\frac{D_p}{D_f}$	4.5918
k_P	$\frac{k_p}{\sigma_c}$	12.8415
γ_P	$\frac{\gamma_p}{f_0}$	0.4747
δ_P	$\frac{\delta_p}{\sigma_c}$	9.4692
$\bar{L}$	-	100
$\overline{\kappa_1}$	-	0.1
$\overline{\kappa_2}$	-	1
$\bar{\lambda}$	-	3

Table 3.2: Values and definitions of the dimensionless parameter values appearing in the Regulated Wound Healing Model in (3.11)–(3.19).

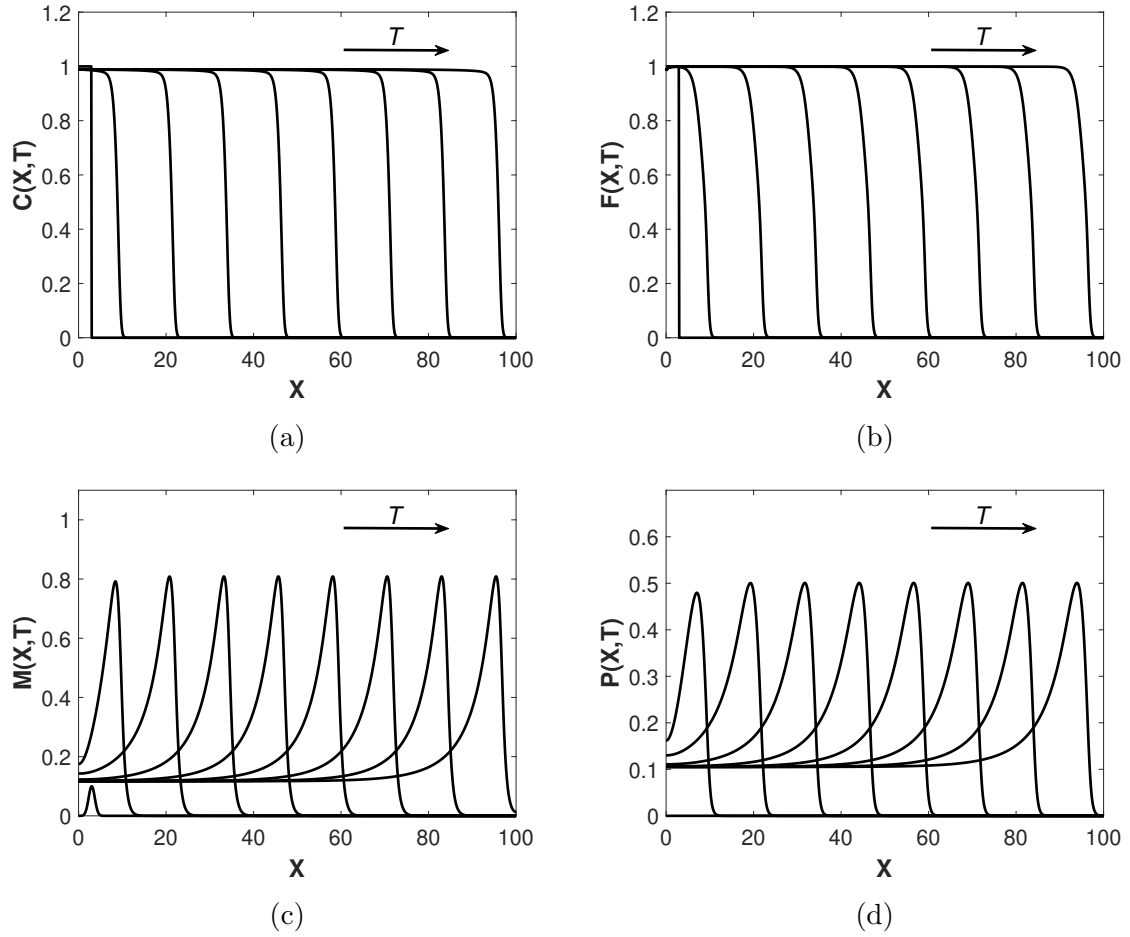


Figure 3.2: Simulation of the RWHM (3.11)–(3.19) at regular time intervals $T = 2$, representing an acute wound, with all parameter values as in Table 2. Evolution of (a) ECM density $C(X,T)$, (b) fibroblast density $F(X,T)$, (c) MMP concentration $M(X,T)$ and (d) TIMP concentration $P(X,T)$ across the spatial domain.

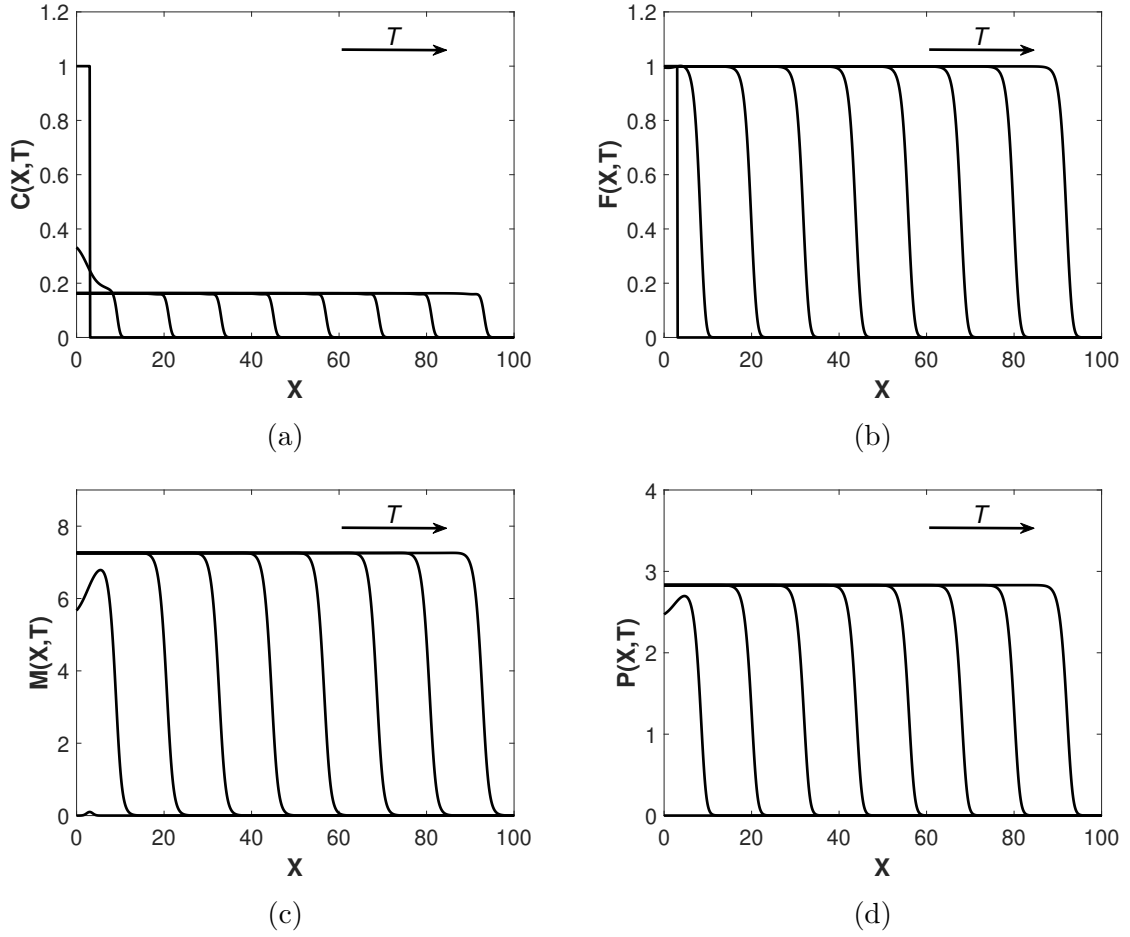


Figure 3.3: Simulation of the RWHM (3.11)–(3.19) at regular time intervals $T = 2$, representing a chronic wound, with $\delta_C = 2.5$, $k_M = 74$ and all other parameter values as in Table 2. Evolution of (a) ECM density $C(X, T)$, (b) fibroblast density $F(X, T)$, (c) MMP concentration $M(X, T)$ and (d) TIMP concentration $P(X, T)$ across the spatial domain.

observe the evolution of ECM density $C(X, T)$ into a travelling wave, where a state close to unity, (i.e. a healed state), invades a wound ($C = 0$). The invasion of a wound by a healed front of tissue suggests the complete healing of an acute wound. We also observe the formation of travelling waves for fibroblasts, $F(X, T)$, where their invading state is close to unity. Additionally, during the wound healing process, we observe travelling waves for MMPs, $M(X, T)$ and TIMPs, $P(X, T)$, as shown in Figures 3.2(c) and (d) respectively. Notably, a spike in the concentrations of MMPs and TIMPs is observed at the edge of the healing wound. We note that this spike occurs naturally and arises across a broad range of initial conditions. We further remark that these travelling waves are consistent with those in Chapter 2, in which we observe travelling waves for dermal cells and MMPs that display the same qualitative features described above. These observations are consistent with the biological literature [6, 28], which suggest that there is an increased expression of MMP concentration at the edge of a healing wound in all stages of the healing of an acute wound.

We now examine the emergence of a chronic wound. Within our model, dysregulated healing processes associated with chronic wounds can be defined by various parameter choices, as their causes and characteristics differ from patient to patient. In line with [6, 11, 183], and for illustrative purposes, we opt to represent this by elevated MMP production and ECM degradation (with the remaining parameters as in Table 2). Numerical simulations of the RWHM, given in Figure 3.3 show the evolution of ECM density into a travelling wave in which the invading state takes an unhealed value, i.e. a value not close to unity. We characterise this unhealed invasion as a chronic wound, in contrast to that observed in acute wounds. Correspondingly, we observe travelling wave profiles in fibroblasts, MMPs and TIMPs. Importantly, the invading state of the MMP wave is elevated in comparison to the acute wound state, as previously observed in Chapter 2 and in accord with clinical studies [6] TIMPs are also elevated, reflecting the production of more TIMPs in response to elevated MMP

levels in an effort to regulate their concentrations.

3.5 Steady States and Bifurcation Analysis

From the numerical solutions of the RWHM seen in Figure 3.2, we observe the emergence of travelling wave solutions. Therefore, in this section, we examine the uniform steady states of our system corresponding to the far-field states of the travelling wave solutions. In particular, the non-zero far-field ECM density, $\bar{C}$, will determine whether or not a wound heals to completion. The steady states of the system $(\bar{C}, \bar{F}, \bar{M}, \bar{P})$ are given by setting the temporal and spatial derivatives in the RWHM to zero:

$$\sigma_C F(1 - C)(1 + f_{\max} M \exp(1 - M)) - \bar{\eta} C M - \delta_C C = 0, \quad (3.20)$$

$$\sigma_F F(1 - F) - \delta_F F = 0, \quad (3.21)$$

$$\frac{k_M F}{\gamma_M + F}(1 - C) - \bar{\mu} M P - \delta_M M = 0, \quad (3.22)$$

$$\frac{k_P F M}{\gamma_P + F} - \bar{\mu} M P - \delta_P P = 0. \quad (3.23)$$

While the above equations cannot be solved analytically for non-trivial solutions, it is clear that we always have the trivial steady state $(C, F, M, P) = (0, 0, 0, 0)$ for all parameter sets.

As discussed in Section 1.1.3, one potential cause for the emergence of chronic wounds is elevated ECM degradation due to oxidative stress, which may be caused by uncontrolled blood sugars in diabetes patients [184]. Therefore, we numerically solve the steady state equations to determine $(\bar{C}, \bar{F}, \bar{M}, \bar{P})$ and examine how $\bar{C}$ changes

with the scaled degradation rate of the ECM, δ_C .

It is important to note that ECM dysregulation, δ_C , reflects a distinct biological process from the apoptotic cell death considered in Chapter 2, Section 2.4. While both phenomena may be triggered by uncontrolled blood glucose levels as discussed in Section 1.1.3, they affect wound healing through different mechanisms. Elevated blood sugar can dysregulate apoptosis in cells such as fibroblasts, as explored in the previous chapter, whereas it can also induce excessive oxidative stress, leading to increased ECM degradation, which is the focus of this chapter. Despite being driven by a common underlying cause and modelled using similar mathematical frameworks, these processes represent biologically distinct aspects of chronic wound pathology.

Bifurcation diagrams are presented in Figure 3.4, where we note the presence of a bistable region bounded by two saddle-node bifurcation points. This phenomenon indicates that if δ_C is increased sufficiently, the invading state of the travelling waves will transition to the lower, unhealed branch. In Section 2.4, we presented a similar bifurcation diagram, showing how the steady states of dermal cells change with cell apoptotic rate δ_N , which also exhibited the existence of such a bistable region. It was demonstrated that if the invading state takes a value on the lower chronic wound branch, then a successful treatment will require substantial reduction in δ_N so as to pass beneath the bistable region and transition back up to the healed branch, meaning that the wound is more difficult to heal, and demonstrating the presence of a hysteresis loop [185]. The bistability indicates the persistence of multi-stable travelling wave profiles, and the profile that we obtain depends on initial conditions corresponding to either a healed or chronic wound. These dynamics were demonstrated through numerical simulations, providing valuable insights into the maintenance of chronic wounds.

We now extend this bifurcation analysis by examining the influence of TIMP production, k_P on the bifurcation diagrams for the ECM degradation rate δ_C in

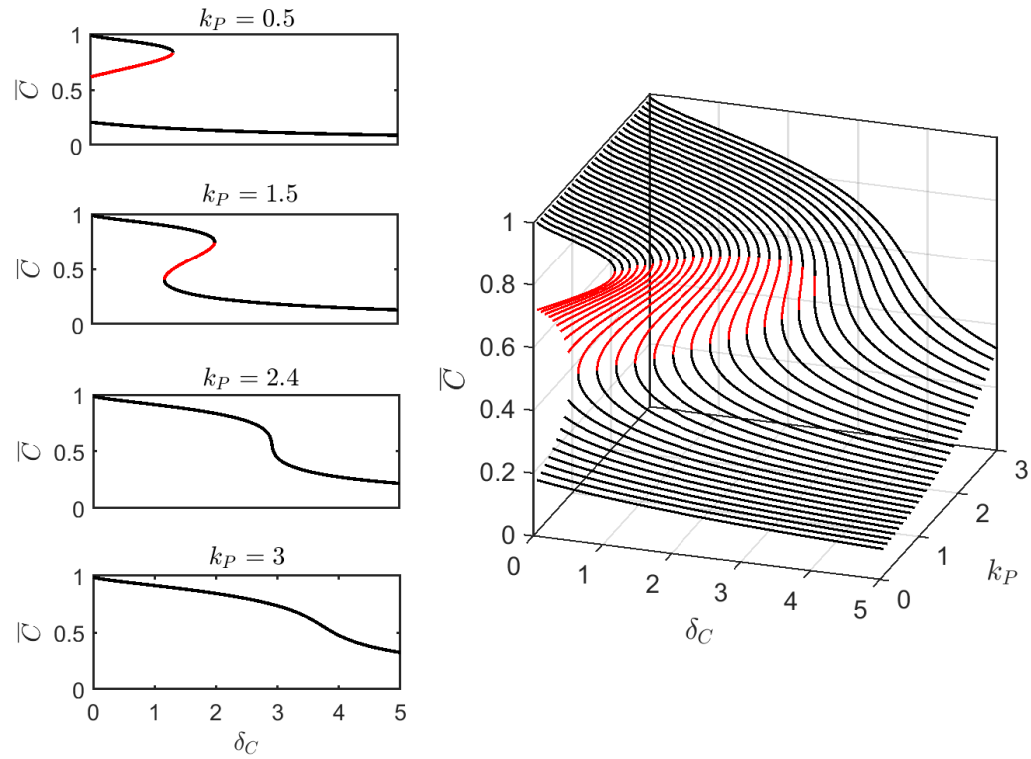


Figure 3.4: Bifurcation diagrams of (3.20)–(3.23) showing the behaviour of the steady state $\bar{C}$ under variation of δ_C and k_P . The solid black lines represent stable branches and the solid red lines represent unstable branches. We also display bifurcation diagrams in the $(\bar{C}, \delta_C)$ -plane for specified values of k_P .

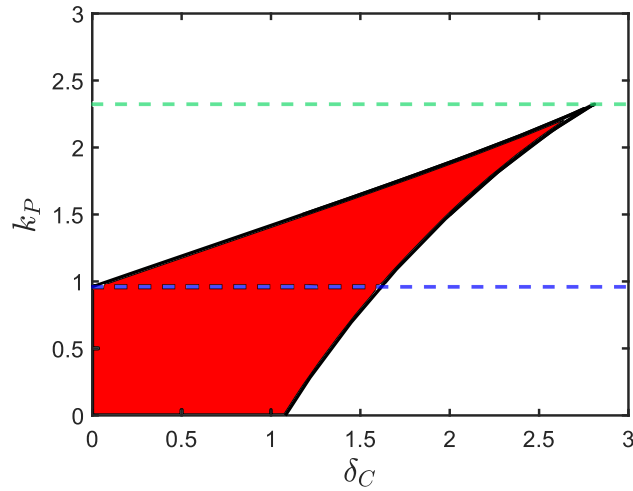


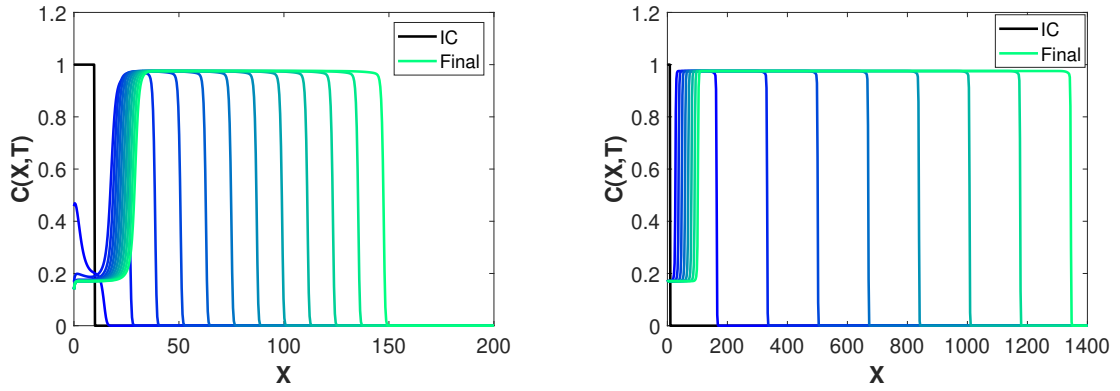
Figure 3.5: Two-parameter continuation of the interaction of the degradation rate of ECM parameter, δ_C and the TIMP production parameter, k_P . The red highlighted region represents the parameter space where bistability is observed. The blue and green dashed lines, at $k_P = 0.960$ and $k_P = 2.323$ respectively, are the thresholds of changing qualitative behaviours.

Figure 3.4. We observe that for small TIMP production, one of the saddle-node bifurcation points exists in the negative δ_C region, therefore, the invading state of the travelling wave cannot return to the healed branch from the lower chronic wound branch in the physically relevant case of positive δ_C . The model initially predicts that at low TIMP production levels, chronic wounds may not be reversed since MMP regulation is insufficient, implying that external treatment may be required to restore healing. While these predictions are evident in the corresponding ODE bifurcation diagram, they must be confirmed in the spatially extended PDE model. To test this, we fix k_P so that one of the saddle nodes lies in the negative δ_C region, and consider three representative values in this regime: $k_P = 0.2$, 0.6 , and 0.9 . For small positive δ_C (e.g. $\delta_C = 0.1$), Figure 3.6 shows the short- and long-term outcomes. In the short term, wounds remain unhealed for $k_P = 0.2$ and $k_P = 0.6$, but heal for $k_P = 0.9$. Over longer times, the wound eventually heals for $k_P = 0.6$, whereas for $k_P = 0.2$ it remains trapped in a chronic configuration consisting of two, locally stable sub-fronts. This behaviour indicates that at very low TIMP levels, MMP degradation

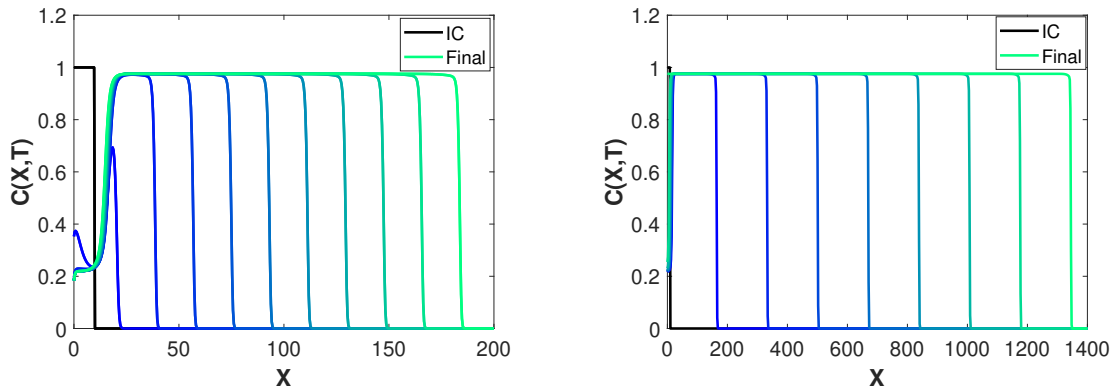
overwhelms ECM recovery, stabilising the chronic front and preventing a return to the healed branch. The wound is therefore effectively irreversible for $k_P = 0.2$. For $k_P = 0.6$, healing occurs only after a long transient, indicating weak reversibility, while for $k_P = 0.9$ recovery is rapid. Reduced TIMP production thus does not eliminate healing altogether but greatly delays it. The ODE bifurcation structure correctly predicts reduced healing capacity at low k_P , but the full PDE dynamics reveal a continuum from irreversible to weakly reversible behaviour depending on TIMP levels.

For larger TIMP production, such as $k_P = 1.5$, both saddle nodes occur at positive δ_C , and the wound heals readily as δ_C decreases. This is demonstrated in the continuation experiment of Section 2.6, where δ_C is decreased in time until it reaches zero. In this regime, the wound remains unhealed for $k_P = 0.6$ within the simulated timeframe (Figure 3.7), whereas for $k_P = 1.5$ the wound heals completely. Hence, low TIMP levels lead to chronic wounds that are progressively harder, and in the extreme case ($k_P = 0.2$) effectively impossible, to reverse.

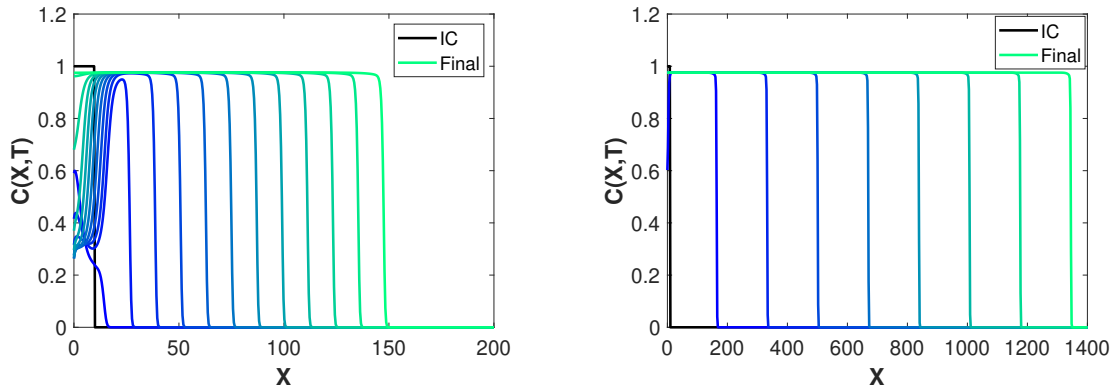
As k_P is increased, we observe dynamics similar to that of the model in Section 2.4 – a hysteresis loop in which it is possible to transition from a chronic wound to a healed wound by decreasing δ_C sufficiently enough such that we are able to return to the upper healed branch. As k_P is increased further, the unstable branch is destroyed, resulting in a single stable branch. The regulatory potential of k_P and δ_C on the healing of the ECM is summarised by the two-parameter continuation of δ_C against k_P in Figure 3.5. From this figure, we deduce that below the value $k_P = 0.960$, weakly-reversible chronic wounds occur, where one of the saddle-node bifurcation points exists in the negative δ_C region, while for values above $k_P = 2.323$, the hysteresis loop is destroyed and we see only a single stable non zero branch for $\overline{C}$. For values of k_P between the above two threshold values, we identify a hysteresis loop representing reversible chronic wounds. This bifurcation analysis suggests that



(a) $k_P = 0.2$



(b) $k_P = 0.6$



(c) $k_P = 0.9$

Figure 3.6: RWHM (3.11)–(3.19) for three representative values of k_P , with $\delta_C = 0.1$ and $\bar{\lambda} = 10, \bar{\kappa}_1 = 10$ and $\bar{\kappa}_2 = 10$ in the initial condition (3.17) specified above. All other parameter values as in Table 3.2. The first panels in each of (a), (b) and (c) represent the short-time solutions for the evolution of ECM density $C(X, T)$ across the spatial domain (time intervals of $T = 2$). The second panels represent the long time solutions (time intervals of $T = 20$).

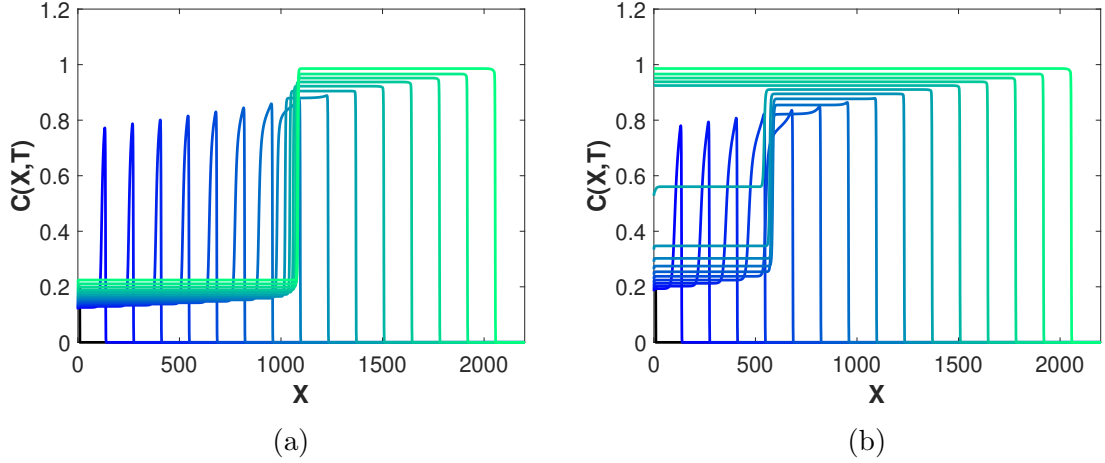


Figure 3.7: Simulations of the RWHM. Wave progression for time-dependent δ_C . Shown are snapshots of $N(X, T)$ at regular time intervals $T=40$. δ_C is decreased from 3 to zero with $\delta_C(T) = 3 - \epsilon T$ with $\epsilon = 0.005$. (a) $k_P = 0.6$ and (b) $k_P = 1.5$. Other parameter values are as given in Table 3.10.

the TIMP production rate significantly contributes to the regulation of the healing of chronic wounds as a result of its MMP inhibiting properties.

For values above $k_P = 2.323$, only a single stable branch exists. In the bistable regime, healed and chronic outcomes are clearly distinguished by the two coexisting equilibria and the travelling wave front between them. In the absence of bistability there is no sharp bifurcation separating outcomes, so we adopt an operational definition. Clinically, chronic wounds are usually defined not by absolute tissue density but by persistent inflammation and failure to progress through the normal healing stages. In our model, N represents tissue density, and we therefore classify outcomes as healed if the invading state remains close to full density ($N > 0.8$) while MMP concentrations remain near baseline ($M < 2$, nondimensional), representing limited inflammation. Although N is not tensile strength, it is biologically well established that measures of wound healing seldom return to 100% of their pre-injury values, for example, tensile strength typically plateaus around 80%. This motivates our choice of 0.8 as a pragmatic cutoff, not as a literal mapping, but as an analogy to capture the idea that healing corresponds to substantial though not complete restoration. The

dual criterion for N and M is not intended as an exact clinical standard but as a representative threshold consistent with biological understanding of wound chronicity, and the specific numerical values are illustrative rather than definitive.

3.6 Parameter Sensitivity and Model Reduction

In this section, we conduct a parameter sensitivity analysis of the RWHM. Currently, there are 19 dimensionless parameters; we seek to retain only those representing the mechanisms of greatest significance. This reduction in the number of parameters allows us to simplify the RWHM and enhance our understanding of the key mechanisms driving wound healing which may aid in developing effective therapeutic strategies. Since the modelling literature currently lacks a thorough exploration of TIMPs as regulators in wound healing, we cannot predict *a priori* which processes are of the highest importance and therefore, a sensitivity analysis is valuable in identifying whether, and to what extent, precise values of parameters and retention of certain processes are important to model predictions. This is particularly important in light of the uncertainty regarding parameter values discussed above. Additionally, reducing the model will make it less computationally expensive, which will be of benefit for extensions to higher spatial dimension that will allow for future exploration of wound geometries of potentially more direct clinical applicability.

We adopt the Extended Fourier Amplitude Sensitivity Test (eFAST), which is based on variance decomposition techniques [186–188], to quantify the individual influences of the parameters, as well as their combined interactions on designated sensitivity metrics. A brief explanation of the method follows, though the interested reader is directed to Saltelli et al. [186] and references therein for detail.

The eFAST method, developed by Saltelli et al. [186], following the original Fourier Amplitude Sensitivity Test (FAST) proposed by Cukier et al. [189], is based

on variance decomposition methods. The input parameters are varied, resulting in changes to the model's output. These changes are measured using the variance metric:

$$s^2 = \frac{\sum_{i=1}^N (y_i - \bar{y})^2}{N - 1}, \quad (3.24)$$

where N is the sample size, y_i is the i^{th} model output and $\bar{y}$ is the sample mean. The algorithm partitions the total variance of the model output to identify how much of it is influenced by each parameter. To achieve this, each parameter is varied using a sinusoidal function of distinct frequency (i.e. a search curve). The resulting output variations are apportioned using a Fourier spectral analysis, determining the model's sensitivity to each parameter to be quantified. The algorithm is executed N_R times (this resampling undertaken to circumvent inefficiency in the method associated with the symmetries of trigonometric functions), using different search curves for each iteration. Thus, the total number of model simulations, N , is given by $N = N_S \times N_R \times k$ where N_S is the total number of samples per search curve and k is the number of parameters being analysed.

We consider the metric (3.24) based on three model outputs: the far-field state of ECM (the value of $\bar{C}$ at $x = 0$), the wave speed of the resulting travelling waves, and the maximum MMP concentration. We choose the far-field state of ECM as it determines whether or not a wound heals to completion; the wave speed is indicative of whether the wound is healing at a healthy rate; elevated MMP concentrations may lead to chronic wounds by degrading the ECM and therefore the maximum MMP concentration provides insight into the potential development of chronic wounds.

The eFAST method yields both a first-order sensitivity and a total-order sensitivity, each of which take a value between 0 and 1 that represents the fraction of the output variance attributed to the variation of the given parameter. The first-order sensitivity index assesses how much a single parameter contributes to the output vari-

ance, whereas the total-order index considers not only the parameter itself but also its interactions with all other model parameters.

We plot the eFAST sensitivity analysis of the RWHM for each of the sensitivity metrics specified earlier in Figure 3.8. We remark that this figure also contains the first- and total-order sensitivity of an additional “dummy” parameter that does not affect the model output. It is used as a control to provide a baseline for comparison with the other parameters. Parameters with a total-order sensitivity index less than or equal to that of the dummy parameter are regarded as not significantly influencing the model output, and so we identify these as candidates for omission. Figure 3.8 indicates that χ_F, γ_M, D_P and γ_P fall below the dummy variable threshold for two of the three sensitivity measures considered and so are identified as candidates for exclusion. While the model is sensitive to both χ_F and D_P , as measured by wave speed, D_P is identified to be one of the most influential parameters affecting wave speed. On this basis, we retain D_P and omit the Michaelis constants γ_M and γ_P and the chemotactic constant χ_F . We therefore reduce the model accordingly, by removing the associated processes, to obtain the Regulated Wound Healing Model 2 (RWHM2).

In the reduced model, Michaelis–Menten terms $\frac{k_M F}{\gamma_M + F}$ and $\frac{k_P F}{\gamma_P + F}$ replaced by linear terms $k_M F$ and $k_P F$ respectively. This corresponds to discarding the saturation effect and redefining the rate parameter so that the overall production rate is effectively linear in fibroblast density. The new constants k_M and k_P therefore have different units from the original as the denominators are removed. This simplification retains the qualitative system dynamics, although the healed steady state shifts quantitatively as discussed in the following Section. In principle, one could calibrate k_M so that the reduced model matches the full model at the baseline steady state (by imposing $k_M^{\text{new}} \bar{F} = \frac{k_M \bar{F}}{\gamma_M + \bar{F}}$), however, in the present work we retained the original values of k_M and k_P for simplicity and did not re-fit them after reduction. Since several param-

eters are already estimated from the literature, exact steady state values are not of primary importance, instead, our focus is on preserving the qualitative behaviours of the system.

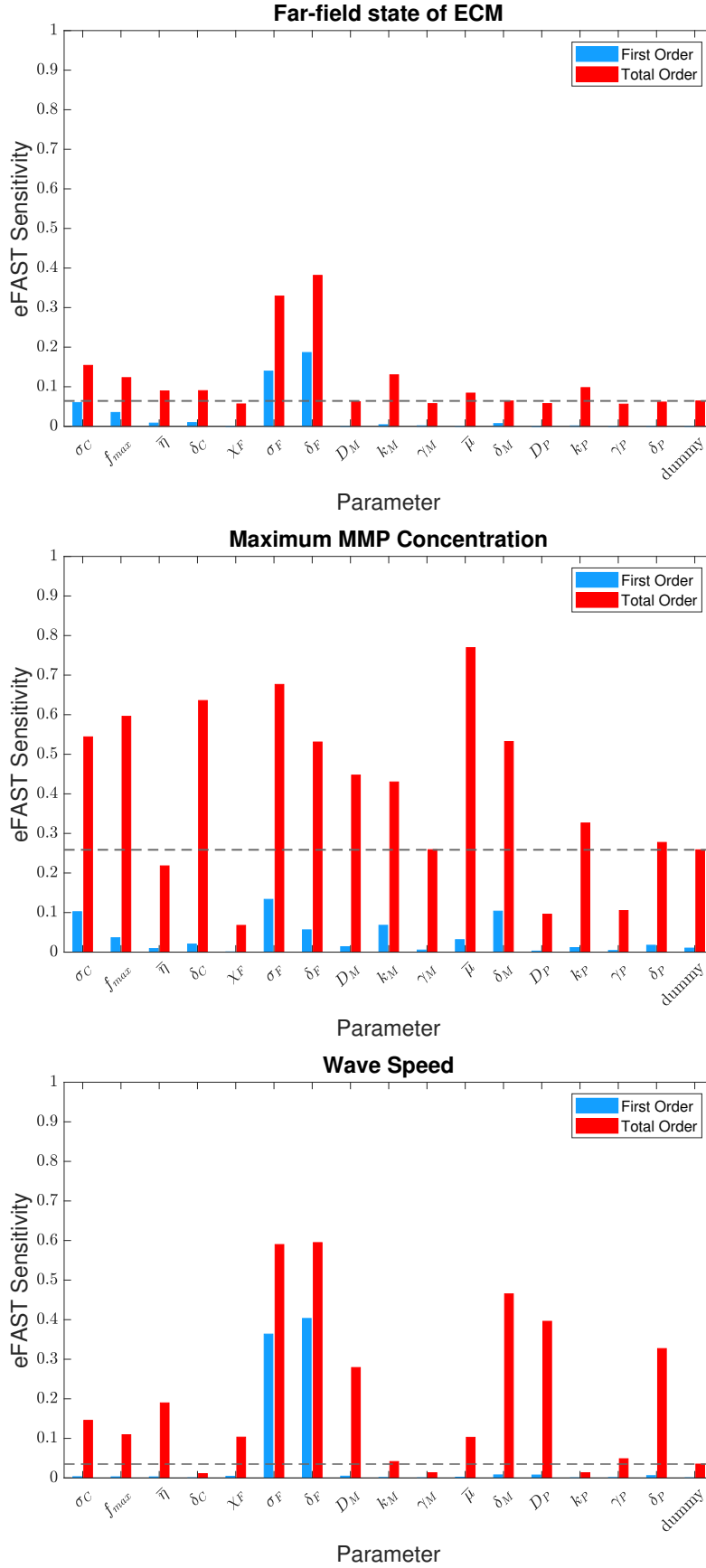


Figure 3.8: eFAST sensitivity analysis of the parameters of the RWHM on the sensitivity metrics (a) far-field state of the ECM; (b) maximum MMP concentration and (c) wave speed. Blue bars represent the first order sensitivities and red bars represent total order sensitivities. The black dashed line illustrates the total order sensitivity of the dummy parameter. Template code adapted from [190] with $N_R = 4$, $k = 17$ and $N_S = 513$.

3.6.1 Reduced Model

The reduced RWHM, denoted as the RWHM2, keeps only the most significant parameters in the RWHM and is defined as follows:

$$\frac{\partial C}{\partial T} = \sigma_C F(1 - C)(1 + f_{\max} M \exp(1 - M)) - \bar{\eta} C M - \delta_C C, \quad (3.25)$$

$$\frac{\partial F}{\partial T} = \frac{\partial^2 F}{\partial X^2} + \sigma_F F(1 - F) - \delta_F F, \quad (3.26)$$

$$\frac{\partial M}{\partial T} = D_M \frac{\partial^2 M}{\partial X^2} + k_M F(1 - C) - \bar{\mu} M P - \delta_M M, \quad (3.27)$$

$$\frac{\partial P}{\partial T} = D_P \frac{\partial^2 P}{\partial X^2} + k_P F M - \bar{\mu} M P - \delta_P P. \quad (3.28)$$

In this subsection, we demonstrate that the RWHM2 displays largely similar qualitative features to the original model.

We present numerical simulations of the RWHM2 in Figure 3.9 using the same methodology as in Section 3.4 and parameter values as in Table 2. From Figure 3.9, we observe travelling wave solutions representing the complete healing of an acute wound, i.e. the invasion of a wound by a healed front of cells and ECM, and an increased expression of MMPs and TIMPs at wound edge. These qualitative observations are consistent with those of the original model as well as experimental studies, as discussed in Section 3.4. As in Section 3.5, we plot a two-parameter bifurcation diagram for the RWHM2 in Figure 3.10. The key qualitative features are consistent with those of the original model shown in Figure 3.5: below a lower threshold, chronic wounds are irreversible; between two thresholds, wounds may be reversed; and above the upper threshold, a single stable branch exists, indicating that wounds are more easily regulated.

From the above analysis, we deduce that the reduced model successfully emulates key qualitative features of the original model while being less computationally

expensive (a runtime of 100.2 seconds compared with 284.6 seconds for the original RWHM). We remark that, between the original and reduced models, there are differences in the precise shapes of the waves and the locations of behavioural shifts. However, these differences are somewhat arbitrary, as we have not performed any fitting to experimental data. Therefore, these minor deviations between the two models are not significant. This reduction in computational cost is particularly advantageous when extending the model to a two-dimensional spatial domain as we will do in Chapter 4, since it allows us to simulate wound healing more efficiently without sacrificing biological realism.

3.7 Conclusions and Discussion

In the previous chapter, we developed a simplified model that we extended in this chapter to incorporate the regulation of MMP activity by TIMPs, and therefore the regulation of chronic wounds. Our model consists of reaction-diffusion equations to describe the interaction of MMPs and TIMPs in both acute and chronic wounds. This extended framework gives rise to travelling wave solutions and is able to replicate key qualitative behaviours observed physiologically. One such property is the increased expression of MMP concentrations at the edge of a healing wound across all stages of acute wound recovery. Furthermore, biological studies suggest that one factor contributing to chronic wound formation is elevated ECM degradation due to oxidative stress. Therefore, we explore the impact of varying the degradation rate of the ECM across different levels of TIMP production to investigate the regulatory effects of TIMPs. Using bifurcation analysis, we observe a region of bistability which indicates that that low TIMP levels lead to chronic wounds that are progressively harder, and in the extreme case effectively impossible, to reverse, while increased TIMP production levels result in reversible chronic wounds, which are better regulated for larger TIMP

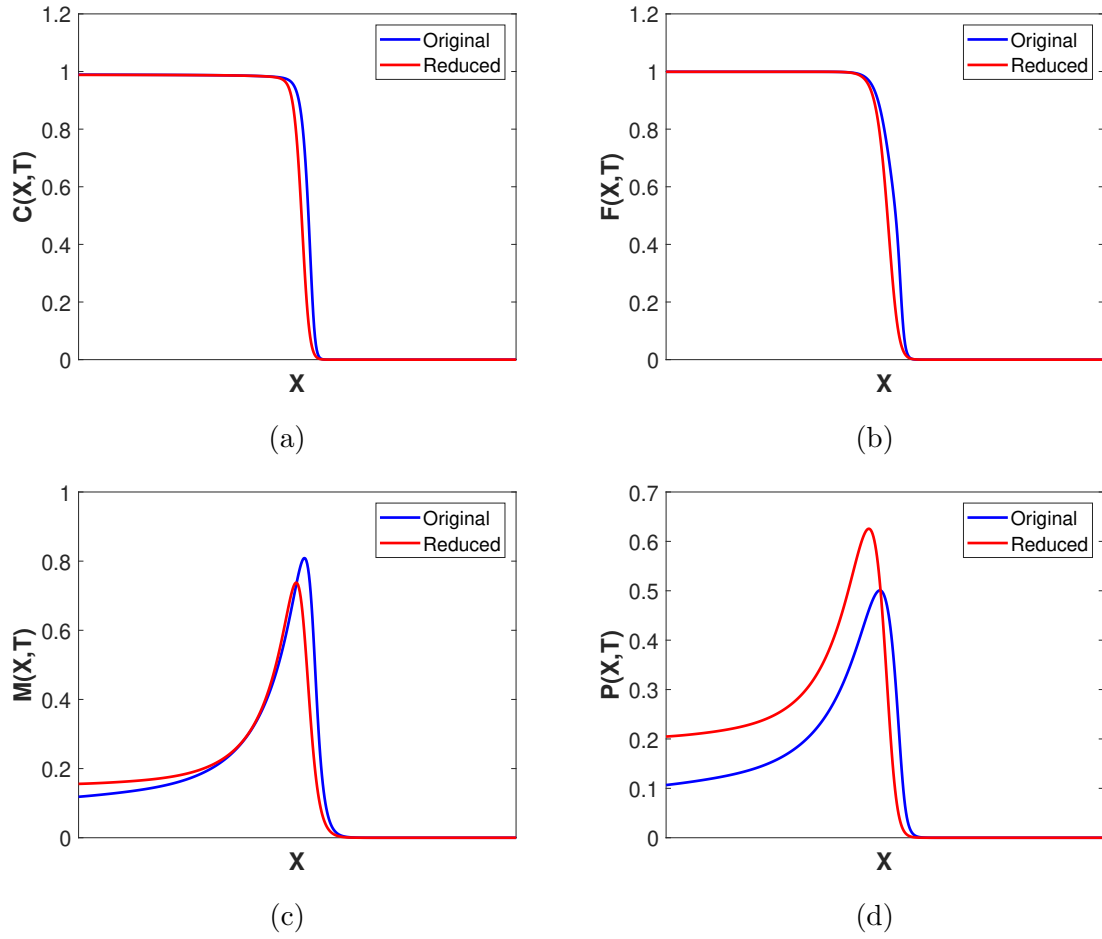


Figure 3.9: Travelling wave profiles representing an acute wound, obtained by numerically simulating the original RWHM (3.11)–(3.19) in blue, and the reduced RWHM2 in red each at a different time snapshot. The specific time points selected are not critical, as the wave shape remains constant once the traveling wave stabilizes. The comparison is presented for illustrative purposes.

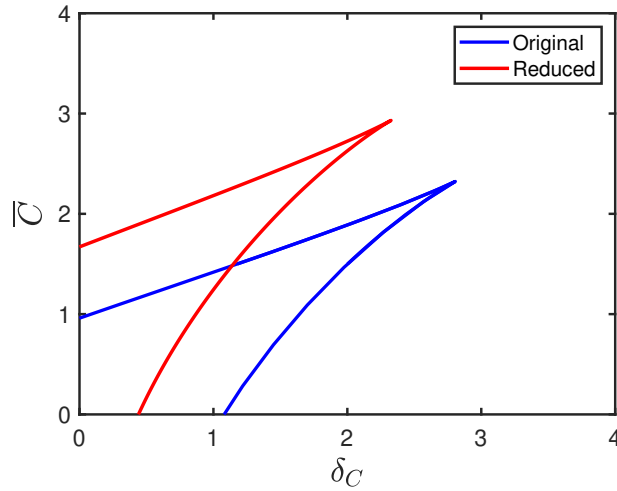


Figure 3.10: Two-parameter continuation showing the interaction of the degradation of ECM parameter, δ_C and the TIMP production parameter, k_P for the RWHM in blue, and the RWHM2 in red. All other parameters are in Table 2.

production values. These results indicate that high TIMP concentrations lead to more effectively regulated chronic wounds, consistent with existing biological research.

The bistability observed has important implications for treatment: once the system enters a chronic wound state within the bistable regime, reversing it may require a significant treatment to return to a healed state. In contrast, if TIMP levels are sufficiently high early on, healing may proceed without interruption. This suggests that early intervention to maintain TIMP levels above a critical threshold could be key to preventing wound chronicity and improving treatment outcomes. In particular, therapeutic strategies that involve the external administration of TIMPs may help to better regulate chronic wounds, especially when endogenous production is insufficient.

The biologically significant features of this model are retained in a simplified model obtained using an eFAST parameter sensitivity analysis, while reducing computational runtime. This simplified model will provide useful in the next chapter, where we extend the reduced model to two-dimensions to consider a more biologically realistic wound domain, before considering the treatment of such a wound with

a hydrogel.

Chapter 4

Two-dimensional model of wound healing

4.1 Introduction

In Chapter 3, we established that elevated TIMP production contributes to improved regulation of chronic wounds. However, directly manipulating intrinsic TIMP expression is not a practical therapeutic approach. A more feasible alternative may involve the external administration of TIMPs via a hydrogel-based delivery system which is an approach currently under investigation [39, 40]. Motivated by this, we extend the RWHM2 model presented in Section 3.6 to two spatial dimensions to capture a more biologically realistic wound domain and to explore the effects of externally supplied TIMPs, conceptually representing a hydrogel treatment containing TIMPs applied to a chronic wound.

In Section 4.2, we extend the RWHM2 model to two dimensions, using analogous initial and boundary conditions to those in Section 3.6 and demonstrate that the solutions remain consistent with those of the original one-dimensional model. Section 4.3 then introduces a Robin boundary condition to represent the application of an

external TIMP source at the wound surface, capturing a simplified version of hydrogel-based treatment. Our findings indicate that such a treatment can restore normal healing dynamics in chronic wounds. In Section 4.4, we simplify the two-dimensional model introduced in Section 4.3 by considering a slender wound domain and perform an asymptotic analysis of the system. This yields analytic expressions for the four key dependent variables and provides explicit spatial profiles of TIMP distribution, revealing the influence of key parameters on wound healing and offering insights into the influence of biological processes on delivery with potential implications for treatment design. Finally, in Section 4.5, we validate the asymptotic solution derived in Section 4.4 by comparing it with numerical simulations of the full two-dimensional RWHM2 model. We then conclude with a discussion of our findings in Section 4.6. The results presented in Sections 4.2 and 4.3 are reported in [162].

4.2 Extension of the RWHM2 to two-dimensions

The dimensionless RWHM2 posed on a two-dimensional spatial domain $(X, Y) \in [0, \bar{L}] \times [0, \bar{h}]$ is as follows:

$$\frac{\partial C}{\partial T} = \sigma_C F(1 - C)(1 + f_{\max} M \exp(1 - M)) - \bar{\eta} C M - \delta_C C, \quad (4.1)$$

$$\frac{\partial F}{\partial T} = D_F \nabla^2 F + \sigma_F F(1 - F) - \delta_F F, \quad (4.2)$$

$$\frac{\partial M}{\partial T} = D_M \nabla^2 M + k_M F(1 - C) - \bar{\mu} M P - \delta_M M, \quad (4.3)$$

$$\frac{\partial P}{\partial T} = D_P \nabla^2 P + k_P F M - \bar{\mu} M P - \delta_P P. \quad (4.4)$$

The domain represents a rectangular strip of tissue, where X denotes the wound width and Y corresponds to the tissue depth. The height h is taken to be small relative to the wound width, reflecting the shallow geometry characteristic of chronic wounds, such

as venous leg ulcers. Thus, in the simulations that follow, we consider a thin wound geometry consistent with this shallow-strip domain. This choice facilitates comparison with the one-dimensional model and enables analytical treatment through asymptotic methods (Section 4.4). We remark that while other two-dimensional geometries, such as circular or square wounds, could be used to explore curvature effects and multidirectional invasion, these are not pursued here as they fall beyond the scope of the present study.

The non-dimensionalisation is obtained using the same scalings in Section 3.3, with the y -coordinate scaled as $Y = \sqrt{\frac{\sigma_c}{D_f}} y$. We remark that we additionally include a diffusive term in the ECM equation (4.1) (with very small diffusivity $D_C = 10^{-2}$) for numerical convenience.

We adopt initial and boundary conditions analogous to those in Section 3.6 for the one-dimensional model—that is, a wound where a small concentration of MMPs is initially present at the wound edge, while TIMPs are absent throughout. Zero-flux boundary conditions are imposed:

$$C(X, Y, 0) = H(\lambda - X), \quad (4.5)$$

$$F(X, Y, 0) = H(\lambda - X), \quad (4.6)$$

$$M(X, Y, 0) = \bar{\kappa} \exp(-(X - \lambda)^2), \quad (4.7)$$

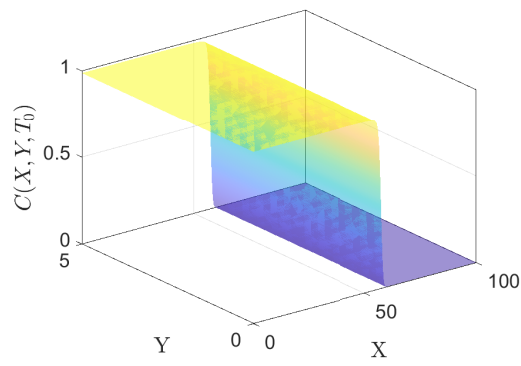
$$P(X, Y, 0) = 0, \quad (4.8)$$

and

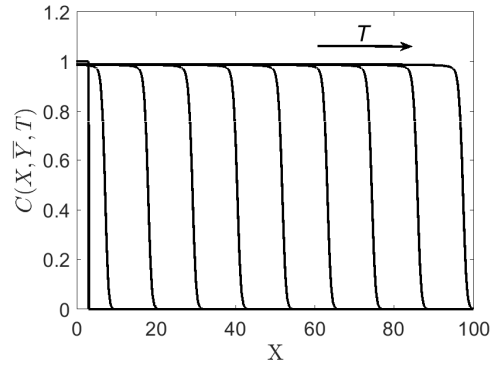
$$\frac{\partial F}{\partial X} = \frac{\partial M}{\partial X} = \frac{\partial P}{\partial X} = 0 \quad \text{for } Y \in [0, \bar{h}] \quad \text{at } X = 0, \bar{L}. \quad (4.9)$$

$$\frac{\partial F}{\partial Y} = \frac{\partial M}{\partial Y} = \frac{\partial P}{\partial Y} = 0 \quad \text{for } X \in [0, \bar{L}] \quad \text{at } Y = 0, \bar{h}. \quad (4.10)$$

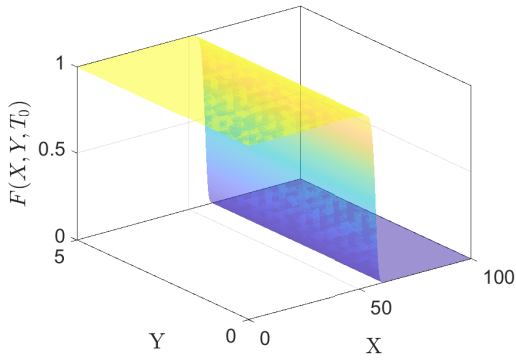
We present numerical simulations of this system and show that these results are consistent with the one-dimensional model. These simulations also provide validation that the additional diffusivity term does not significantly alter the model dynamics through wave speed comparisons later in this section. We solve the 2D system (4.1)–(4.4) with initial and boundary data (4.5)–(4.10) using MATLAB’s PDE toolbox and with parameter values given in Table 2 (See Section 3.3). As shown in Figure 4.1, the simulations yield travelling waves representing the complete healing of a wound, i.e. the invasion of a wound by a healed front of cells, with an increased expression of MMP and TIMP concentrations at the edge of the healing wound as in the 1D case. We note that the travelling waves exhibit no Y -dependence, as expected from the initial and boundary data (4.5)–(4.10). Since we only observe variations in the X -direction, and to confirm consistency with the original 1D model, we compute Y -averaged wave profiles from the 2D simulations, shown in Figure 4.1, generated using MATLAB’s `interpolatesolution` function. The resulting profiles closely match those from the 1D model and yield the same dimensionless wave speed of 5.6, accurate to one decimal place, and the transient wave profiles remain unchanged with peak MMP and TIMP values differing only by two decimal places. This agreement is also true across a physiologically relevant parameter range confirming both the validity of the 2D numerical solution and the negligible impact of the added diffusion term.



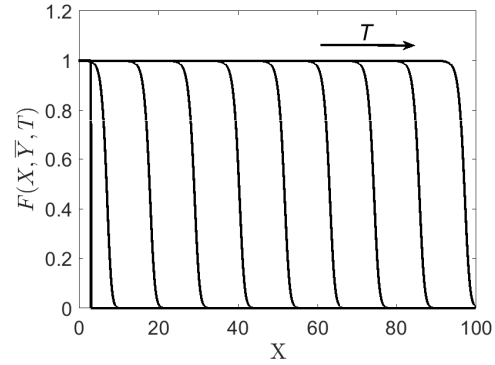
(a)



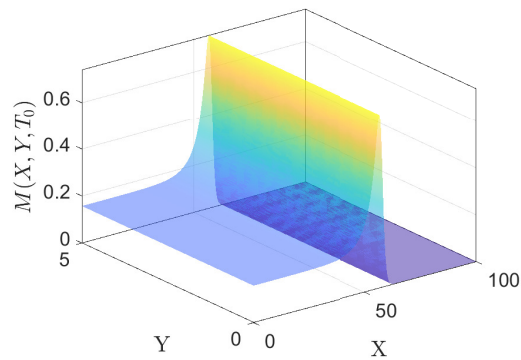
(b)



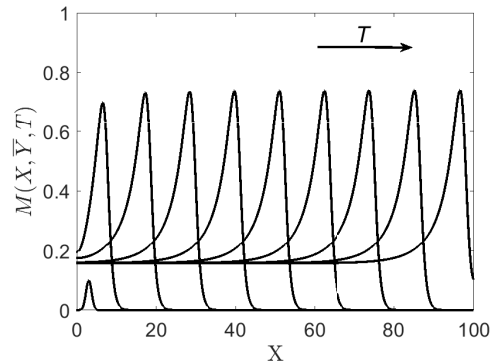
(c)



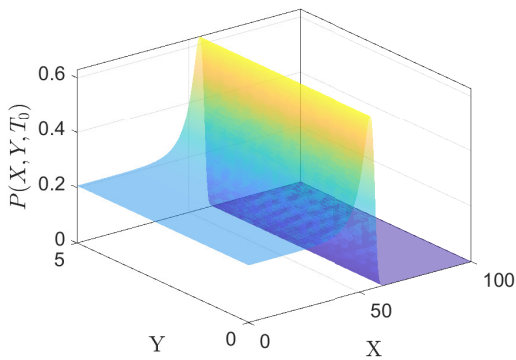
(d)



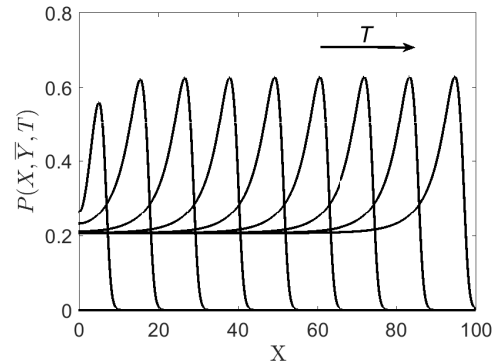
(e)



(f)



(g)



(h)

Figure 4.1: (a) Numerical simulations of the 2D RWHM2 with parameter values as in Table 2 at fixed time $T_0 = 10$ (b) Y -averaged travelling waves at time intervals $T = 2$ across the X domain with parameter values as in Table 2.

4.3 Treatment of a chronic wound with an external source of TIMPs

We now examine the impact of an elevated value of k_M , the MMP production parameter, on wound healing in our 2D model, in the presence and absence of treatment by an external source of TIMPs as guided by the biological literature discussed in Section 1.1.2. In the first case of no treatment, we adopt zero flux boundary conditions as in Section 3.4; treatment via an external source of TIMPs applied to the wound surface is modelled via a Robin boundary condition on the top edge of the 2D wound:

$$\frac{\partial C}{\partial X} = \frac{\partial F}{\partial X} = \frac{\partial M}{\partial X} = \frac{\partial P}{\partial X} = 0 \quad \text{for } Y \in [0, \bar{h}] \quad \text{at } X = 0, \bar{L}, \quad (4.11)$$

$$\frac{\partial C}{\partial Y} = \frac{\partial F}{\partial Y} = \frac{\partial M}{\partial Y} = \frac{\partial P}{\partial Y} = 0 \quad \text{for } X \in [0, \bar{L}] \quad \text{at } Y = 0, \quad (4.12)$$

$$\frac{\partial C}{\partial Y} = \frac{\partial F}{\partial Y} = \frac{\partial M}{\partial Y} = 0 \quad \text{for } X \in [0, \bar{L}] \quad \text{at } Y = \bar{h}, \quad (4.13)$$

$$\frac{\partial P}{\partial Y} = \begin{cases} 0, & \text{for } X \in [0, \bar{L}] \quad \text{at } Y = \bar{h} \quad (\text{no treatment}), \\ \frac{\bar{\rho}}{D_P}(P_{\text{hydrogel}} - P), & \text{for } X \in [0, \bar{L}] \quad \text{at } Y = \bar{h} \quad (\text{treatment}). \end{cases} \quad (4.14)$$

We now detail the procedure for deriving this non-dimensionalised Robin boundary condition. The dimensional Robin boundary condition takes the form:

$$D_p \frac{\partial p}{\partial y} = \rho(p_{\text{hydrogel}} - p), \quad (4.15)$$

where, p_{hydrogel} denotes the TIMP concentration in the hydrogel and ρ governs the rate of exchange between the hydrogel and the wound. A large value of ρ means that

TIMPs diffuse more readily from the hydrogel into the wound. The concentration gradient $(p_{\text{hydrogel}} - p)$ drives the flux across the boundary, where p represents the TIMP concentration in the wound. If $p < p_{\text{hydrogel}}$ TIMP flows into the wound, whereas if $p > p_{\text{hydrogel}}$, the model would suggest a reverse flux into the hydrogel. To ensure a sustained release of TIMPs into the tissue and prevent reverse diffusion, p_{hydrogel} is set to a sufficiently high value, effectively modelling the hydrogel as an idealised reservoir. Using the scalings $Y = \sqrt{\frac{\sigma_c}{D_f}} y$ and $P = \frac{p}{m_{\text{thresh}}}$, we obtain the non-dimensional Robin boundary condition as in (4.14) where $\bar{\rho} = \frac{\rho}{\sqrt{\sigma_c D_f}}$ and $P_{\text{hydrogel}} = \frac{p_{\text{hydrogel}}}{m_{\text{thresh}}}$ with $\rho = 1.894 \times 10^{-4}$ and $p_{\text{hydrogel}} = 3.6 \times 10^{-7}$, thereby obtaining $\bar{\rho} = 1$.

We consider a wound that is initially symmetric about the centreline ($X = \frac{\bar{L}}{2}$), resulting in the following initial conditions:

$$C(X, Y, 0) = \beta H(\lambda - X) + \beta H(X - (\bar{L} - \lambda)), \quad (4.16)$$

$$F(X, Y, 0) = H(\lambda - X) + H(X - (\bar{L} - \lambda)), \quad (4.17)$$

$$M(X, Y, 0) = \bar{\kappa} \exp(-(X - \lambda)^2) + \bar{\kappa} \exp(-(X - (\bar{L} - \lambda))^2), \quad (4.18)$$

$$P(X, Y, 0) = 0, \quad (4.19)$$

and we assess wound closure as an indicator of healing. We note that the chosen values of β and $\bar{\kappa}$ are different in the two cases that we will consider. In the case of no treatment, we use $\beta = 1$ and $\bar{\kappa} = 0.1$, for consistency with the results presented in Section 3.2 in the case of the 1D RWHM. This parameter choice leads to the emergence of a chronic wound, with travelling wave solutions characterised by a far-field ECM concentration of approximately 0.2 and an elevated peak MMP concentration around 8.5, representing dysregulated healing. To assess the effect of treatment via an external source of TIMPs (implemented using the Robin boundary condition (4.14)), we use these far-field values in our initial conditions with $\beta = 0.2$ and $\bar{\kappa} = 8.5$. This

ensures the development of travelling waves characteristic of the chronic wound and allows us to evaluate whether treatment leads to the healing of a chronic wound.

In order to obtain numerical simulations of the 2D RWHM2, we use MATLAB's PDE toolbox and consider a domain where $\bar{L} = 100$ and $\bar{h} = 3$. This aspect ratio allows the 2D numerical simulations to be well approximated by the 1D RWHM2 as expected, provided that analogous zero-flux boundary conditions (but not Robin boundary conditions) are applied. Firstly, we present numerical solutions of the 2D RWHM2 in the case of elevated MMP production levels and no treatment. Figure 4.2 illustrates the development of travelling waves representing chronic wounds that are independent of Y . In these simulations, the invading state for $C(X, Y, T)$ maintains an unhealed value, while the wound is characterised by elevated MMP concentrations. Eventually, $C(X, Y, T)$ settles at this unhealed value and $M(X, Y, T)$ settles at a large value which we characterise as an unhealthy, chronic wound state. We now aim to investigate the therapeutic potential of a hydrogel containing TIMPs administered to a chronic wound, characterised by a Robin boundary condition on the top edge of the 2D wound as given in (4.14). We present illustrative simulations of the 2D RWHM2 in Figure 4.3 to show the treatment of an existing chronic wound, modelled using initial conditions representing a chronic wound as well as elevated MMP production levels, with an external source of TIMPs. The travelling waves for $C(X, Y, T)$ settle at a healed state $\bar{C}$, as the additional TIMPs effectively regulate the elevated MMP concentrations, causing $M(X, Y, T)$ to settle at a reduced and healthy value $\bar{M}$. This regulation ultimately allows the wound to heal fully. We also observe that all variables, with the exception of $P(X, Y, T)$, exhibit minimal variation in the Y -direction; we display a comparison of these Y -averaged travelling waves for $C(X, Y, T)$ in Figure 4.4. We can easily see in this figure that elevated MMP production results in a chronic wound with the far-field state of C settling at a chronic value. Conversely, an external source of TIMPs results in the complete healing of such a wound, with the

far-field state of C settling at a healed value. These results align with the biological literature, indicating that TIMPs regulate elevated MMP concentrations and promote better healing of chronic wounds and therefore support the use of hydrogels containing TIMPs as an effective treatment for chronic wounds.

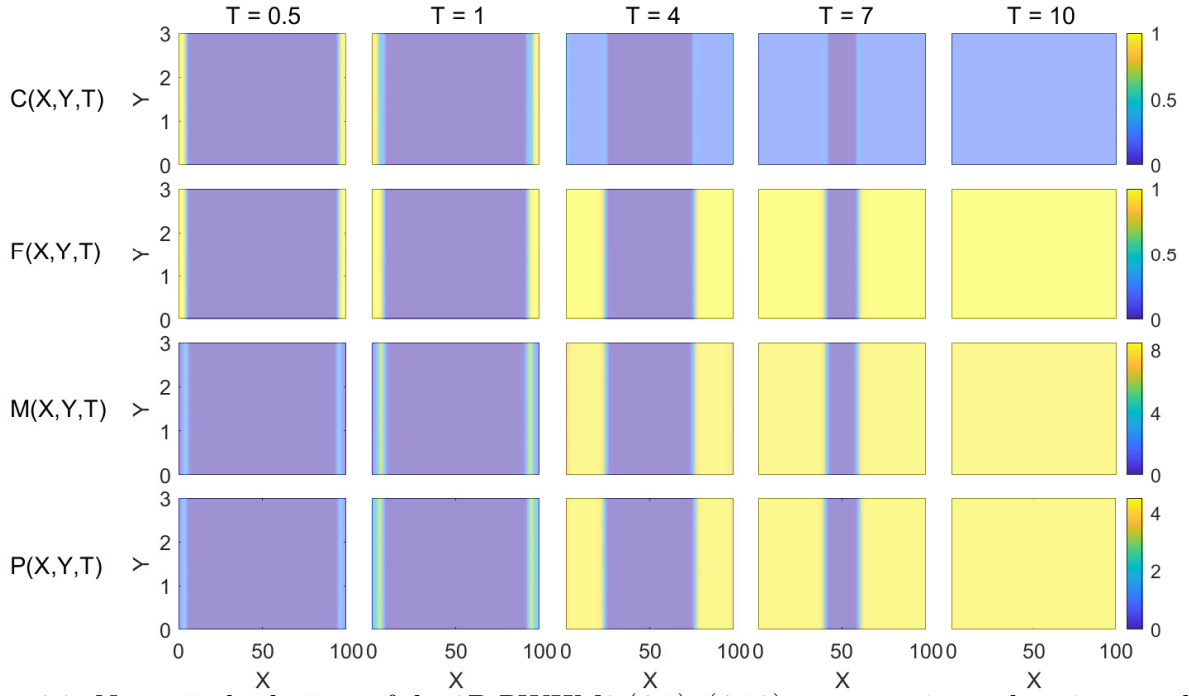


Figure 4.2: Numerical solutions of the 2D RWHM2 (4.1)–(4.19), representing a chronic wound, at the time points shown. $k_M = 90$, $\bar{X} = 100$, $\bar{Y} = 3$, $\beta = 1$ and $\bar{\kappa} = 0.1$. All other parameter values as are as in Table 2. Initial conditions as in (4.16)–(4.19) representing a symmetric synthetic wound and zero flux boundary conditions as in (4.11)–(4.14)

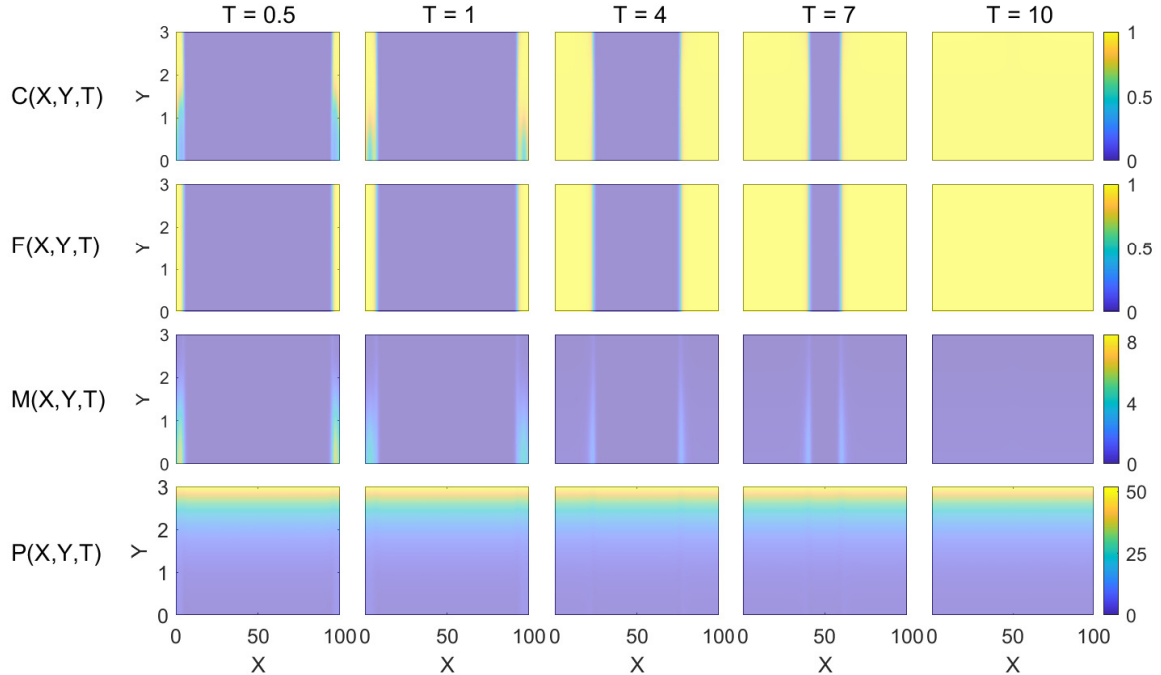


Figure 4.3: Numerical solutions of the 2D RWHM2 (4.1)–(4.19) representing the healing of a chronic wound, at at the time points shown. $k_M = 90$, $\bar{X} = 100$, $\bar{Y} = 3$, $\beta = 0.2$ and $\bar{\kappa} = 8.5$. All other parameter values as are as in Table 2. Initial conditions as in (4.16)–(4.19) representing a symmetric synthetic wound and zero flux boundary conditions as in (4.11)–(4.14), noting that we apply the Robin boundary condition as defined in (4.14), with $\bar{p} = 1$ and $P_{\text{hydrogel}} = 400$. We also note the colourbar limits change for $P(X, Y, T)$ compared with that in Figure 4.2.

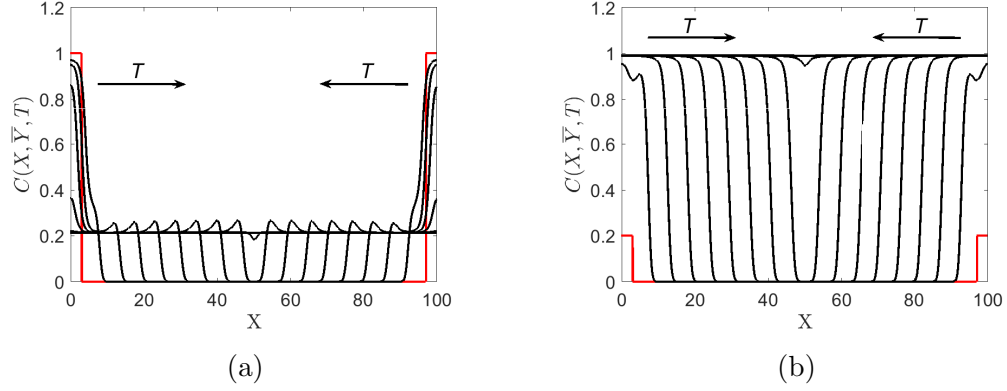


Figure 4.4: Y -averaged travelling waves across the X domain with $K_M = 90$, $\bar{X} = 100$, $\bar{Y} = 3$ and all other parameter values as in Table 2. (a) Initial conditions as in (4.16)–(4.19), with $\beta = 1$ and $\bar{\kappa} = 0.1$ and zero flux boundary conditions as in (4.11)–(4.14). (b) Initial conditions as in (4.16)–(4.19) with $\beta = 0.2$ and $\bar{\kappa} = 8.5$ and zero flux boundary conditions as in (4.11)–(4.14), except for $P(X, Y, T)$ on the top edge of the wound, noting that we apply the Robin boundary condition in (4.14), with $\bar{\rho} = 1$ and $P_{\text{hydrogel}} = 400$.

4.4 Slender Domain Approximation

In this section, we consider a slender domain approximation of the full PDE system of the 2D RWHM2 from Section 4.3, (4.1)–(4.4) and (4.11)–(4.19). The chronic wound is modelled with elevated levels of MMP production to simulate dysregulated healing, and the influx of TIMPs is modelled with a Robin boundary condition – a simplified representation of a hydrogel treatment. To reduce the complexity of the original two-dimensional system, we adopt a slender domain approximation, modelling the hydrogel as a long, thin structure. This geometric simplification is motivated by realistic wound morphologies, such as venous leg ulcers, which are often shallow and elongated in shape and enables an analytical examination of how the hydrogel facilitates wound healing. We focus on the long-term dynamics of the system, motivated by numerical simulations of the full model that suggest valuable insights into whether or not the wound heals, emerge after the initial transient phase has settled and the solution approaches its long-term state.

As in Section 4.2, we consider a two-dimensional spatial domain $(X, Y) \in [0, \bar{L}] \times [0, \bar{h}]$. We exploit the small aspect ratio $\epsilon = \frac{\bar{h}}{\bar{L}} \ll 1$ which represents the ratio between vertical and horizontal lengthscales. We introduce a rescaled variable $X = \frac{1}{\epsilon} \hat{X}$, following which, the hydrogel domain is mapped onto $(\hat{X}, Y) = [0, \bar{h}] \times [0, \bar{h}]$, where $\bar{h}$ is an $O(1)$ dimensionless constant. We note that this choice does not yield a unit square, as the variables X and Y were previously scaled using the approach in Chapter 3, rather than being non-dimensionalised with respect to the domain's length scales. Under this transformation, we obtain:

$$\frac{\partial^2}{\partial X^2} = \epsilon^2 \frac{\partial^2}{\partial \hat{X}^2}$$

Before presenting the governing equations in this domain, we consider the choice of scaling for the parameter values in the 2D RWHM2. We use the scalings outlined in Table 4.1 where each parameter is denoted as an $O(1)$ constant multiplied by a power of ϵ representing its order (e.g., $\delta_C = \hat{\delta}_C \epsilon$). Table 4.1 summarises the asymptotic order for each parameter and nearly all dimensionless values are consistent with those in Table 3.2, except that for $f_{\max}$ to enable asymptotic simplification and analytic tractability, while the qualitative features remain consistent. Also, the order of k_M , is consistent with Section 4.3 to model dysregulated healing in a chronic wound. We remark that the choice of parameter values in our model introduces some ambiguity in certain cases. Some parameters, may be interpreted in various ways depending on the chosen scaling approach. Consequently, there exists an overlap in the range where different asymptotic classifications may apply.

By applying the asymptotic scalings for the parameters, as detailed in Table 4.1, to the two-dimensional RWHM2 model, we obtain the following system of scaled equations. For simplicity and without loss of generality, we omit the hat notation for

Parameter, p	Order
σ_C	$O(1)$
$f_{\max}$	$O(\epsilon^{-1})$
$\bar{\eta}$	$O(1)$
δ_C	$O(\epsilon^2)$
σ_F	$O(\epsilon^{-1/2})$
δ_F	$O(\epsilon)$
D_M	$O(\epsilon^{-1/2})$
k_M	$O(\epsilon^{-1})$
$\bar{\mu}$	$O(1)$
δ_M	$O(1)$
D_P	$O(\epsilon^{-1/2})$
k_P	$O(\epsilon^{-1/2})$
δ_P	$O(\epsilon^{-1/2})$
P_{hydrogel}	$O(\epsilon^{-1})$
$\bar{\rho}$	$O(1)$

Table 4.1: Orders of the dimensionless parameter values appearing in the 2D RWHM2.

the $O(1)$ constants:

$$\epsilon \frac{\partial C}{\partial T} = \sigma_C F(1 - C)(\epsilon + f_{\max} M \exp(1 - M)) - \epsilon \bar{\eta} C M - \epsilon^3 \delta_C C, \quad (4.20)$$

$$\epsilon^{1/2} \frac{\partial F}{\partial T} = \epsilon^{5/2} \frac{\partial^2 F}{\partial X^2} + \epsilon^{1/2} \frac{\partial^2 F}{\partial Y^2} + \sigma_F F(1 - F) - \epsilon^{3/2} \delta_F F, \quad (4.21)$$

$$\epsilon \frac{\partial M}{\partial T} = \epsilon^{5/2} D_M \frac{\partial^2 M}{\partial X^2} + \epsilon^{1/2} D_M \frac{\partial^2 M}{\partial Y^2} + k_M F(1 - C) - \epsilon \bar{\mu} M P - \epsilon \delta_M M, \quad (4.22)$$

$$\epsilon^{1/2} \frac{\partial P}{\partial T} = \epsilon^2 D_P \frac{\partial^2 P}{\partial X^2} + D_P \frac{\partial^2 P}{\partial Y^2} + k_P F M - \epsilon^{1/2} \bar{\mu} M P - \delta_P P. \quad (4.23)$$

with boundary conditions:

$$\frac{\partial F}{\partial X} = \frac{\partial M}{\partial X} = \frac{\partial P}{\partial X} = 0 \quad \text{for } Y \in [0, \bar{h}] \quad \text{at } X = 0, \bar{h}, \quad (4.24)$$

$$\frac{\partial F}{\partial Y} = \frac{\partial M}{\partial Y} = \frac{\partial P}{\partial Y} = 0 \quad \text{for } X \in [0, \bar{h}] \quad \text{at } Y = 0, \quad (4.25)$$

$$\frac{\partial F}{\partial Y} = \frac{\partial M}{\partial Y} = 0 \quad \text{for } X \in [0, \bar{h}] \quad \text{at } Y = \bar{h}, \quad (4.26)$$

$$\epsilon^{1/2} D_P \frac{\partial P}{\partial Y} = \bar{\rho}(P_{\text{hydrogel}} - \epsilon P), \quad \text{for } X \in [0, \bar{h}] \quad \text{at } Y = \bar{h}. \quad (4.27)$$

We assume the following asymptotic expansions:

$$C \sim C_0 + \epsilon^{1/2} C_{1/2} + \epsilon C_1 + \epsilon^{3/2} C_{3/2} + O(\epsilon^2)$$

$$F \sim F_0 + \epsilon^{1/2} F_{1/2} + \epsilon F_1 + \epsilon^{3/2} F_{3/2} + O(\epsilon^2)$$

$$M \sim M_0 + \epsilon^{1/2} M_{1/2} + \epsilon M_1 + \epsilon^{3/2} M_{3/2} + O(\epsilon^2)$$

$$P \sim \frac{1}{\epsilon^{1/2}} P_{-1/2} + P_0 + \epsilon^{1/2} P_{1/2} + \epsilon P_1 + \epsilon^{3/2} P_{3/2} + O(\epsilon^2)$$

The inclusion of an $O(\epsilon^{-1/2})$ term in the expansion for P is motivated by numerical simulations presented in Section 4.3, which indicate that P attains a significantly larger value than the other variables, particularly as $\epsilon \rightarrow 0$. In the asymptotic analysis that follows, we substitute the expansions above into the governing equations (4.20)–

(4.27), equate coefficients of $O(\epsilon^k)$ and consider the limit $\epsilon \rightarrow 0$ to systematically derive the leading-order behaviour and higher-order corrections for C, F, M and P .

4.4.1 Leading Order Solutions

We begin by examining the expansion of the exponential term in equation (4.20):

$$\begin{aligned} \exp(1 - M) &\sim \exp + (1 - M_0)\exp(-(\epsilon^{1/2}M_{1/2} + \epsilon M_1 + \epsilon^{3/2}M_{3/2})) \\ &\sim \exp(1 - M_0) \left[1 - \epsilon^{1/2}M_{1/2} + \epsilon \left(\frac{M_{1/2}^2}{2} - M_1 \right) + \epsilon^{3/2} \left(M_{1/2}M_1 - M_{3/2} - \frac{M_{1/2}^3}{6} \right) \right. \\ &\quad \left. + \epsilon^2 \left(\frac{M_1^2}{2} + M_{1/2}M_{3/2} - M_2 - \frac{M_{1/2}^2M_1}{2} + \frac{M_{1/2}^4}{24} \right) \right]. \end{aligned}$$

At leading order, $O(1)$ for C, F , and M , and $O(\epsilon^{-1/2})$ for P , the asymptotic system (4.20)–(4.27) reduces to:

$$k_M F_0(1 - C_0) = 0, \quad (4.28)$$

$$\sigma_C f_{\max} F_0(1 - C_0) M_0 \exp(1 - M_0) = 0, \quad (4.29)$$

$$\sigma_F F_0(1 - F_0) = 0, \quad (4.30)$$

$$D_P \frac{\partial^2 P_{-1/2}}{\partial Y^2} = \delta_P P_{-1/2}, \quad (4.31)$$

with boundary conditions

$$\frac{\partial F_0}{\partial X} = \frac{\partial M_0}{\partial X} = \frac{\partial P_{-1/2}}{\partial X} = 0 \quad \text{for } Y \in [0, \bar{h}] \quad \text{at } X = 0, \bar{h}, \quad (4.32)$$

$$\frac{\partial F_0}{\partial Y} = \frac{\partial M_0}{\partial Y} = \frac{\partial P_{-1/2}}{\partial Y} = 0 \quad \text{for } X \in [0, \bar{h}] \quad \text{at } Y = 0, \quad (4.33)$$

$$\frac{\partial F_0}{\partial Y} = \frac{\partial M_0}{\partial Y} = 0 \quad \text{for } X \in [0, \bar{h}] \quad \text{at } Y = \bar{h}, \quad (4.34)$$

$$D_P \frac{\partial P_{-1/2}}{\partial Y} = \bar{\rho} P_{\text{hydrogel}} \quad \text{for } X \in [0, \bar{h}] \quad \text{at } Y = \bar{h}. \quad (4.35)$$

To avoid trivial solutions for F in (4.30) corresponding to a dysregulated state, we must have $F_0 = 1$. Then equation (4.28) implies that $C_0 = 1$, and (4.29) is identically satisfied. Finally, at leading order $O(\epsilon^{-1/2})$, equation (4.31) yields the general solution:

$$P_{-1/2} = A_{-1/2}(X, T) \sinh\left(\sqrt{\frac{\delta_P}{D_P}} Y\right) + B_{-1/2}(X, T) \cosh\left(\sqrt{\frac{\delta_P}{D_P}} Y\right). \quad (4.36)$$

By implementing the above boundary conditions for $P_{1/2}$, we arrive at the solution:

$$P_{-1/2} = \frac{\bar{\rho} P_{\text{hydrogel}}}{\sqrt{D_P \delta_P} \sinh\left(\sqrt{\frac{\delta_P}{D_P}} \bar{h}\right)} \cosh\left(\sqrt{\frac{\delta_P}{D_P}} Y\right). \quad (4.37)$$

Since (4.29) is automatically satisfied, we do not yet have a leading order solution for M and thus we proceed to the next order. Using the above results, at $O(\epsilon^{1/2})$ for C, F and M and $O(1)$ for P , the asymptotic system (4.20)–(4.27) reduces to:

$$D_M \frac{\partial^2 M_0}{\partial Y^2} = k_M C_{1/2} + \bar{\mu} M_0 P_{-1/2}, \quad (4.38)$$

$$\sigma_C f_{\max} \exp(1 - M_0) C_{1/2} M_0 = 0, \quad (4.39)$$

$$\sigma_F F_0 F_{1/2} = 0, \quad (4.40)$$

$$D_P \frac{\partial^2 P_0}{\partial Y^2} = \bar{\mu} M_0 P_{-1/2} + \delta_P P_0, \quad (4.41)$$

with boundary conditions:

$$\frac{\partial F_{1/2}}{\partial X} = \frac{\partial M_{1/2}}{\partial X} = \frac{\partial P_0}{\partial X} = 0 \quad \text{for } Y \in [0, \bar{h}] \quad \text{at } X = 0, \bar{h}, \quad (4.42)$$

$$\frac{\partial F_{1/2}}{\partial Y} = \frac{\partial M_{1/2}}{\partial Y} = \frac{\partial P_0}{\partial Y} = 0 \quad \text{for } X \in [0, \bar{h}] \quad \text{at } Y = 0, \quad (4.43)$$

$$\frac{\partial F_{1/2}}{\partial Y} = \frac{\partial M_{1/2}}{\partial Y} = 0 \quad \text{for } X \in [0, \bar{h}] \quad \text{at } Y = \bar{h}, \quad (4.44)$$

$$\frac{\partial P_0}{\partial Y} = -\bar{\rho}P_{-1/2} \quad \text{at } Y = \bar{h}. \quad (4.45)$$

Equation (4.39) reveals that either $C_{1/2} = 0$ or $M_0 = 0$. Implementing (4.38) then shows that $(C_{1/2}, M_0) = (0, 0)$ or $(M_0, 0)$. Using the latter and substituting the expression for $P_{-1/2}$ (4.37), (4.38) simplifies to:

$$D_M \frac{\partial^2 M_0}{\partial Y^2} = \bar{\mu} M_0 \frac{\bar{\rho} P_{\text{hydrogel}}}{\sqrt{D_P \delta_P} \sinh\left(\sqrt{\frac{\delta_P}{D_P}} \bar{h}\right)} \cosh\left(\sqrt{\frac{\delta_P}{D_P}} Y\right), \quad (4.46)$$

which is a modified Mathieu equation [191], which we solve to obtain the leading order solution for M , subject to the zero-flux boundary conditions for M_0 given in (4.32)–(4.34). Solutions to this class of equations are known as modified Mathieu functions, denoted Ce_n and Se_n which are real-valued for real arguments, with Ce_n and Se_n being even and odd functions, respectively. We rewrite (4.46) in the following form:

$$\frac{\partial^2 M_0}{\partial Y^2} = C_1 M_0 \cosh(C_2 Y), \quad (4.47)$$

where the constants are given by $C_1 = \frac{\bar{\mu} \bar{\rho} P_{\text{hydrogel}}}{D_M \sqrt{D_P \delta_P} \sinh\left(\sqrt{\frac{\delta_P}{D_P}} \bar{h}\right)}$ and $C_2 = \sqrt{\frac{\delta_P}{D_P}}$. The general solution to equation (4.46) is then:

$$M_0(Y) = D Ce_n\left(\frac{C_2 Y}{2}, \frac{2C_1}{C_2^2}, 0\right) + E Se_n\left(\frac{C_2 Y}{2}, \frac{2C_1}{C_2^2}, 0\right). \quad (4.48)$$

We impose the boundary conditions $M'_0(0) = M'(\bar{h}) = 0$ and use the fact that Ce_n and Se_n are even and odd functions respectively, implying that their derivatives are odd and even, respectively. Also noting that any odd function vanishes at zero, we conclude that the only consistent solution is $M_0 = 0$.

4.4.2 Two-term Solutions

We now proceed to derive the correction terms for each of the variables. Following the approach used in Section 4.4.1, and employing the result $M_0 = 0$, we solve equation (4.41) subject to the boundary conditions (4.43) and (4.45). This yields the $O(1)$ solution for P_0 :

$$P_0 = \frac{-\bar{\rho}^2 P_{\text{hydrogel}} \coth\left(\sqrt{\frac{\delta_P}{D_P}} \bar{h}\right)}{D_P \delta_P \sinh\left(\sqrt{\frac{\delta_P}{D_P}} \bar{h}\right)} \cosh\left(\sqrt{\frac{\delta_P}{D_P}} Y\right) \quad (4.49)$$

Using the result $F_0 = 1$, equation (4.40) implies that $F_{1/2} = 0$.

Since we have not yet obtained a non-zero correction for C , F or M , we proceed to the next order. At $O(\epsilon)$ and using the previously determined results, equation (4.20) is identically satisfied and therefore provides no new information. The equation for M (4.22) yields:

$$D_M \frac{\partial^2 M_{1/2}}{\partial Y^2} = k_M C_1 + \bar{\mu} M_{1/2} P_{-1/2}, \quad (4.50)$$

subject to zero flux boundary conditions. In order to solve the above, we require the solution for C_1 . Before addressing this, we consider equation (4.21) at $O(\epsilon)$ which gives:

$$\sigma_F F_0 F_1 = 0, \quad (4.51)$$

which again yields $F_1 = 0$ upon using $F_0 = 1$. To obtain the correction term for C , we examine (4.20) at the next order $O(\epsilon^{3/2})$ which gives:

$$M_{1/2}(\sigma_C f_{\max} e C_1 + \bar{\eta}) = 0. \quad (4.52)$$

This equation implies that either $M_{1/2} = 0$ or $C_1 = -\frac{\bar{\eta}}{\sigma_C f_{\max} e}$. Assuming $M_{1/2} = 0$, and proceeding analogously as previously, we would find that $C_1 = M_1 = 0$. However, numerical results indicate that M must be at least of $O(\epsilon)$, thereby ruling out this

trivial solution. As a result, we must have the correction term for C ,

$$C_1 = -\frac{\bar{\eta}}{\sigma_C f_{\max} e}. \quad (4.53)$$

We now turn attention back to $M_{1/2}$. Substituting the expression for C_1 into equation (4.50), we obtain the following inhomogeneous second-order equation for $M_{1/2}$:

$$D_M \frac{\partial^2 M_{1/2}}{\partial Y^2} - \bar{\mu} M_{1/2} \frac{\bar{\rho} P_{\text{hydrogel}}}{\sqrt{D_P \delta_P} \sinh\left(\sqrt{\frac{\delta_P}{D_P}} \bar{h}\right)} \cosh\left(\sqrt{\frac{\delta_P}{D_P}} Y\right) = -k_M \frac{\bar{\eta}}{\sigma_C f_{\max} e}. \quad (4.54)$$

which satisfies zero flux boundary conditions at $Y = 0$ and $\bar{h}$. An analytical solution to (4.54) is not available, so we employ MATLAB's BVP4c solver to compute numerical simulations for $M_{1/2}(Y)$ which we present in Section 4.5.

Since no correction term for F has yet been obtained, we now consider the equation (4.21) at $O(\epsilon^{3/2})$. Using the result $F_0 = 1$, we find:

$$F_{3/2} = -\frac{\delta_F}{\sigma_F}. \quad (4.55)$$

4.4.3 Summary and Discussion

Here, we present the asymptotic expansions for each of our variables C, F, M and P from above and discuss the insights that we obtain from each:

$$C \sim 1 - \epsilon \frac{\bar{\eta}}{\sigma_C f_{\max} e}, \quad (4.56)$$

$$M \sim \epsilon^{1/2} M_{1/2}, \quad (4.57)$$

$$F \sim 1 - \epsilon^{3/2} \frac{\delta_F}{\sigma_F}. \quad (4.58)$$

$$P \sim \frac{\bar{\rho} P_{\text{hydrogel}} \cosh\left(\sqrt{\frac{\delta_P}{D_P}} Y\right)}{\sqrt{D_P \delta_P} \sinh\left(\sqrt{\frac{\delta_P}{D_P}} \bar{h}\right)} \left[\frac{1}{\epsilon^{1/2}} - \frac{\bar{\rho} \coth\left(\sqrt{\frac{\delta_P}{D_P}} \bar{h}\right)}{\sqrt{D_P \delta_P}} \right] \quad (4.59)$$

where $M_{1/2}$ is the solution to the BVP (4.54) subject to zero flux boundary conditions at $Y = 0$ and $\bar{h}$. The expansion for C (4.56) indicates that, in the case of a chronic wound treated with an influx of TIMPs, the wound successfully heals and the ECM density C remains at its carrying capacity at leading order. The fact that C does not reach exactly unity is due to the continued, low-level degradation of ECM by MMPs, as reflected by the parameter $\bar{\eta}$ in the $O(\epsilon)$ correction term. This residual degradation results in a small but persistent loss of ECM. However, as discussed in Section 1.1.2, such controlled degradation is both natural and beneficial, facilitating necessary tissue remodelling during healing. Moreover, the asymptotic expression reveals that increased ECM production parameters, specifically, higher values of σ_C (the ECM production rate) and f_{max} (the healing contribution of MMPs) lead to values of C even closer to unity. This aligns with biological intuition: higher production enhances ECM restoration.

Despite the fact that we do not have an analytic form for the expansion for M , we may still deduce valuable qualitative insights from (4.54) which we discuss below. At leading order, the asymptotic expansion yields $M_0 = 0$, indicating that MMP activity is strongly suppressed in the presence of an externally delivered source of TIMPs, due to the inhibition action of TIMPs on MMPs. The next-order correction $M_{1/2}(Y)$, satisfies the inhomogeneous second-order ODE given in Equation (4.54). While this equation cannot be solved analytically, several key features of the MMP profile may be inferred. The constant term on the right-hand side of the equation acts as a uniform source that drives the production of $M_{1/2}(Y)$ across the domain. The magnitude of this term increases with both k_M (the rate of MMP production) and $\bar{\eta}$ (the rate of ECM degradation by MMPs), while decreases with σ_c

and $f_{\max}$. Consequently, larger values of k_M or $\bar{\eta}$ amplify MMP concentrations, while larger σ_c or $f_{\max}$ suppress them. This aligns with biological expectations: increased ECM degradation implies more wounded tissue and thus greater MMP recruitment, while enhanced ECM production reflects healing and leads to reduced MMP activity. The homogeneous component of the equation contains a spatially varying inhibitory term, proportional to $M_{1/2}(Y)$, the rate of MMP inhibition by TIMPs, $\bar{\mu}$ and the the leading-order TIMP profile, $P_{-1/2}(Y)$. This term represents the local inhibition of MMPs by TIMPs and further captures the regulatory role of TIMP delivery in controlling elevated MMP levels within chronic wounds. The presence of the hyperbolic cosine function in the TIMP profile indicates that TIMP concentration peaks at $Y = 1$, the site of hydrogel application, and decays toward the interior. Therefore, we expect the MMP profile will exhibit higher concentrations near $Y = 0$, where TIMP levels are lowest, and be lowest at $Y = 1$, where TIMPs are most concentrated.

The expansion for F (4.58) indicates that fibroblast density remains extremely close to its carrying capacity, with the correction term being small, of $O(\epsilon^{3/2})$, implying minimal deviation from carrying capacity. Biologically, this suggests that fibroblast production remains largely unaffected under the model assumptions. The correction term further reveals that fibroblast density decreases slightly as the apoptotic rate δ_F increases and increases toward carrying capacity as the mitotic rate σ_F increases—both consistent with biological expectations. Notably, this expansion highlights that fibroblast dynamics in the model are governed solely by parameters within the fibroblast equation itself. As such, disturbances or dysregulation in other components of the system do not directly influence fibroblast behaviour within our model.

From the expression (4.59), we observe that, up to and including $O(1)$, the TIMP concentration scales linearly with both P_{hydrogel} and $\bar{\rho}$. This is biologically intuitive as increasing the concentration of TIMP within the hydrogel and enhancing the rate

of TIMP delivery from the hydrogel to the wound will result in increased TIMP concentrations within the wound. Thus, if a chronic wound requires greater TIMP delivery, these parameters are readily tunable during hydrogel design. For instance, P_{hydrogel} can be adjusted directly by loading the hydrogel with more TIMPs, while $\bar{\rho}$ may be increased by, for example, designing hydrogels with higher porosity or more easily erodible structures.

The spatial profile of TIMPs is governed by the hyperbolic cosine term $\cosh\left(\sqrt{\frac{\delta_P}{D_P}}Y\right)$, which implies that TIMP concentration is maximal at $Y = 1$, the site of hydrogel application, and decays toward the wound bed, reaching its minimum at $Y = 0$, the region furthest from the hydrogel. The steepness of this decay is determined by the ratio $\sqrt{\frac{\delta_P}{D_P}}$: increasing D_P leads to a flatter concentration profile, corresponding to more sustained and spatially uniform delivery, while increasing δ_P sharpens the gradient, leading to more rapid decay of TIMP levels away from the hydrogel.

The denominator term $\sinh\left(\sqrt{\frac{\delta_P}{D_P}}\right)$ introduces an overall exponential suppression, which is stronger with increased decay of TIMPs, δ_P and is weaker with increased diffusion of TIMPs, D_P . Taken together with the term $\sqrt{D_P\delta_P}$, we see that increasing δ_P consistently reduces the overall TIMP concentration, as expected for enhanced degradation. In contrast, the dependency on D_P is more nuanced: increasing D_P initially increases P due to the reduction in the hyperbolic sine function, but this is opposed by the growing $\sqrt{\delta_P}$ term in the denominator. To deduce the behaviour of P with D_P , we define $\lambda = \sqrt{\frac{\delta_P}{D_P}}$ and consider $\lambda \ll 1$, i.e. $D_P \gg 1$. Using the Taylor expansions $\sinh(\lambda) \approx \lambda$ and $\coth(\lambda) \approx \frac{1}{\lambda} + \frac{\lambda}{3}$, we obtain the limiting form

$$P(Y) \sim \frac{\bar{\rho}P_{\text{hydrogel}}}{\delta_P} \left[\frac{1}{\epsilon^{1/2}} - \frac{\bar{\rho}}{\delta_P} \right] \quad (4.60)$$

Thus, TIMP concentration increases with D_P until the influence of $\sqrt{D_P}$ dominates at which point the solution plateaus and approaches the limiting value (4.60). This corresponds to a flattened profile, which may result in premature depletion of TIMPs

or a lack of spatial targeting. While both D_P and δ_P themselves are not directly tunable parameters, as they depend on the intrinsic properties of TIMPs in the tissue environment, understanding their influence is still valuable for predicting delivery dynamics and informing hydrogel design. Finally, the correction term involving the hyperbolic cotangent function is always positive and acts to slightly reduce the total magnitude of P .

The asymptotic analysis provides clear mechanistic insight into the healing process under TIMP treatment and summary is as follows. It shows that ECM density is restored close to its carrying capacity, with only a small deficit caused by natural low-level degradation. Fibroblasts remain essentially at carrying capacity, their behaviour determined only by their intrinsic birth and death rates. MMPs are strongly suppressed by TIMPs, vanishing at leading order and persisting only as a small correction that is largest deeper in the wound, where TIMP concentrations are lowest. TIMPs themselves attain their highest levels at the hydrogel–tissue interface and decay with depth, with the shape of the profile governed by diffusion and degradation rates. The analysis highlights that hydrogel loading and release rate linearly increase TIMP levels, while diffusion and degradation tune how far TIMPs penetrate into the wound.

4.5 Comparison of asymptotic and full solutions

In this section, we evaluate the accuracy of the asymptotic solutions derived in Section 4.4 by comparing them against numerical solutions of the 2D RWHM2 model. First, we compare the numerical solution of $P(X, Y, T)$ with the asymptotic solution (4.59), which includes both the leading-order term of $O(\epsilon^{-1/2})$ and the $O(1)$ correction term. We choose representative values for the parameter values in the asymptotic system (4.20)–(4.27), consistent with those in Table 2.12. These representative parameter

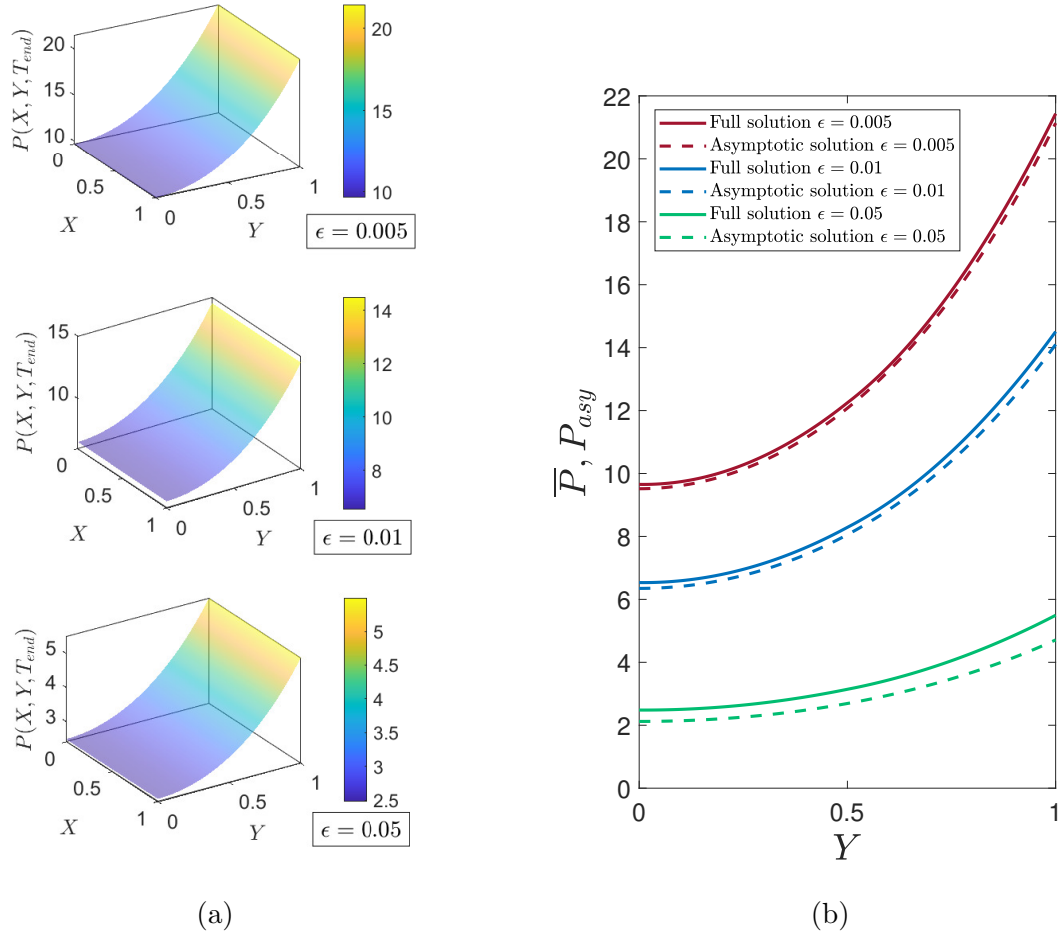


Figure 4.5: (a) Numerical simulation of the variable P from the 2D RWHM2, (4.1)–(4.4) and (4.11)–(4.19), with parameter values as in Table 4.2 at the final simulation time $T_{\text{end}} = 20$ for $\epsilon = 0.005, 0.01, 0.05$. (b) Comparison between the X -averaged numerical solutions for P at $T = T_{\text{end}}$, denoted by $\bar{P}(Y, T_{\text{end}})$ (solid lines), and the corresponding asymptotic approximations $P_{\text{asy}}(Y)$ (dashed lines), as given in equation (4.59) for $\epsilon = 0.005, 0.01, 0.05$.

values are given in Table 4.2. Figure 4.5(a) shows the profile of $P(X, Y, T_{\text{end}})$ for different values of ϵ , representing a snapshot at the final time point $T = 20$, since we are interested in the long-term dynamics of the system. We observe that while the overall shape of the profile remains consistent across different values of ϵ , the minimum and maximum concentrations vary. This variation reflects how changes in ϵ influence the underlying biological processes, as expected. To facilitate a meaningful comparison between the numerical and asymptotic solutions at T_{end} , we consider

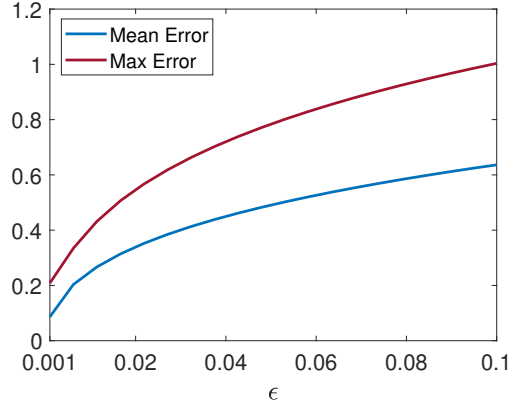


Figure 4.6: Evolution of the mean absolute error (blue) and the maximum absolute error (red) for P as defined in (4.61), with varying the asymptotic parameter ϵ .

the X -averaged solution denoted by $\bar{P}(Y, T_{\text{end}})$. This is appropriate because the asymptotic solution depends only on Y and the numerical simulations confirm that, after initial transients, P is approximately constant in X . Figure 4.5(b) presents a comparison between the X -averaged numerical solution $\bar{P}(Y, T_{\text{end}})$ and the asymptotic solution $P_{\text{asy}}(Y)$ for different values of ϵ . Upon inspection of the figure, it appears that the asymptotic solutions for P provide a good approximation to the numerical solutions and the offset due to the $O(\epsilon^{1/2})$ error reduces for lower ϵ . We quantify the accuracy of the asymptotic approximation by defining the absolute error at each Y node as

$$\text{Absolute Error}(Y) = |\bar{P}(Y, T_{\text{end}}) - P_{\text{asy}}(Y)|, \quad (4.61)$$

and examine how the mean and maximum absolute error vary with ϵ in Figure 4.6. As expected, the error decreases with smaller ϵ , and the trend is consistent with an $O(\epsilon^{1/2})$ scaling. This confirms the validity of the asymptotic approximation for P , which closely captures the qualitative behaviour of the full solution, including its functional form and trends. Moreover, the asymptotic solution provides analytical insight into the system by revealing parameter dependencies that are otherwise difficult to extract numerically, as discussed in Section 4.4.3.

Parameter, p	value
σ_C	0.93
$f_{\max}$	0.4
$\bar{\eta}$	0.45
δ_C	5.46
σ_F	0.82
δ_F	0.76
D_M	0.46
k_M	0.9
$\bar{\mu}$	1.77
δ_M	0.77
D_P	0.46
k_P	1.28
δ_P	0.95
P_{hydrogel}	1
$\bar{\rho}$	1
$\bar{h}$	1

Table 4.2: Representative values for the dimensionless $O(1)$ parameters appearing in the asymptotic system (4.20)–(4.27).

For completeness, we now consider the asymptotic solutions for C , M , and F alongside the corresponding full numerical solutions to assess the accuracy and consistency of the approximations. Based on the expansions (4.56)–(4.58), we expect the errors to scale as $O(\epsilon^{3/2})$, $O(\epsilon)$, and $O(\epsilon^2)$ for C , M , and F , respectively. As before, we compare the X -averaged numerical solutions of the full system at $T = T_{\text{end}}$ with the corresponding asymptotic expansions. The asymptotic forms of C and F , denoted C_{asy} and F_{asy} , are spatially constant, while M varies with Y , and is therefore written as $M_{\text{asy}}(Y)$. As previously discussed, there is no closed-form expression for the $O(\epsilon^{1/2})$ correction term $M_{1/2}$, which satisfies the boundary value problem (4.54) with zero-flux boundary conditions. To construct the full asymptotic approximation for M , we solve this BVP numerically using MATLAB’s `bvp4c` solver. Figure 4.6 shows the comparison between the full and asymptotic solutions for C_{asy} and $M_{\text{asy}}(Y)$, confirming the expected accuracy and validating the asymptotic forms. We omit the plot for F_{asy} , as the difference from the full solution is so small that it cannot be meaningfully visualised without substantial magnification. However, we have verified that it remains consistent with the predicted $O(\epsilon^2)$ behaviour. These results validate the asymptotic expansions and demonstrate that their accuracy improves as $\epsilon \rightarrow 0$.

While the model captures the essential balance between ECM, fibroblasts, MMPs, and TIMPs, we remark the following limitations. Some biological processes are neglected. The wound geometry is fixed and does not evolve to capture contraction and as discussed in Chapter 1.2 and immune cell dynamics are not explicitly represented. The hydrogel is treated as an idealised reservoir with constant release, rather than a material that may deplete, swell, or erode. Finally, the model ignores tissue mechanics and anisotropy. These assumptions make the mathematics tractable and allow asymptotic simplification, but they necessarily reduce biological realism.

A further limitation of the asymptotic reduction is that some parameters present in the full PDE model do not appear at leading order, or even in the first corrections,

of the asymptotic solutions. Most notably, the MMP degradation rate δ_M and the ECM degradation rate δ_C drop out entirely from the expansions at the orders we derived. Biologically, this reflects the fact that in the treated regime MMPs are suppressed to such an extent that their natural decay is irrelevant, while the ECM recovers to carrying capacity so rapidly that its explicit decay rate due to oxidative stress become negligible. Similarly, fibroblast proliferation and apoptosis rates do not appear at leading order, since fibroblasts remain pinned near carrying capacity; they enter only in a small higher-order correction. ECM degradation by MMPs also contributes only as a small deficit at order.

By contrast, TIMP-related parameters the hydrogel concentration and release rate, the diffusion and decay rates of TIMP, and the inhibition rate of MMP by TIMP dominate the asymptotic solutions. These parameters set the shape and amplitude of the TIMP profile and therefore govern the suppression of MMPs and recovery of ECM. The asymptotics therefore highlight a mechanistic simplification: in the TIMP-treated regime, wound healing dynamics are controlled primarily by delivery and transport of TIMP, while the intrinsic kinetics of fibroblasts, MMPs, and ECM degradation play only a secondary role and may be neglected to leading order.

Finally, The asymptotic analysis assumes that TIMP delivery is sufficiently strong that TIMP concentrations in the wound scale as $O(\epsilon^{-1/2})$. In practice, this means that the hydrogel must release TIMPs at a rate high enough to suppress MMPs at leading order. If TIMP delivery is too weak, the balance breaks down: MMPs remain elevated, ECM is degraded, and the wound remains chronic. If TIMP delivery is very high, the present model predicts complete suppression of MMPs and full healing, although in reality additional effects may then need to be considered, such as MMP–TIMP binding saturation or depletion of the hydrogel reservoir. The results of the asymptotic analysis are therefore valid in an intermediate regime where TIMP levels are sufficient to regulate MMPs but not so large that additional biology

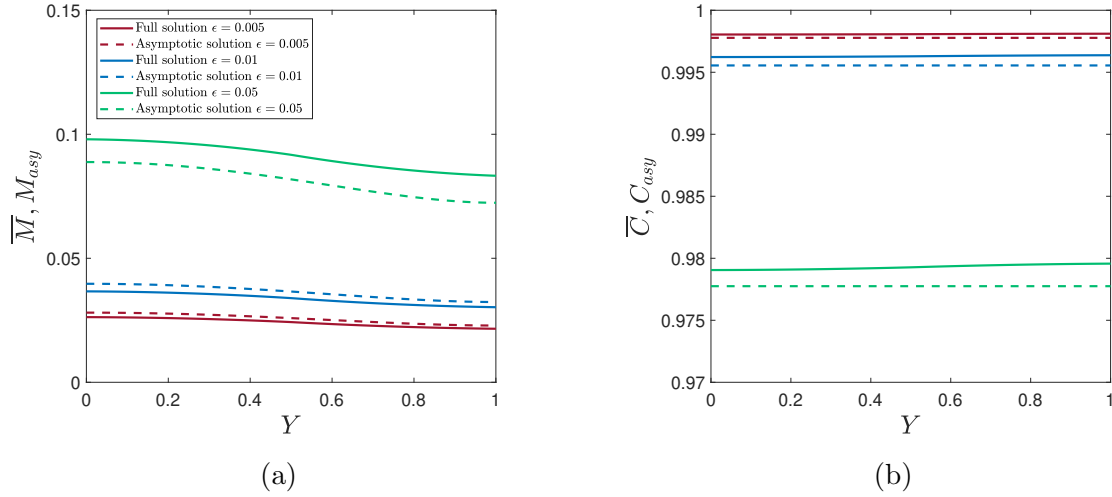


Figure 4.7: Comparison between the X -averaged numerical solutions at $T = T_{\text{end}}$ and the corresponding asymptotic approximations for $\epsilon = 0.05, 0.01$, and 0.005 : (a) $\bar{M}(Y, T_{\text{end}})$ (solid lines) compared with $M_{\text{asy}}(Y)$ (dashed lines), as given in equation (4.57); and (b) $\bar{C}(Y, T_{\text{end}})$ (solid lines) compared with $C_{\text{asy}}(Y)$ (dashed lines), as given in equation (4.56).

must be accounted for.

4.6 Conclusions and Discussion

In this chapter, we extended the RRWHM2 to a two-dimensional spatial domain in order to model a more realistic wound geometry and to explore the efficacy of a hydrogel treatment. Simulations of the 2D model yield the same wave speed as that of the 1D model, thereby affirming the validity of our 2D numerical solution. We also find that elevated MMP production levels lead to the emergence of chronic wounds, consistent with biological studies. We consider such chronic wounds and employ a Robin boundary condition to model an external source of TIMPs such as supplied by the application of a hydrogel on the wound surface. Simulation results demonstrate the reversal of the chronic wound, thereby reaffirming the regulatory properties of TIMPs and supporting the use of TIMP-containing hydrogels as a viable treatment option for chronic wounds.

We then sought analytical insight into the behaviour of TIMP delivery within our model. To achieve this, we introduce a slender domain approximation to simplify the system, which enables an asymptotic analysis aimed at providing deeper insights into the model. By utilising a small parameter that represents the aspect ratio of the domain, we derive an approximate solution that captures the leading-order behaviour of TIMP concentration within the wound. Through this process, we gain valuable understanding of how key parameters influence TIMP delivery dynamics, their regulatory impact, and the healing progression of the wound.

We observe that introducing an influx of TIMPs into the wound leads to complete healing of the ECM, with a small correction term related to the low-level degradation of ECM by MMPs which is consistent with a healthy wound environment, as discussed in Section 1.1.2. Similarly, fibroblasts exhibit healthy behaviour, remaining at carrying capacity at leading order, with a small correction term dependent on the ratio of fibroblast production and apoptotic rates. It is noteworthy that fibroblast dynamics are influenced solely by processes within their own equation. We also find that MMP concentration is small at leading order, indicating significant suppression due to the TIMP dynamics. Furthermore, we find that TIMP concentration increases linearly with both hydrogel loading and delivery rate, highlighting these as viable biologically tunable parameters for enhancing treatment. The spatial profile of TIMP concentration is shaped by diffusion and degradation: higher diffusivity flattens the profile and increases TIMP delivery up to a saturation point, while greater degradation consistently suppresses the overall concentration. These insights into the delivery dynamics have important implications for treatment design and underscore the influence of biological processes on TIMP delivery.

We then validate our solutions by comparing them with numerical simulations of the full 2D RWHM2 model. It is important to note that modelling the delivery of a source of TIMPs as a Robin boundary condition serves as a proof of concept, offering a

simplified representation of a hydrogel containing TIMPs. In the subsequent chapter, we propose a more detailed and realistic model to better capture the behaviour of such a hydrogel.

Chapter 5

Hydrogel Model

5.1 Introduction

In Chapter 4, we demonstrated that TIMPs play a crucial role in regulating the elevated MMP concentrations characteristic of chronic wounds. We further showed that applying an external source of TIMPs, modelled using a Robin boundary condition, can promote wound healing, thereby offering a potential therapeutic strategy. In this chapter, we advance this approach by developing a more biologically realistic model of a TIMP-containing hydrogel treatment, in a two-dimensional spatial domain, allowing for a more detailed exploration of the hydrogel’s physical and chemical properties. This treatment strategy is currently under experimental investigation and holds promise for improving outcomes in patients with chronic wounds. The following analysis is informed by experimental data provided by our collaborators in the biological sciences, as detailed in [40].

We begin in Section 5.2.1 by outlining the key physiological properties of the hydrogel, which underpin the development of a two-dimensional mathematical model in Section 5.2.2 to capture the essential system dynamics. Section 5.2.3 presents a non-dimensionalisation of the model to simplify analysis and reduce parameter complexity.

Two key parameters remain unknown: the rate at which the hydrogel is degraded by MMPs, and a minimum diffusivity parameter that captures the porosity-dependent spatial variation in the diffusion of MMPs and TIMPs. Through parameter estimation using experimental data, we observe that multiple combinations of these parameters yield similarly low error values, indicating a case of parameter non-identifiability. We attribute this to the absence of spatially resolved measurements. To address this, Section 5.3.2 introduces a synthetic dataset to investigate how different spatial data resolutions affect identifiability. This analysis provides guidance for future experimental design aimed at accurately characterising the delivery dynamics of the hydrogel and its potential therapeutic efficacy. Our findings are summarised and discussed in Section 5.4.

5.2 Model Development

5.2.1 Hydrogel Physiology and Experimental Details

In Section 1.1.5, we discussed the various mechanisms through which hydrogels can deliver therapeutic agents. In the current study, we focus on the hydrogels presented in [40] which contain cross-linked bonds that are specifically sensitive to the presence of MMPs. These cross-links are cleaved when they interact with MMPs, and upon cleavage, TIMPs are released from the hydrogel matrix. This release occurs via diffusion, driven by concentration gradients, enabling TIMPs to inhibit MMP activity and to regulate elevated MMP concentrations within the wound environment. Consequently, the hydrogels in this study demonstrate both chemically controlled and diffusion-controlled delivery mechanisms.

The experiments we consider [40] involve a hydrogel without the presence of a wound. In this setup, the hydrogel is placed in a solution containing a reservoir of fixed concentration of MMPs, which is continuously replenished to maintain a constant

reservoir. The cumulative release of 20 kDa fluorescent dextran (FD20) is measured at 24-hour, 48-hour, and 72-hour time points. FD20 serves as a proxy for the degradation kinetics of the hydrogel’s cross-linkers, as it allows us to track the breakdown of the material over time. Therefore, the amount of FD20 released is used to quantify the proportion of cross-linking bonds that have been cleaved by the MMPs. The hydrogels do not contain TIMPs in the experiment that we consider, allowing the interaction dynamics between MMPs and the hydrogel to be studied in isolation. Results are presented for three distinct cases, each with a different concentration of MMPs in the surrounding reservoir. Although the authors in [40] present experiments that include TIMPs, the available experimental details are insufficient to support reliable quantitative analysis. As such, we do not use these data for parameter estimation but retain TIMPs in the model formulation for completeness. Further discussion is provided in the future work section in Chapter 6.

5.2.2 Mathematical Model

We develop a mathematical framework to describe the behaviour of the hydrogel outlined in Section 5.2.1. The model captures the dynamics of TIMPs embedded within the hydrogel, the degradation of the hydrogel’s cleavable linkers by MMPs, and the subsequent inhibition of MMPs by released TIMPs.

The hydrogel is disc-shaped, with a diameter of 5mm and a thickness of 1.5mm. To reduce computational complexity while retaining the essential transport and degradation dynamics, we approximate the three-dimensional geometry by a two-dimensional Cartesian slice through the disc’s centre. This vertical cross-section spans the hydrogel’s full thickness along the y -axis and the disc’s radius along the x -axis. This two-dimensional simplification is justified by the radial symmetry of the disc and the uniform exposure to the surrounding medium, ensuring that the dynamics in the cross-section are representative of the whole. We therefore formulate a reaction-

diffusion model on the domain $(x, y) \in [0, L] \times [0, h]$, where x denotes the radial coordinate (with $L = 2.5\text{mm}$) and y the vertical coordinate (with $h = 1.5\text{mm}$), as illustrated in Figure 5.1(b). While a cylindrical coordinate system would be a natural choice given the disc geometry, we adopt a two-dimensional Cartesian slice for numerical stability. In practice, simulations using a cylindrical domain in MATLAB's PDE toolbox introduced significant numerical artefacts, particularly near the axis. The Cartesian approximation, justified by radial symmetry and uniform boundary exposure, provided stable and representative results.

We examine the system's temporal evolution over time t . The model comprises three key variables: the density of cleavable linkers $l(x, y, t)$, MMP concentration $m(x, y, t)$, and TIMP concentration $p(x, y, t)$, and is summarised schematically in Figure 5.1(a). We begin by considering the dynamics of the cleavable linkers that form the hydrogel scaffold. These linkers are degraded by MMPs at a rate k_{ml} , but MMPs are not consumed in this process and remain active to continue hydrogel degradation. As in the model introduced in Section 3, MMPs are inhibited upon binding to TIMPs, effectively removing them from the system at a rate μ . MMPs also undergo natural decay at rate δ_m . Similarly, TIMPs are consumed through MMP inhibition at rate μ , and degrade naturally at rate δ_p .

Both MMPs and TIMPs are allowed to diffuse through the hydrogel, but their mobility is governed by the local porosity of the hydrogel structure. Specifically, their diffusion is hindered when the cleavable linker density l is high (i.e., low porosity), and enhanced as the hydrogel degrades and porosity increases. To capture this behaviour, we introduce effective diffusion coefficients for MMPs and TIMPs, denoted D_m^{eff} and D_p^{eff} , respectively, where:

$$D_m^{\text{eff}} = D_m \epsilon(l) \quad D_p^{\text{eff}} = D_p \epsilon(l). \quad (5.1)$$

Here, $\epsilon(l)$ is a function of the linker density representing the porosity of the hydrogel,

following the framework from [155], in which porosity evolves spatially and temporally as the polymer degrades. This approach introduces two unknown parameters: the critical linker density at which significant molecular diffusion becomes feasible, and the variance which is influenced by the polymer's crystallinity (see Section 1.2.5 for more details). To capture the essential dynamics while maintaining analytical tractability, we adopt a simplified linear model for $\epsilon(l)$:

$$\epsilon(l) = \left(\frac{D_{\min} - 1}{l_0} \right) l + 1. \quad (5.2)$$

This model assumes that diffusivity is maximal, i.e. equal to D_m or D_p when the linker density is zero and decreases linearly to a minimum at the initial linker density l_0 . We represent the minimum diffusivities for MMPs and TIMPs as $D_m \times D_{\min}$ and $D_p \times D_{\min}$ respectively, where $D_{\min}$ is a dimensionless constant representing the ratio of minimum to maximum diffusivity. We assume that $D_{\min}$ is the same for both species, given their similar molecular sizes and diffusion characteristics, reducing the number of unknown parameters in the model. Combining the above modelling elements yields the following set of PDEs describing the spatiotemporal evolution of $l(x, y, t)$, $m(x, y, t)$ and $p(x, y, t)$:

$$\frac{\partial l}{\partial t} = -k_{ml}lm, \quad (5.3)$$

$$\frac{\partial m}{\partial t} = \nabla \cdot (D_m^{\text{eff}}(l)\nabla m) - \mu mp - \delta_m m, \quad (5.4)$$

$$\frac{\partial p}{\partial t} = \nabla \cdot (D_p^{\text{eff}}(l)\nabla p) - \mu mp - \delta_p p. \quad (5.5)$$

The initial conditions are selected to represent a hydrogel where the cleavable linkers are uniformly distributed with density l_0 . The hydrogel is assumed to be fully saturated with TIMPs at a uniform concentration p_0 , while no MMPs are present. These assumptions result in the following initial conditions for the model:

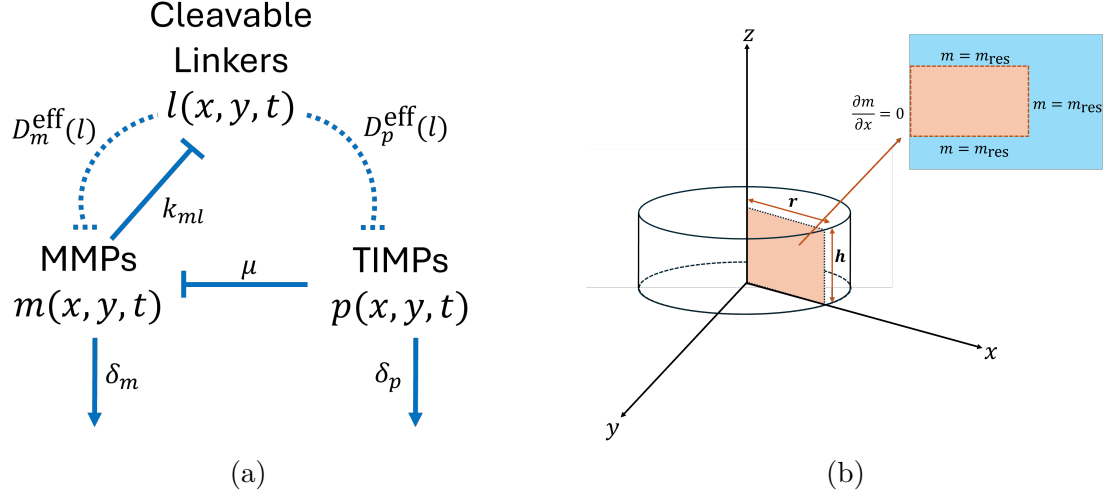


Figure 5.1: (a) Schematic of the interactions of cleavable linkers, MMPs and TIMPs in a hydrogel. Labelled rates are defined as in the system (5.3)–(5.5), (b) diagram of the domain $(x, y) \in [0, L] \times [0, h]$, where x denotes the radial coordinate (with $L = 2.5\text{mm}$) and y the vertical coordinate (with $h = 1.5\text{mm}$).

$$l(x, y, 0) = l_0, \quad (5.6)$$

$$m(x, y, 0) = 0, \quad (5.7)$$

$$p(x, y, 0) = p_0. \quad (5.8)$$

Since the hydrogel is submerged in a reservoir where the MMP concentration is continuously replenished, we impose Dirichlet boundary conditions for m on the outer three edges of the domain at a fixed concentration m_{res} representing the MMP level in the reservoir. The hydrogel is modelled as a central cross-sectional slice of a cylinder; hence, by symmetry, we apply a zero-flux boundary condition for m at $x = 0$, the central axis of the cylinder. For TIMPs, we impose zero-flux boundary conditions. While TIMPs may diffuse out in biologically permissive environments (such as a wound bed), we simplify the model by assuming that TIMPs remain confined within the hydrogel. This assumption allows us to focus on the internal hydrogel dynamics without the added complexity of external interactions.

$$m(x, y, t) = m_{\text{res}} \quad \text{at} \quad x = L, y = 0, h, \quad (5.9)$$

$$\frac{\partial}{\partial x} m(x, y, t) = 0 \quad \text{at} \quad x = 0, \quad (5.10)$$

$$\frac{\partial}{\partial x} p(x, y, t) = 0 \quad \text{at} \quad x = 0, L, \quad (5.11)$$

$$\frac{\partial}{\partial y} p(x, y, t) = 0 \quad \text{at} \quad y = 0, h. \quad (5.12)$$

5.2.3 Non-dimensionalisation

In this section, we nondimensionalise the system (5.1)–(5.12) to reduce the number of governing parameters and simplify the model formulation. To this end, we rescale time using the characteristic timescale of MMP decay and introduce the following dimensionless variables:

$$L = \frac{l}{l_0}, \quad M = \frac{m}{m_{\text{res}}}, \quad P = \frac{p}{m_{\text{res}}}, \quad X = \sqrt{\frac{\delta_m}{D_m}} x, \quad Y = \sqrt{\frac{\delta_m}{D_m}} y, \quad T = \delta_m t. \quad (5.13)$$

We note that we scale x and y to normalise the maximum MMP diffusivity to 1, rather than using the physical length scales. This avoids introducing very small dimensionless diffusion coefficients, which may lead to numerical stiffness. Applying these scalings to (5.1)–(5.5), we obtain the following dimensionless PDEs defined on the domain $(X, Y) \in [0, \bar{L}] \times [0, \bar{h}]$:

$$\frac{\partial L}{\partial T} = -k_{LM} L M \quad (5.14)$$

$$\frac{\partial M}{\partial T} = \nabla(\bar{\epsilon}(L) \nabla M) - \bar{\mu} M P - M \quad (5.15)$$

$$\frac{\partial P}{\partial T} = \nabla(D_P \bar{\epsilon}(L) \nabla P) - \bar{\mu} M P - \delta_P P \quad (5.16)$$

where $D_P = \frac{D_p}{D_m}$ and the effective diffusivity function $\bar{\epsilon}(L)$ is defined as:

$$\bar{\epsilon}(L) = \left(D_{\min} - 1 \right) L + 1. \quad (5.17)$$

The nondimensionalised initial and boundary conditions corresponding to the original system (5.3)–(5.12) are given by:

$$L(X, Y, 0) = 1, \quad (5.18)$$

$$M(X, Y, 0) = 0, \quad (5.19)$$

$$P(X, Y, 0) = P_0. \quad (5.20)$$

and

$$M(X, Y, T) = 1 \quad \text{at} \quad X = \bar{L} \text{ and } Y = 0, \bar{h}, \quad (5.21)$$

$$\frac{\partial}{\partial X} M(X, Y, T) = 0 \quad \text{at} \quad X = 0, \quad (5.22)$$

$$\frac{\partial}{\partial X} P(X, Y, T) = 0 \quad \text{at} \quad X = 0, \bar{L}, \quad (5.23)$$

$$\frac{\partial}{\partial Y} P(X, Y, T) = 0 \quad \text{at} \quad Y = 0, \bar{h}. \quad (5.24)$$

We refer to the dimensionless system (5.14)–(5.24) as the hydrogel model. The original dimensional parameters used in equations (5.1)–(5.12) are listed in Table 5.1, while Table 5.2 provides the values for the corresponding nondimensional parameter groupings. Note that the diffusion coefficients D_m and D_p , the inhibition rate μ and the decay rates δ_m and δ_p are as previously specified in Table 3.1 in Chapter 3. Two additional parameters, namely, the diffusive ratio $D_{\min}$ and the rate of linker degradation by MMPs k_{ml} remain unknown and are therefore indicated by dashes in Table 5.1. We note that the experimental data include three different values of m_{res} , and

Parameter	Description	Value	Reference
k_{ml}	Rate of degradation of cleavable linkers by MMPs	–	
$D_{\min}$	Ratio of minimum to maximum diffusivities	–	
D_m	Diffusion coefficient for MMPs	$2.16 \times 10^{-2} \text{ mm}^2 \text{ days}^{-1}$	[163]
μ	Inhibition rate of MMPs by TIMPs	$0.876 \text{ nM}^{-1} \text{ days}^{-1}$	[174]
δ_m	MMP decay rate	0.3384 days^{-1}	[167]
D_p	Diffusion coefficient for TIMPs	$2.16 \times 10^{-2} \text{ mm}^2 \text{ days}^{-1}$	[163]
δ_p	TIMP decay rate	4.1592 days^{-1}	[168]
r	Horizontal length of the hydrogel	2.5mm	[40]
h	Vertial length of the hydrogel	1.5mm	[40]

Table 5.1: Dimensional parameter values appearing in (5.1)–(5.12).

since three of the dimensionless parameters are defined in terms of m_{res} , their values depend on the specific experimental condition. For this reason, we do not list their values in the parameter table. Additionally, although the initial hydrogel density is not directly available from the experiments, this does not affect the model, as the linker density is scaled to unity in the non-dimensional formulation.

5.3 Parameter Estimation for the Hydrogel Model

5.3.1 Estimating k_{ML} and $D_{\min}$ from experimental data

The experimental data used in this study were originally reported in [40], in which hydrogels without TIMPs were submerged in solutions containing MMPs. This experiment was repeated for three different MMP concentrations in the reservoir: 20nM, 30nM, and 60nM and the cumulative release of FD20 was measured at three time points: 24, 48, and 72 hours, which correspond to the nondimensionalised time points

Parameter	Definition	Value
k_{ML}	$\frac{k_{ml}m_{\text{res}}}{\delta_m}$	*
$D_{\min}$	-	
$\bar{\mu}$	$\frac{\mu m_{\text{res}}}{\delta_m}$	*
D_P	$\frac{D_p}{D_m}$	1
δ_P	$\frac{\delta_p}{\delta_m}$	12.29
$\bar{L}$	$\sqrt{\frac{\delta_m}{D_m}} L$	9.895
$\bar{h}$	$\sqrt{\frac{\delta_m}{D_m}} h$	5.94
P_0	$\frac{p_0}{m_{\text{res}}}$	*

Table 5.2: Non-dimensional parameter values appearing in the hydrogel model (5.14)–(5.24). Parameters marked with * depend on the rescaled MMP reference concentration m_{res} and therefore vary between experiments.

	Hydrogel Density (Proportion)		
MMP conc (nM)	24h	48h	72h
20	0.56 ± 0.13	0.38 ± 0.12	0.18 ± 0.11
30	0.47 ± 0.15	0.30 ± 0.13	0.16 ± 0.12
60	0.33 ± 0.05	0.08 ± 0.07	0.015 ± 0.015

Table 5.3: Experimental data representing the remaining hydrogel density over time for different MMP concentrations.

$T = 0.34, 0.69, 1.02$, respectively, yielding a total of nine data points. The release of FD20 is used as an indirect measure of hydrogel degradation. The unreleased fraction of FD20 is taken to represent the proportion of hydrogel remaining and assuming cleavable linkers are uniformly distributed throughout the hydrogel matrix; this value serves as a proxy for the remaining linker density. The data is summarised Table 5.3 which represent the mean of three biological replicates, with the variability quantified by the corresponding standard deviations [40].

Although the full model accounts for TIMP dynamics, focusing on experiments without TIMPs allows us to isolate the effects of MMP-mediated degradation, thereby

simplifying the system and reducing confounding influences during parameter estimation. Furthermore, as discussed in Section 3.3, the biological parameter values used in our model, such as diffusion coefficients and degradation rates, are sourced from literature rather than derived experimentally within our specific setup. These values originate from studies involving various tissue types and MMP/TIMP isoforms. While they serve as reasonable approximations, they are not tailored to our exact system. Therefore, restricting the fitting process to data from the simpler, TIMP-free experiments helps ensure that parameter estimates reflect the dominant processes with greater accuracy and minimal bias from external variability. The non-dimensionalised system is thus reduced to:

$$\begin{aligned}\frac{\partial L}{\partial T} &= -k_{ML}LM, \\ \frac{\partial M}{\partial T} &= \nabla \cdot (\bar{\epsilon}(L)\nabla M) - \bar{\mu}MP - M,\end{aligned}$$

with

$$\bar{\epsilon}(L) = \left(D_{\min} - 1\right)L + 1,$$

subject to initial conditions

$$L(X, Y, 0) = 1,$$

$$M(X, Y, 0) = 0,$$

and boundary conditions

$$M(X, Y, T) = 1 \quad \text{at} \quad X = \bar{L} \text{ and } Y = 0, \bar{h},$$

$$\frac{\partial}{\partial X}M(X, Y, T) = 0 \quad \text{at} \quad X = 0,$$

We refer to the above as the reduced hydrogel model. In this system, two key unknown parameters are estimated: the rate of hydrogel degradation k_{ML} and the diffusivity ratio $D_{\min}$. The MMP concentration m is nondimensionalised by m_{res} ,

fixing the Dirichlet boundary condition for M at 1 across experiments. Since m_{res} varies, the nondimensional degradation rate $K_{ML} = \frac{k_{ml}m_{\text{res}}}{\delta_m}$ also changes. To account for this, we fit the dimensional parameter k_{ml} and compute k_{ML} within the model using the corresponding m_{res} value. To estimate both k_{ml} and D_{min} , we use MATLAB's PDE Toolbox to simulate the nondimensionalised system, and we employ the `lsqcurvefit` function to carry out the fitting. We construct a custom PDE function that accepts both the nondimensional time points T and the MMP reservoir concentration m_{res} as inputs. Within this function, the two unknown parameters to be estimated, (k_{ml}, D_{min}) , are represented as a vector $\mathbf{p}$. The model simulates the evolution of the nondimensional linker density, which takes values between 0 and 1. At each specified time point T^i and for each reservoir concentration m_{res}^j , we compute the spatial integral of $L(x, y, T^i; m_{\text{res}}^j)$ over the domain Ω , representing the total remaining cleavable linker content within the hydrogel. This is then normalised by the initial total linker content (at $T = 0$), resulting in the proportion of remaining linker density. The expression compared to the experimental data is:

$$L_{\text{model}}(T^i, m_{\text{res}}^j; \mathbf{p}) = \frac{\iint_{\Omega} L(x, y, T^i; m_{\text{res}}^j) dx dy}{\bar{L} \times \bar{h}}. \quad (5.25)$$

The `lsqcurvefit` function minimises the difference between model predictions and experimental measurements by reducing the sum of squared residuals. The residuals are defined as the difference between model-predicted and experimentally observed linker densities at each time point and for each reservoir concentration. Specifically, the objective function minimised by `lsqcurvefit` is given by:

$$\min_{\mathbf{p}} \sum_{i=1}^{N_T} \sum_{j=1}^{N_m} (L_{\text{model}}(T^i, m_{\text{res}}^j; \mathbf{p}) - L_{\text{exp}}^{i,j})^2, \quad (5.26)$$

where $\mathbf{p} = (k_{ml}, D_{\text{min}})$ denotes the vector of unknown parameters, T_i represents the nondimensionalised time points, m_{res}^j the MMP reservoir concentrations, L_{model} the

model output, and $L_{\text{exp}}^{i,j}$ the corresponding experimental values.

To assess the robustness of our parameter estimation, we first define a measure of fit quality: the Root Mean Squared Error (RMSE). This metric quantifies how well the model output matches the experimental data and is defined as the square root of the mean of the squared residuals:

$$\text{RMSE} = \sqrt{\frac{1}{N_T \times N_m} \sum_{i=1}^{N_T} \sum_{j=1}^{N_m} (L_{\text{model}}(T^i, m_{\text{res}}^j; \mathbf{p}) - L_{\text{exp}}^{i,j})^2}. \quad (5.27)$$

We now present our results. Using the RMSE (5.27), we evaluated the sensitivity of the fitting procedure to different initial guesses. While this led to varying estimates for the unknowns, the associated RMSE values were nearly identical across fits.

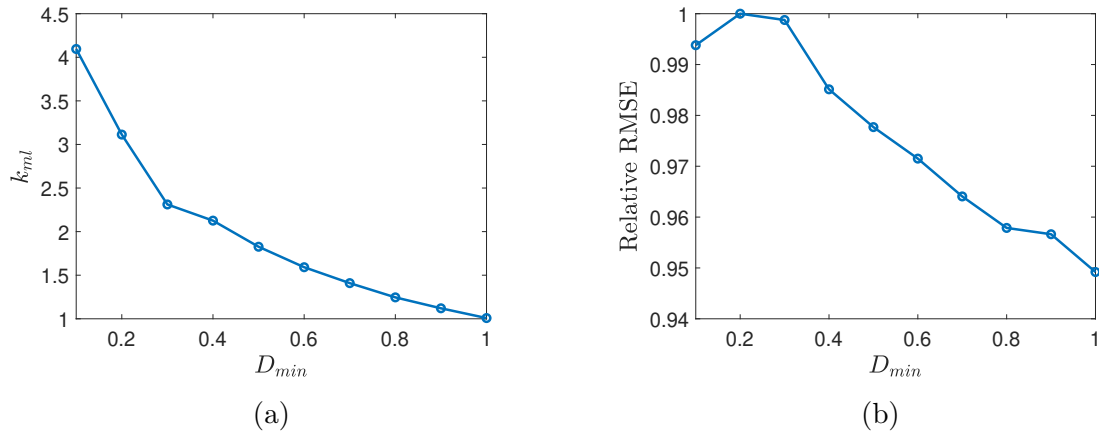


Figure 5.2: (a) Best-fit estimates of k_{ml} for fixed values of $D_{\min}$ obtained using the reduced hydrogel model, the model output given in (5.25) and the objective function defined in (5.26). (b) Corresponding RMSE values (as defined in (5.27), normalised by the maximum) for each $(D_{\min}, k_{ml})$ combinations specified in (a).

To explore this compensatory behaviour more systematically, we fixed a range of values for $D_{\min}$ and estimated the corresponding optimal k_{ml} for each case. In this approach, $D_{\min}$ was held constant during each fitting routine, reducing the number of unknown parameters to one. We then repeated the process across several $D_{\min}$ values, effectively generating a plot of best-fit k_{ml} estimates in Figure 5.2(a). From this figure,

we see that as $D_{\min}$ decreases, the fitted value of k_{ml} increases, indicating a strong trade-off between hydrogel porosity and MMP-mediated hydrogel degradation. To determine which parameter combination yields the best fit, we calculated the RMSE values, normalised by the maximum of the RMSE values. In Figure 5.2(b), we observe that the plotted $(D_{\min}, k_{ml})$ combinations all yield errors within 5% of the maximum RMSE, clearly illustrating parameter non-identifiability. A global minimum of the error does not by itself establish identifiability; the optimum may sit within a broad, flat valley where many parameter combinations fit almost equally well. We therefore judge identifiability by the sharpness of the minimum: how rapidly the error increases as we move away from the optimum. We examine this in detail in the next section.

These results reveal that the model is able to fit the experimental data equally well using fundamentally different mechanisms — either through faster MMP diffusion and reduced degradation, or via slower diffusion compensated by more rapid hydrogel degradation. This behaviour is demonstrated in Figure 5.3 where we compare the experimental data with model outputs corresponding to two combinations of $(D_{\min}, k_{ml})$ selected from Figure 5.2(a), where despite relying on different underlying mechanisms, both align well with the observed data, despite fundamentally different underlying mechanisms.

This minimal variation arises from the fact that the metric used, namely, the proportion of remaining hydrogel, is spatially averaged and lacks resolution of local gradients. This is further supported by the observation that, although the proportion remaining profiles appear very similar and close to experimental data across different parameter combinations, the spatial distributions of linker density vary significantly. As illustrated in Figure 5.4, higher diffusivity values produce more uniform inhibition and degradation, whereas lower diffusivity values lead to steeper spatial gradients. We remark that in both Figure 5.4(a) and (b), the hydrogel degradation appears strongly reaction-dominated. Exploratory simulations examining the effect of the

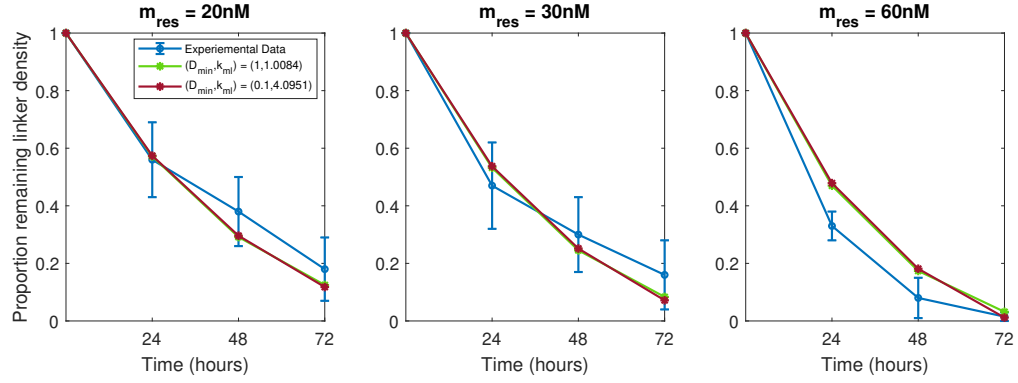


Figure 5.3: Comparison between experimental data from Table 5.3 and model predictions from the reduced hydrogel model (5.25), showing the proportion of remaining linker density. Simulations use parameter values from Table 5.2 for the three experimental conditions: $m_{\text{res}} = 20\text{nM}$, 30nM and 60nM .

maximum MMP diffusivity D_M indicate that larger values lead to a more diffusion-dominated regime, with degradation spreading more uniformly through the hydrogel. The dimensional value of D_M used here was estimated from experimentally reported MMP diffusivities in cellular and tissue environments [?], which are of a similar order of magnitude but may differ from those in synthetic hydrogels. Consequently, while higher diffusivities could in principle yield more spatially uniform degradation, they were not explored further in the present study. Investigating how the hydrogel properties or MMP mobility influence this transition between reaction- and diffusion-dominated regimes would be an interesting direction for future work.

The differences observed in Figure 5.4 are crucial because they imply that the correct parameter set cannot be uniquely identified from spatially averaged measurements alone. Consequently, at this stage, we can only provide a lower bound estimate for k_{ml} corresponding to $D_{\min} = 1$, with $k_{ml} = 1.0084\text{nM}^{-1}\text{h}^{-1}$. These results highlight the importance of spatially resolved experimental data to better constrain the model and achieve more accurate parameter estimates. With such data, both $D_{\min}$ and k_{ml} may be independently estimated with greater confidence. In the next section, we explore, using a synthetic data set, the data required for unique estimation of the

unknown parameters k_{ml} and $D_{\min}$, and in turn resolve the identifiability issue.

We note that while the choice of hydrogel geometry can, in principle, influence parameter identifiability, the sensitivity to geometry in this study is limited. The 2D Cartesian slice is effectively equivalent to an axisymmetric cylinder under the boundary conditions considered, since both preserve radial symmetry and capture the dominant gradient direction. More broadly, identifiability is governed not by the coordinate system itself but by factors such as the extent of reservoir exposure and the aspect ratio of the hydrogel, which determine the strength of spatial gradients in MMP and linker density. Altering the geometry (for example, using a disc versus a thin slab) would modify the steepness and location of these gradients, and hence change the optimal positions for data collection. Nevertheless, the primary limitation of the current dataset lies in the absence of spatial resolution: bulk averages mask the diffusion–reaction trade-off, and this issue would remain irrespective of geometry. While the most informative spatial sampling points may differ across geometries, the addition of any spatially resolved data would substantially enhance identifiability. We therefore proceed with our chosen two-dimensional slice geometry.

5.3.2 Synthetic Data Set

Due to the lack of spatial resolution resulting in parameter non-identifiability in the experimental data described previously, we now consider the type of data required to reliably estimate the unknown parameters k_{ml} and $D_{\min}$. We adopt a synthetic data approach similar to that outlined in [192]. Specifically, we select representative values for parameters k_{ml} and $D_{\min}$ from the parameter space explored in Figure 5.2. We simulate our PDE hydrogel model using these parameter values and extract synthetic datasets for three different scenarios: measurements taken at 2, 3, and 4 spatial points, each observed at time points of 24h, 48h, and 72h, mirroring the original experimental sampling schedule. As before, simulations are performed for

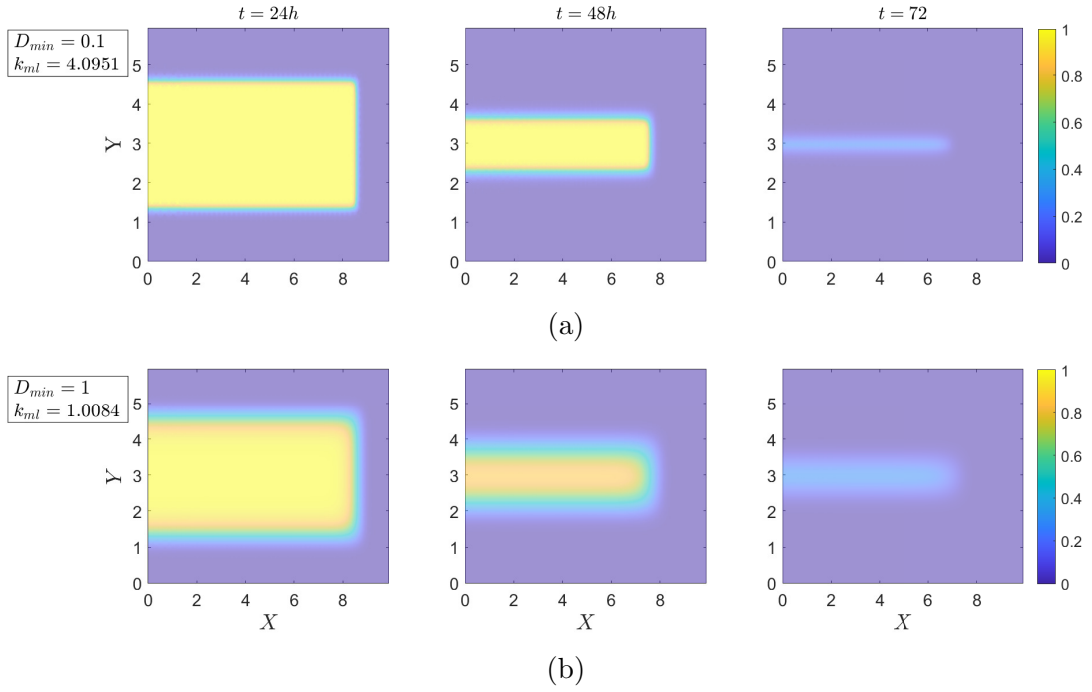


Figure 5.4: Spatial profiles for hydrogel linker density $L(X, Y, T)$ from the reduced hydrogel model with parameter values as in Table 5.2, $M_{\text{res}} = 20$ at time points $T = 0.34, 0.69, 1.02$ corresponding to $t = 24h, 48h, 72h$ for (a) $(D_{\min}, k_{ml}) = (0.1, 4.0951)$ and (b) $(D_{\min}, k_{ml}) = (1, 1.0084)$.

MMP reservoir concentrations of 20 nM, 30 nM, and 60 nM. The spatial measurement points are denoted p_1, p_2, p_3 , and p_4 . The spatial locations of these points are:

$$p_1 = \left(0, \frac{\bar{h}}{2}\right), \quad p_2 = \left(\bar{L}, \frac{\bar{h}}{2}\right), \quad p_3 = \left(\frac{\bar{L}}{3}, \frac{\bar{h}}{2}\right), \quad p_4 = \left(\frac{2\bar{L}}{3}, \frac{\bar{h}}{2}\right). \quad (5.28)$$

The spatial measurement points are all positioned halfway along the vertical (y) direction to maintain consistency in sampling height across the domain. This uniform y -coordinate minimises variability due to vertical differences, allowing us to focus on spatial variations along the horizontal (x) axis, thus making it easier to interpret spatial patterns without confounding effects from vertical gradients. To capture the spatial profile effectively, the points are evenly distributed along the x -direction, ensuring comprehensive coverage of the hydrogel length. We take $(k_{ml}, D_{\min}) = (1.8265, 0.5)$ which serve as demonstrative inputs. We note that:

- 2-point data uses only p_1 and p_2 ,
- 3-point data includes p_1, p_2 and p_3 ,
- 4-point data includes p_1, p_2, p_3 and p_4 .

To generate the synthetic datasets, we take the simulated hydrogel density values at each point and add 5% arbitrary Gaussian noise to mimic experimental variability. For a range of parameter pairs $(k_{ml}, D_{\min})$, $D_{\min} \in [0.1, 1]$, $k_{ml} \in [0.1, 4]$, we run numerical simulations of the PDE model and extract model predictions at the same spatial and temporal points. We then compute the RMSE between the model outputs and the noisy synthetic data for each parameter combination. This procedure is repeated separately for the 2-point, 3-point, and 4-point cases, allowing us to assess how the spatial resolution of data affects parameter identifiability. The RMSE is defined, similar to before, as:

$$\text{RMSE}(k_{ml}, D_{\min}) = \sqrt{\frac{1}{N} \sum_{i=1}^N \left(y_i^{\text{model}}(k_{ml}, D_{\min}) - y_i^{\text{synthetic}} \right)^2} \quad (5.29)$$

where N is the total number of data points (combining all spatial locations, time points, and MMP concentrations into a single vector), $y_i^{\text{model}}(k_{ml}, D_{\min})$ is the model output at the i^{th} data point for the given parameters, $y_i^{\text{synthetic}}$ is the corresponding synthetic data value (with noise) and the RMSE is computed separately for 2, 3, and 4 spatial point subsets.

Figure 5.5 presents the RMSE contour plots for the three different configurations of synthetic data used to estimate the parameters $(k_{ml}, D_{\min})$, normalised with the maximum RMSE across all three. In addition to the spatial data cases, we also include a fourth contour plot corresponding to the non-spatial case, where only the percentage of hydrogel remaining is considered, thus replicating the original data format presented in the previous section. In this non-spatial synthetic scenario, we generate data using the same prescribed parameter values for $(k_{ml}, D_{\min})$, but record only the overall hydrogel density at each time point. The synthetic data is generated following the same procedure as for the spatial cases, but evaluated using the objective function defined in Equation (5.26) in Section 5.3.1, for total hydrogel density rather than spatially resolved profiles. The resulting RMSE values are then computed for a range of parameter combinations $(k_{ml}, D_{\min})$, as previously described. Since this objective function is fundamentally different, being based on total hydrogel density rather than spatially resolved measurements, it is normalised by its own maximum. As a result, a different colour scale is used, and direct numerical comparisons with the other panels are not meaningful. Instead, the emphasis is placed on the qualitative structure of the error surface.

We note that in Figure 5.5, lighter regions correspond to lower RMSE values,

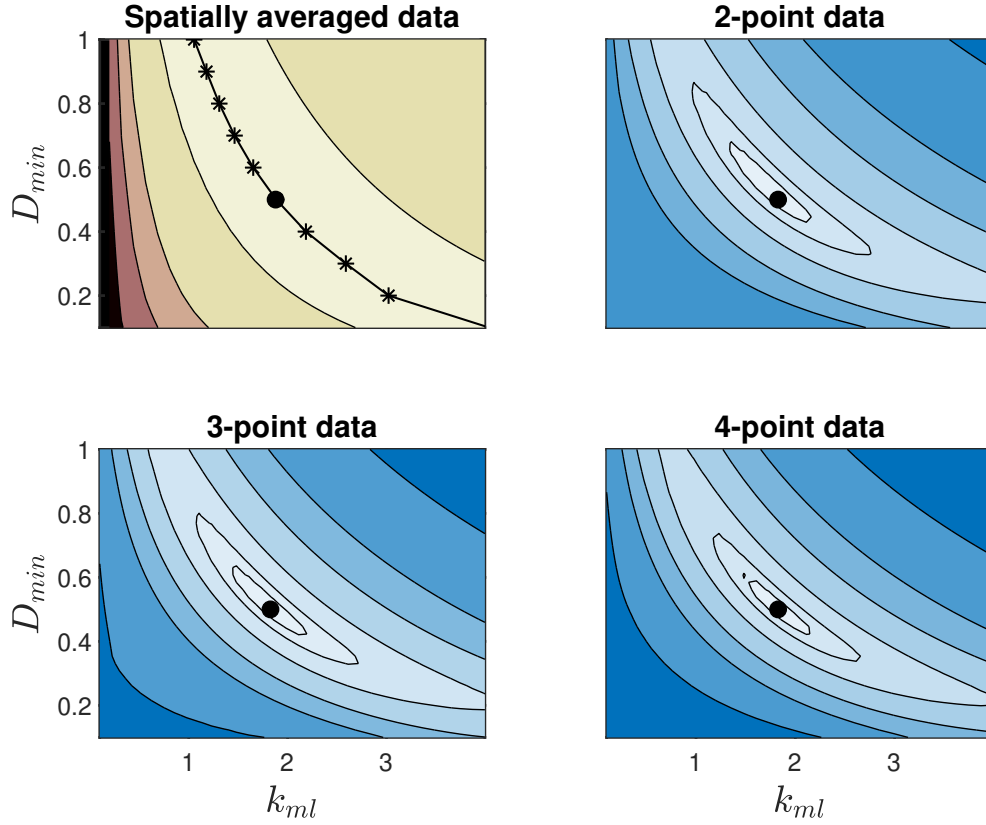


Figure 5.5: Contour plots of the normalised RMSE for four cases: no spatial data (first panel), two-point data (second), three-point data (third), and four-point data (fourth). The RMSE for the non-spatial case (defined in (5.27)) is normalised by its own maximum, while the RMSE for spatial cases (defined in (5.29)) is normalised by the maximum across all three. Lighter regions indicate lower RMSE values, and darker regions indicate higher values. Contour levels (from outermost to innermost, i.e., darker to lighter) represent normalised RMSE bands: 100% – 75%, 75% – 50%, 35% – 50%, 20% – 35%, 10% – 20%, 5% – 10% and finally, 5% – 0%. We note that the first panel also plots the best-fit estimates of k_{ml} for fixed values of D_{min} obtained using the model output given in (5.25) and the objective function defined in (5.26) similar to Figure 5.2.

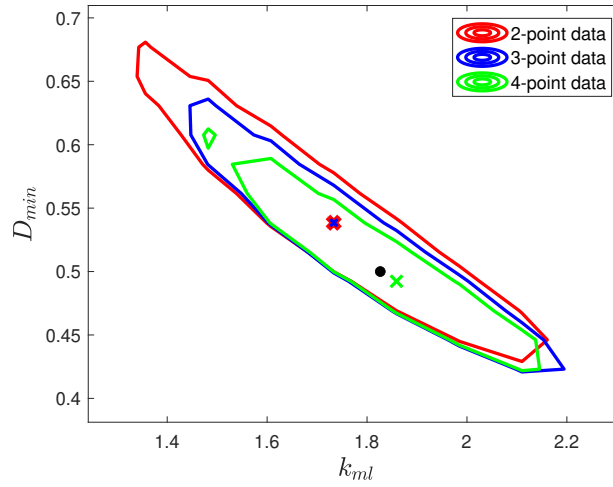


Figure 5.6: Regions of parameter space where the normalised RMSE lies within 5% of the maximum, based on the two-point, three-point, and four-point data shown in Figure 5.5. The red, blue, and green crosses denote the $(D_{\min}, k_{ml})$ values associated with the minimum RMSE for the two-point, three-point, and four-point datasets, respectively.

while darker regions indicate higher RMSE values. The contour levels, defined in terms of the normalised RMSE, are listed in the caption of Figure 5.5. As shown in the panel corresponding to hydrogel remaining percentage, this non-spatial dataset leads to significant parameter non-identifiability, consistent with expectations from the previous section. The RMSE surface notably exhibits a wide valley of low-error regions, which indicates that many parameter combinations produce similarly good fits to the data. Similarly for in Figure 5.2, we also plot best-fit k_{ml} for fixed values of $D_{\min}$ which shows as before that as $D_{\min}$ decreases, the fitted value of k_{ml} increases, indicating a strong trade-off between hydrogel porosity and MMP-mediated hydrogel degradation, reflecting a compensatory trade-off between degradation and diffusion: higher degradation rates (k_{ml}) can be offset by lower diffusivity ($D_{\min}$), and vice versa. Without spatially resolved measurements, the model lacks sufficient information to distinguish these effects, preventing unique identification of the true parameter values. Furthermore, no parameter combinations fall within the 10% RMSE region, further underscoring the strong non-identifiability. Notably, the parameter pair yielding the

lowest error is $(k_{ml}, D_{\min}) = (0.98, 1)$, which deviates substantially from the true parameter values.

However, even the addition of data from just two spatial points dramatically improves identifiability. In the second panel of Figure 5.5, representing 2-point data, the RMSE landscape tightens significantly around the true parameter pair (indicated by the black dot), forming more localised error contours. This indicates that spatial data begins to meaningfully constrain the solution space, reducing ambiguity in parameter estimation. Adding a third and fourth spatial point (third and fourth panels respectively) further refines the parameter estimation, with increasingly sharper minima and tighter 5% error contours centred around the true values. A key feature highlighted is the larger area for higher error values (darker regions) for 3-point and 4-point data which reflects the fact that the RMSE values at parameter combinations far from the true values are substantially higher when more spatial data is used. That is, as the spatial resolution increases, the model becomes more sensitive to deviations from the true parameter set, producing larger errors for poor estimates.

While there are improvements in parameter identifiability when increasing from 2-point to 3-point to 4-point data, the relative gain from three to four spatial points appears to be marginal, indicating diminishing returns beyond two spatial locations. To quantify this, we plot the regions of the parameter space that fall within 5% error for each spatial resolution in Figure 5.6. We find that while the size of this region decreases as more spatial points are added, thereby better constraining the true parameter values, the reduction is modest, shrinking from 1.56% of the domain for 2-point data to 1.02% for 4-point data. In the same figure, we also display the parameter pair corresponding to the minimum error for each case. While all three are close to the true value, both the 2-point and 3-point datasets yield the same minimum at $(k_{ml}, D_{\min}) = (1.73, 0.54)$, while the 4-point data gives a slightly more accurate estimate of $(k_{ml}, D_{\min}) = (1.86, 0.49)$. These results suggest that adding

a third spatial point provides little to no additional benefit, and while 4-point data offers some improvement, the marginal gains must be carefully weighed against the added experimental complexity and effort required to obtain higher spatial resolution.

Overall, this analysis highlights the importance of spatially resolved data in improving parameter identifiability. While data without spatial resolution is insufficient to resolve the trade-off between degradation and diffusivity, the inclusion of measurements from just two spatial locations is enough to yield accurate and robust parameter estimates. Additional spatial data enhances precision further, but with less pronounced benefit, suggesting that from a practical experimental standpoint, acquiring data at two well-chosen spatial positions is likely to offer the optimal balance between accuracy and experimental effort. These results reinforce the value of designing experiments that include some degree of spatial resolution, even if limited, in order to effectively constrain model parameters.

5.4 Conclusions and Discussion

Hydrogel-based delivery systems are commonly used to aid chronic wound healing by releasing biochemical agents into the wound environment. One promising strategy under investigation is the incorporation of TIMPs into hydrogels to modulate the elevated concentrations of MMPs typically present in chronic wounds. While this has been explored experimentally, there has been limited focus on modelling such hydrogel–TIMP–MMP interactions in the mathematical biology literature.

In Chapter 4, we introduced a simplified representation of a hydrogel, thus in this chapter, we developed a more biologically realistic model of the hydrogel itself, explicitly incorporating both diffusion-controlled and chemically-controlled mechanisms of TIMP release. The model focuses on the internal hydrogel dynamics, including the degradation of cleavable linkers by MMPs and the subsequent release of TIMPs, which

in turn inhibit MMP activity. Two key unknown parameters arise: the degradation rate of cleavable linkers by MMPs and a porosity-related diffusivity parameter. To estimate these parameters, we used experimental data from [40], specifically from a system where an empty (TIMP-free) hydrogel was placed in a reservoir containing MMPs. The cumulative release of FD20 was measured over time and used as a proxy for hydrogel density loss. We implemented a parameter fitting procedure in MATLAB using `lsqcurvefit`, treating the spatially averaged hydrogel density as the quantity of interest. However, our results revealed significant parameter non-identifiability: multiple mechanistic scenarios, such as higher porosity with reduced MMP-mediated degradation or lower porosity with larger degradation produced nearly identical error values, and thus can equally explain the observed data. This trade-off arises due to the lack of spatial resolution in the data, which only reflects average hydrogel degradation rather than spatial concentration profiles. Indeed, although these different parameter combinations yield similar bulk degradation outcomes, they produce markedly different spatial distributions: higher diffusivity results in more uniform degradation, whereas lower diffusivity leads to sharper spatial gradients in hydrogel density.

To explore this further, we generated a synthetic data set by fixing true values for the degradation rate and diffusivity, then adding noise to simulate experimental uncertainty. We extracted synthetic measurements of average hydrogel density as well as data from 2, 3, and 4 spatial points at each time step. By computing fitting errors across a grid of parameter combinations, we observed that using only average density data led to parameter non-identifiability as expected. Adding spatial information from just two distinct points dramatically improved identifiability, allowing us to recover the true parameter values within a tight error range. Furthermore, including 3 or 4 spatial points marginally increased accuracy and tightened the confidence region around the true values, but with diminishing returns. From a practical standpoint,

we conclude that two-point spatial data provides sufficient information to resolve both parameters reliably. While more spatial points improve precision, the gains may not justify the additional experimental complexity. Importantly, we demonstrate that spatially resolved data is essential for accurate parameter inference in models where diffusion and reaction interplay drives system behaviour. This would help guide the design of future hydrogel experiments, improving understanding of the underlying mechanisms and enabling exploration of wound treatment dynamics with such hydrogels.

We note that the non-identifiability observed here arises from the lack of spatial data rather than the chosen geometry; while different geometries may shift the optimal sampling points, the addition of spatial data would improve identifiability in any case.

Chapter 6

Conclusions and Future Work

In this thesis, we have developed mathematical models to describe the behaviour of MMPs in chronic wound development, as well as the role of TIMPs as inhibitors of MMP activity and, thus as regulators of healing. In the UK, approximately 2.2 million people are affected by chronic wounds, significantly impacting patients' quality of life and placing a substantial burden on the NHS. The primary motivation for this work has been to explore the therapeutic potential of TIMPs as a treatment for chronic wounds — an emerging and novel approach which is underexplored in mathematical modelling literature. In particular, we focus on the delivery of TIMPs to a wound through hydrogel treatment, a treatment strategy that is currently under experimental investigation and shows promise for improving patient outcomes. Accordingly, we also incorporate experimental data to inform modelling of such hydrogel-based therapies.

6.1 Conclusions

In Chapter 1, we examine the stages of wound healing, discussing the characteristics of both acute and chronic wounds, the factors contributing to pathological healing, and key properties of chronic wounds that inform the modelling approaches developed

later in this thesis. We also review previous mathematical models of wound healing, with particular emphasis on reaction-diffusion frameworks that build upon the seminal work of Sherratt and Murray. These modelling approaches, along with analytical techniques such as travelling wave and bifurcation analysis employed in the literature, provide a foundation for the methodologies used throughout this thesis.

One key characteristic of chronic wounds is the presence of elevated levels of MMPs. MMPs are essential for healing in many ways such as by facilitating fibroblast migration, enabling tissue remodelling and proliferation and promoting the activation of growth factors such as TGF- β , which promotes ECM production and contributes to angiogenesis. However, the overexpression of MMPs is detrimental to healing as it leads to excessive degradation of the ECM. Therefore, to investigate the dynamics of MMPs in both acute and chronic wound healing, we develop a two-variable reaction-diffusion model in Chapter 2, capturing the interaction between MMPs and dermal cells during the healing process. Our mathematical model gives rise to travelling wave solutions and, in particular, is able to emulate key qualitative features of the wound healing process observed in experimental and clinical studies. One such property is that under parameter regimes representing healthy healing, we observe acute wounds healing to completion with an increased expression in MMP concentration at the edge of the healing wound.

To contrast acute wounds with chronic wounds, we consider the effect of varying the apoptotic rate of dermal cells. Small apoptotic rates correspond to healthy biological functioning and thus we observe the complete healing of a wound. Provided initial concentrations of MMPs are kept low, an increase in apoptotic rate also results in the healing of a wound, which suggests that in order to avoid risk of the emergence of a chronic wound, baseline levels of MMPs must be kept minimal. Elevated apoptotic rate beyond a threshold value leads to the emergence of a chronic wound, with increased levels of MMPs invading the wound which prevents its healing. Moreover, the

presence of a bistable region in the bifurcation diagrams for the apoptotic rate gives rise to multistable travelling wave profiles. The specific profile obtained depends on the initial condition, reflecting the system's history dependence. As a result, reversing a chronic wound to a fully healed state requires reducing the apoptotic rate below a critical threshold. This highlights the importance of regulating apoptosis in chronic wound treatment, potentially through interventions such as blood sugar control in diabetic patients. Crucially, successful healing demands a substantial reduction in the apoptotic rate to exit the bistable region and transition back to a healed state, making recovery significantly more challenging than by simply decreasing apoptotic rate slightly.

We also consider the effect of varying MMP production rate on the healing of a wound. Similar to the analysis for apoptotic rate, we obtain threshold values at which chronic wounds persist and may be reversed. However, contrasting from the variation of the apoptotic rate, we observe a change in the qualitative features of the travelling wave solutions at a different threshold value. At this new threshold, occurring in the middle of the multistable regime, a wound transitions from healing completely to only being partially healed, before reaching a regime where chronic wounds persist.

In this preliminary model, the role of TIMPs in the wound healing process was not included. Additionally, the interaction between fibroblasts and the extracellular matrix was simplified by coupling their effects into a single equation. In Chapter 3, we address these limitations by extending the model. The extended model incorporates the regulatory role of TIMPs in the healing process. It consists of reaction-diffusion equations as before, but now includes four equations: fibroblast activity and ECM dynamics are modelled separately, and a fourth equation is introduced to capture the behaviour of TIMPs. This extended framework, continues to give rise to travelling wave solutions and is able to replicate key qualitative behaviours observed physio-

logically. One such property is the increased expression of MMP concentrations at the edge of a healing wound across all stages of acute wound recovery. Furthermore, biological studies suggest that one factor contributing to chronic wound formation is elevated ECM degradation due to oxidative stress. Therefore, we explore the impact of varying the degradation rate of the ECM across different levels of TIMP production to investigate the regulatory effects of TIMPs. Using bifurcation analysis, we observe a region of bistability which indicates that low TIMP levels lead to chronic wounds that are progressively harder, and in the extreme case effectively impossible, to reverse, while increased TIMP production levels result in reversible chronic wounds, which are better regulated for larger TIMP production values. These results indicate that high TIMP concentrations lead to more effectively regulated chronic wounds, consistent with existing biological research.

The bistability observed in this extended model has important implications for treatment: once the system enters a chronic wound state within the bistable regime, reversing it may require a significant treatment to return to a healed state. In contrast, if TIMP levels are sufficiently high early on, healing may proceed without interruption. This suggests that early intervention to maintain TIMP levels above a critical threshold could be key to preventing wound chronicity and improving treatment outcomes. In particular, therapeutic strategies that involve the external administration of TIMPs may help to better regulate chronic wounds, especially when endogenous production is insufficient.

The biologically significant features of this model are retained in a simplified model obtained using an eFAST parameter sensitivity analysis, while reducing computational runtime. In Chapter 4, we extended this reduced model to a two-dimensional spatial domain in order to model a more realistic wound geometry and to explore the efficacy of a hydrogel treatment. Simulations of the 2D model yield the same wave speed as that of the 1D model, thereby affirming the validity of our 2D numerical

solution. We also find that elevated MMP production levels lead to the emergence of chronic wounds, consistent with biological studies. We consider such chronic wounds and employ a Robin boundary condition to model an external source of TIMPs such as supplied by the application of a hydrogel on the wound surface. Simulation results demonstrate the reversal of the chronic wound, thereby reaffirming the regulatory properties of TIMPs and supporting the use of TIMP-containing hydrogels as a viable treatment option for chronic wounds.

We then sought analytical insight into the behaviour of TIMP delivery within our model. To achieve this, we introduce a slender domain approximation to simplify the system, which enables an asymptotic analysis aimed at providing deeper insights into the model. By utilising a small parameter that represents the aspect ratio of the domain, we derive an approximate solution that captures the leading-order behaviour of TIMP concentration within the wound. Through this process, we gain valuable understanding of how key parameters influence TIMP delivery dynamics, their regulatory impact, and the healing progression of the wound.

We observe that introducing an influx of TIMPs into the wound leads to complete healing of the ECM, with a small correction term related to the low-level degradation of ECM by MMPs which is consistent with a healthy wound environment, as discussed in Section 1.1.2. We also find that MMP concentration is small at leading order, indicating significant suppression due to the TIMP dynamics. Furthermore, we find that TIMP concentration increases linearly with both hydrogel loading and delivery rate, highlighting these as viable biologically tunable parameters for enhancing treatment. The spatial profile of TIMP concentration is shaped by diffusion and degradation: higher diffusivity flattens the profile and increases TIMP delivery up to a saturation point, while greater degradation consistently suppresses the overall concentration. These insights into the delivery dynamics have important implications for treatment design and underscore the influence of biological processes on TIMP

delivery.

We then validate our asymptotic solutions by comparing them with numerical simulations of the full 2D model. It is important to note that modelling the delivery of a source of TIMPs as a Robin boundary condition serves as a proof of concept, offering a simplified representation of a hydrogel containing TIMPs. In Chapter 5, we propose a more detailed and realistic model to more accurately capture the chemical behaviour of a hydrogel that is currently under development [40]. The model incorporates both diffusion-controlled and chemically-controlled mechanisms of TIMP release. The model focuses on the internal hydrogel dynamics, including the degradation of cleavable linkers by MMPs and the subsequent release of TIMPs, which in turn inhibit MMP activity. Two key unknown parameters arise: the degradation rate of cleavable linkers by MMPs and a porosity-related diffusivity parameter. To estimate these parameters, we used experimental data from [40], specifically from a system where an empty (TIMP-free) hydrogel was placed in a reservoir containing MMPs. The cumulative release of FD20 was measured over time and used as a proxy for hydrogel density loss. We implemented a parameter fitting procedure in MATLAB using `lsqcurvefit`, treating the spatially averaged hydrogel density as the quantity of interest. However, our results revealed significant parameter non-identifiability: multiple mechanistic scenarios, such as higher porosity with reduced MMP-mediated degradation or lower porosity with larger degradation produced nearly identical error values, and thus can equally explain the observed data. This trade-off arises due to the lack of spatial resolution in the data, which only reflects average hydrogel density rather than spatial concentration profiles. Indeed, although these different parameter combinations yield similar bulk degradation outcomes, they produce markedly different spatial distributions: higher diffusivity results in more uniform degradation, whereas lower diffusivity leads to sharper spatial gradients in hydrogel density.

To explore this further, we generated a synthetic data set by fixing true values

for the degradation rate and diffusivity, then adding noise to simulate experimental uncertainty. We extracted synthetic measurements of average hydrogel density as well as data from 2, 3, and 4 spatial points at each time step. By computing fitting errors across a grid of parameter combinations, we observed that using only average density data led to parameter non-identifiability as expected. Adding spatial information from just two distinct points dramatically improved identifiability, allowing us to recover the true parameter values within a tight error range. Furthermore, including 3 or 4 spatial points marginally increased accuracy and tightened the confidence region around the true values, but with diminishing returns. From a practical standpoint, we conclude that two-point spatial data provides sufficient information to resolve both parameters reliably. While more spatial points improve precision, the gains may not justify the additional experimental complexity. Importantly, we demonstrate that spatially resolved data is essential for accurate parameter inference in models where diffusion and reaction interplay drives system behaviour. This would help guide the design of future hydrogel experiments, improving understanding of the underlying mechanisms and enabling exploration of wound treatment dynamics with such hydrogels.

6.2 Future Work

In Chapters 2, 3, and 4, we developed models of wound healing. In these models, we capture the positive contribution of MMPs to healing through a reaction term. However, MMPs influence healing through multiple mechanisms, including facilitating fibroblast migration [6]. Future work could extend the current framework to incorporate this additional role by modifying the fibroblast transport term to include an MMP-dependent effective diffusivity, thereby capturing this added biological complexity and further enhancing the realism of the model.

Additionally, in the models presented, several biological processes have been represented through effective terms. For example, while MMPs promote healing by activating TGF- β , which subsequently stimulates ECM production [29], our models have not explicitly included TGF- β or its intermediaries. Instead, we used an effective reaction term to represent the net positive contribution of MMPs to ECM healing. Future work may consider explicitly incorporating TGF- β into the system to model these interactions directly, avoiding the use of proxy terms and providing a more faithful representation of the underlying biology.

While our models address both acute and chronic wound healing, we define chronic wounds primarily by elevated MMP production and ECM degradation, starting from parameter sets corresponding to acute wound healing. In reality, chronic wounds may arise through a variety of pathological processes [183], which may correspond to a broader and more diverse range of parameter values than those considered here. Future studies could explore these variations to better capture the full spectrum of chronic wound behaviours. In particular, it would be valuable to investigate whether chronic wounds arising from different pathological mechanisms exhibit distinct healing dynamics and whether treatment strategies should be tailored accordingly.

Finally, our models exhibit travelling wave solutions for both acute and chronic wound scenarios. The bistability in the bifurcation diagrams suggests the presence of multistable travelling waves, therefore, future work could include a detailed stability analysis of these travelling waves to gain deeper insights into their dynamics and potential clinical implications.

In Chapter 5, we developed a model of a hydrogel currently under investigation as a treatment for chronic wounds, where diffusivity of MMPs and TIMPs was dependent on the hydrogel linker density. We examined the nature of spatial data required to overcome parameter non-identifiability and accurately estimate the unknown pa-

rameters in the hydrogel model. In this analysis, spatial measurement locations were fixed, and the potential influence of their positioning on parameter estimation was not explored. Future research should investigate whether the choice of measurement locations impacts estimation accuracy and, if so, determine the optimal spatial placement. Additionally, a simplified linear form was used to represent the effective diffusivity; however, the true relationship may be nonlinear. Further work is needed to explore alternative functional forms and identify the most appropriate representation to improve the biological realism of the model.

In the analysis of the hydrogel model, we focused on a reduced version of the hydrogel model corresponding to experimental data obtained from a hydrogel formulation that did not contain TIMPs. Future work should extend this analysis to the full model, which includes the effects of TIMPs, once appropriate spatial data becomes available. This would enable more accurate estimation of the parameter representing MMP inhibition by TIMPs. In Chapters 2–4, we used a representative value for this parameter based on biological literature. However, in practice, this value originates from studies involving various tissue types and MMP/TIMP isoforms and while they serve as reasonable approximations, they are not tailored to our exact system. Applying the same fitting methodology to the full model with corresponding spatial data would allow for a more robust estimate of the inhibition parameter and would provide an opportunity to validate previous parameter estimates and deepen understanding of the spatial interplay between degradation and inhibition within the hydrogel environment.

Once parameters have been estimated, future work should also focus on coupling the wound model from Chapter 4 with the hydrogel model from Chapter 5 to investigate the use of these specific hydrogels as a treatment for chronic wounds. This could be achieved by modelling two adjacent domains: one representing the wound and the other representing the hydrogel and applying appropriate continuity conditions

for both flux and concentration at the interface as demonstrated in Figure 6.1. Such a coupled framework would provide important insights into chronic wound management, allowing investigation into factors such as the optimal loading capacity of the hydrogel and predictions of healing time.

While this thesis has made an initial exploration into experimental data for these hydrogel experiments, future work could place greater emphasis on integrating experimental results with the modelling framework to refine parameter estimates and improve model validation. Furthermore, the modelling framework could be extended into three spatial dimensions to capture a more biologically realistic representation of wound healing. This would allow investigation into whether wound shape and geometry influence healing dynamics and whether treatment strategies, such as the application of TIMP-containing hydrogels, should be tailored accordingly. Since we adopted a two-dimensional Cartesian system for numerical stability, future work should incorporate a cylindrical coordinate system to more accurately reflect the geometry of the hydrogel itself and explore whether hydrogel shape affects healing outcomes, potentially identifying optimal geometries for clinical application.

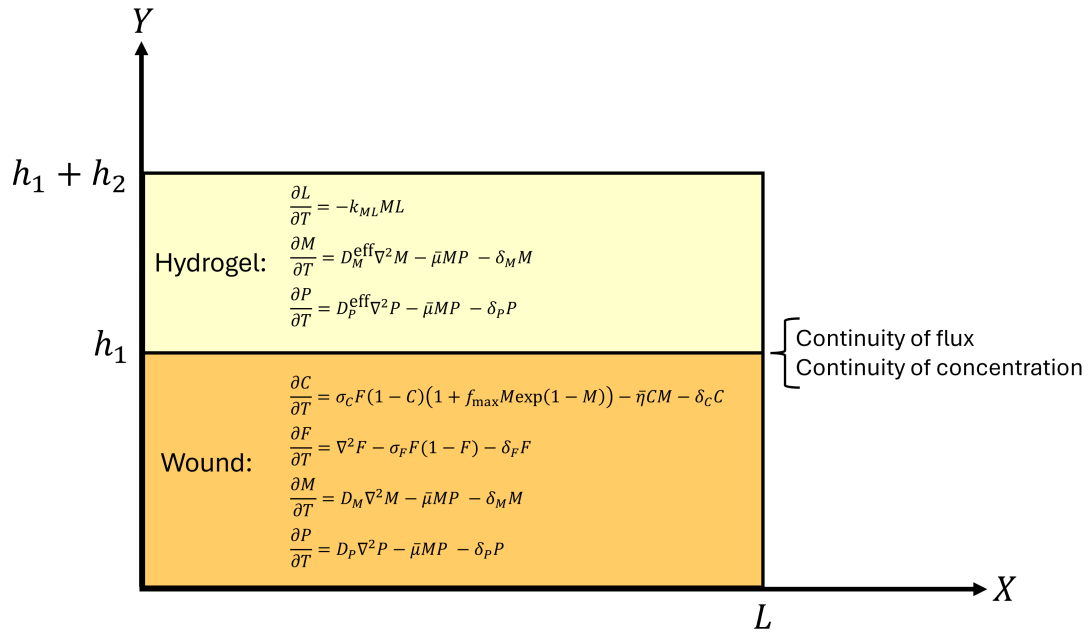


Figure 6.1: Schematic illustrating a potential model extension that integrates the hydrogel model with the regulated wound healing model for future work.

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