

THE FRiND MODEL: A MATHEMATICAL MODEL FOR REPRESENTING
IMMUNOLOGICAL INTERACTIONS IN MUSCULAR DYSTROPHY PATHOGENESIS.

by

MATTHEW T. HOUSTON

(Under the Direction of Juan B. Gutierrez)

ABSTRACT

Muscular dystrophy (MD) describes generalized progressive muscular weakness due to the wasting of muscle fibers. The progression of the disease is affected by known immunological and mechanical factors, and possibly other unknown mechanisms. These dynamics have begun to be elucidated in the last two decades, and mathematical models have been created to understand these dynamics. This dissertation introduces a new mathematical model, the FRiND model. This model describes how the immune system repairs muscle tissue. The FRiND model will be mathematical analyzed and results verified by biological studies in the literature. This model could be the foundation for further understanding of immunological muscle repair.

INDEX WORDS: Immunology, Tissue Repair, Tissue Regeneration, Muscular Dystrophy, Mathematical Models, Systems of Ordinary Differential Equations, Law of Mass Action, Law of Generalized Mass Action

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DEDICATION

This dissertation is dedicated to my wife, Melissa, with gratitude and love for her support and dedication. She has been instrumental in the completion of this work towards my Ph.D.

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TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	v
LIST OF FIGURES	viii
LIST OF TABLES	xi
CHAPTER	
1 A REVIEW OF MATHEMATICAL MODELS FOR MUSCULAR DYSTROPHY: A SYSTEMS BIOLOGY APPROACH	1
2 THE FRiND MODEL	30
2.1 INTRODUCTION TO MUSCLE REPAIR BY THE IMMUNE SYSTEM . .	30
2.2 THE FRiND MODEL	31
2.3 DISCUSSION	38
3 MATHEMATICAL ANALYSIS OF THE FRiND MODEL	40
3.1 STEADY STATES OF THE FRiND MODEL	40
3.2 STABILITY ANALYSIS OF THE STEADY STATES	45
3.3 IDENTIFIABILITY OF THE FRiND MODEL.	55
3.4 DISCUSSION	58
4 RESULTS	59
4.1 ACUTE MUSCLE DAMAGE	59
4.2 CHRONIC MUSCLE DAMAGE	61
4.3 INFLAMMATORY MACROPHAGE DEPLETION BY DIPHTHERIA . . .	64
4.4 DISCUSSION	66

5	DISCUSSION	69
APPENDIX		
A	ABM.M	87
B	FIGURES_4_1.M	89
C	FIGURES_4_2.M	97
D	FIGURES_4_3_AND_4_4.M	104
E	FIGURES_4_5_AND_4_6.M	113
F	FIGURES_4_7.M	122
G	SENSITIVITYMATRIX.M	129

LIST OF FIGURES

1.1	Conceptual linkage between the sections of this article. Most types of MD are genetic disorders. Vulnerabilities due to genetic mutation result in increased stress-strain on muscles during contraction. Mitochondrial and immunological responses continue for extended periods; this causes a chronic immunological response that perpetuates MD progression. This figure was created by Sanaz Haghani.	4
1.2	Location of Genes Implicated in MD. A diagram of cell structure proteins implicated in MD pathogenesis. The corresponding SNPs for each type of MD shown here are given in Table 1.1. The proteins affected by many types of MD have yet to be elucidated. This figure was created by Sanaz Haghani and Amanda N Cameron.	7
1.3	Sarcomere Regional Classification. Muscle contraction occurs when calcium ions allow crossbridge sites on myosin to bind with actin and slide actin into the H zone. This action called a powerstroke is followed by ATP binding to the crossbridge allowing the crossbridge to release the actin; Rebinding and powerstroke may then occur again until calcium ions depart the muscle [22, 32]. This figure was created by Sanaz Haghani and Amanda N Cameron.	8
2.1	Muscle Tissue Repair. This figure shows the process of muscle repair. Phagocytosis of damaged tissue (D) by macrophages gives regenerating tissue (R). Fibrous connective tissue (F) is laid by some types of macrophages over the regenerating tissue. Fibrous tissue binds with differentiate myoblasts to form normal muscle tissue (N).	32

2.2	Formation of Nascent Myofibers. This figure shows the creation of nascent myofibers. Pro-inflammatory macrophages promote activation and proliferation of myoblasts (M_b). Exposure to resolution macrophages promotes differentiation of myoblasts (M_d) which eventually bind together into myofibers. .	33
2.3	Macrophage Interactions. This figure describes the interaction of different type of macrophages during muscle repair. Inflammatory signals from resident macrophages are released after tissue is damaged prompting invasion by pro-inflammatory macrophages (M_1). Phagocytosis of damaged tissue by pro-inflammatory macrophages promotes inhibiting macrophage phenotype (M_{2c}) which starts deactivating (M_3) pro-inflammatory macrophages. Resolution macrophages (M_{2a}) appear after further phagocytosis of damaged tissue by inhibiting macrophages. Conflicting signals by pro- and anti-inflammatory macrophages force some macrophages into a phenotypeless state (M_3). . . .	34
4.1	Time Course of FRiND Model. This figure shows the time course of the A) muscle tissue equations, B) macrophage equations, and C) myocytes equations. Actual data in Table 2.1 is given as x's on the graphs.	60
4.2	Phase Portrait of $N \times F$ plane. This figure shows the time course in the $N \times F$ phase plane where vector arrows show direction of flow and the red circle gives position of $(N(t), F(t))$. The blue line $F = N - 100$ acts as a barrier where the red circle cannot cross since $N(t) + F(t) = 100$	62
4.3	Non-fading periodic damage. These figures show the time course for the FRiND model with $f(t) = a \sin^2(\pi t)$ with a randomly selected each day. A shows the results for muscle tissue. B shows the results for macrophages. C shows the time course of myocytes.	63

4.4	Phase portrait of $N \times F$ plane for non-fading periodic damage. This figure shows the time course in the $N \times F$ phase plane where vector arrows show direction of flow and the red circle gives position of $(N(t), F(t))$. The blue line $F(t) = N(t) - 100$ acts as a barrier where the red circle cannot cross since $N(t) + F(t) \leq 100$	64
4.5	Terminating Periodic Damage. These figures show the time course for the FRiND model with $f(t) = \frac{a \sin^2(\pi t)}{t+1}$. <i>A</i> shows the results for muscle tissue. <i>B</i> shows the results for macrophages. <i>C</i> shows the time course of myocytes. . .	65
4.6	Phase Portrait of $N \times F$ plane for fading periodic damage. This figure shows the time course in the $N \times F$ phase plane where vector arrows show direction of flow and the red circle gives position of $(N(t), F(t))$. The blue line $F(t) = N(t) - 100$ acts as a barrier where the red circle cannot cross since $N(t) + F(t) = 100$	66
4.7	Diphtheria Treated Mice. These figures show the time course for the FRiND model with M_1 macrophages depleted by DT. The results are consistent with Arnold et al. (2007) [105]. <i>A</i> shows the results for muscle tissue. <i>B</i> shows the results for macrophages. <i>C</i> shows the time course of myocytes.	67

LIST OF TABLES

1.1	SNPs Implicated in MD. SNPs Implicated in different types of MD. SNP IDs correspond to SNPedia.com [10].	6
1.2	Propensity Equations for Tam et al. (2013). This table lists the equations used in Tam et al. (2013)[70] to update the number of mtDNA in the mitochondria where $r_{max} + 1$ – is the maximum copy number for amplification of mtDNA, M_{mito} – is the number of mitochondria in a cell at a given time k_D – the rate of autophagy, and $V_{F,max}$ – is the maximum propensity of fission.	16
2.1	Data Used to Estimate Parameters. This table gives the data used to estimate the parameters for the FRiND model. The data was gleamed from Mounier et al. (2013)[93] and Ogawa et al. (2015)[95].	36
2.2	Estimated Parameters for the FRiND Model. This table shows the estimated parameters for the FRiND model using an acute lognormal damage with data from 2.1.	37

CHAPTER 1

A REVIEW OF MATHEMATICAL MODELS FOR MUSCULAR DYSTROPHY: A SYSTEMS BIOLOGY APPROACH

Published on 1/2018 [29]

INTRODUCTION

Muscular dystrophy (MD) describes generalized progressive muscular weakness due to the wasting of muscle fibers. While this is an umbrella term used to describe a wide range of muscle wasting diseases, the two types that have been most extensively mathematically modeled are Duchenne's (DMD) and Becker's (BMD) [15, 35], with DMD being the most common childhood form of MD in males. Children affected by this X-linked recessive disorder have an average life expectancy in the mid-twenties, typically becoming fully wheelchair dependent by their teens [8]. Most forms of MD leave striated muscle cells with reduced contractile abilities, leading to a wide range of phenotypic expression in patients from fatigue to drooping eyelids.

Previous research attributes pathogenesis of DMD and BMD to either absent or partial forms of the dystrophin protein [26]. From a cellular perspective, the basic contractile unit of the muscle is the sarcomere. The dystrophin protein is located between the sarcolemma, the outer membrane of the sarcomere, and outer layer of myofilaments, providing a scaffold for muscular contraction. Weakness typically begins in extremity muscles, propagating in a proximal–distal direction, until ultimately affecting the diaphragmatic muscles responsible

for breathing [6, 8]. Hypertrophy of cardiac muscle cells is an additional complication associated with both DMD and BMD. The loss of function associated with both typically leads to premature death [9].

Epidemiological impacts of MD remain difficult to pinpoint definitively. Each subset of MD contains its own range and pattern of pathogenesis and progression. We can further classify subsets of MD in (ordered in decreasing rates of prevalence): dystrophinopathies, laminopathies, dystroglycanopathies, sarcoglycanopathies, and alternative congenital forms [3, 50]. In the United States, dystrophinopathies have a prevalence of about 15 cases per 100,000 people [62]. The following list describes the rates of prevalence of MD per 100,000 cases in Northern England [50]:

1. Myotonic muscular dystrophy is the most common adult onset MD. The prevalence rate is about 10.6.
2. With respect to dystrophinopathies that manifest as dystrophin absence or deficiency, DMD and BMD have been most extensively studied. Incidence of dystrophinopathies is about 8.5.
3. Facioscapulohumeral muscular dystrophy (FSHD) has a prevalence rate of about 4.
4. Laminopathies, dystroglycanopathies, and sarcoglycanopathies are typically classified as Limb-Girdle MD (LGMD). The prevalence rate is about 2.3.
5. Collagen VI deficiencies that take form in Ullrich Congenital MD (UCMD, also known as Ullrich Scleroatonic MD) have a prevalence rate around 0.13.
6. Most alternative congenital forms have rates below one.

The goal of this paper is to offer a survey of mathematical models relating to MD, as well as identifying potential opportunities for quantitative research that have yet to be explored within the context of MD. In the context of this paper, mathematical models refer

to quantitative approaches that start from mechanistic principles and result in simplified representations of the phenomena that is studied. The modeling formalisms include algebraic expressions, ordinary and partial differential equations, discrete dynamical systems, agent-based models, etc. Mathematical methods differ from statistical methods in that the latter are applied *posteriori* to broad families of data representations - usually to test hypothesis of significance - whereas mathematical models can be used *a priori*. Clearly, we exclude from this paper statistical analysis of experimental data. However, we include in the scope of the present work strictly computational methods such as network reconstruction.

Each section describes either progression or pathogenesis of several forms of MD. Fig 1 illustrates how the sections relate in some forms of MD. At their roots, most forms of MD have genetic causes; the section Genetic Models presents models of gene-gene interactions intended to discover pathways either specific to a given form of MD or shared between multiple forms of MD. Vulnerabilities caused by genetic mutations result in an increased probability of muscle damage due to normal stress-strain of muscle usage; the section Muscle Models considers simulations of stress-strain during muscle contraction. Muscle contraction requires ATP originated in the mitochondria of which dysfunctions have been implicated in MDs; the section Mitochondrial Models discusses models of cell death initiated by mitochondria as a result of muscle cell damage. Both muscle damage and cell death initiate an acute immune response; this response attempts to regenerate the damaged or dead muscle tissue. Apoptotic signals from immune cells during muscle repair further antagonizes the mitochondrial response. In a patient with some forms of MD, the mitochondrial and immunological responses cause extended periods of inflammation; chronic immune responses have been implicated in the deterioration of muscle fibers in MD patients. The section Immunological Models introduces models of inflammation – both acute and chronic – and tissue repair.

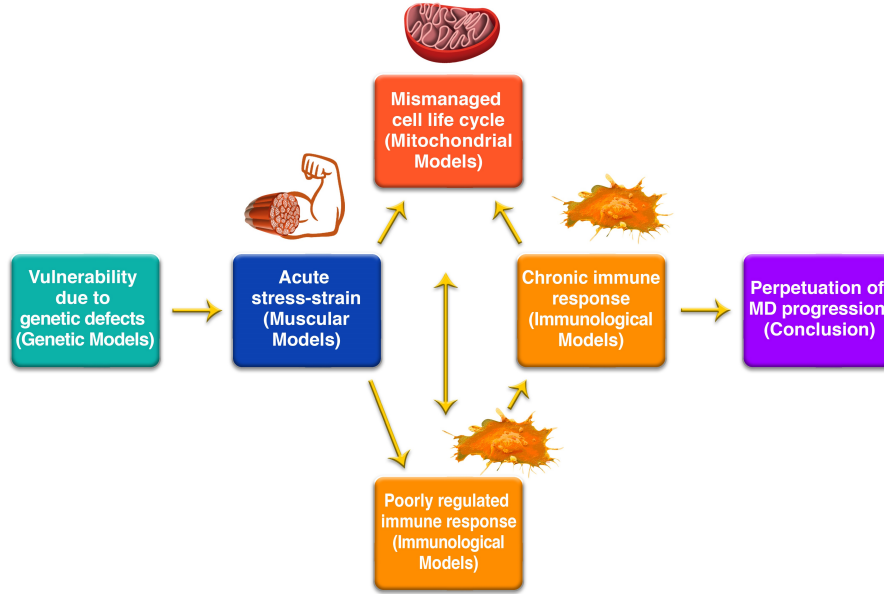


Figure 1.1: **Conceptual linkage between the sections of this article.** Most types of MD are genetic disorders. Vulnerabilities due to genetic mutation result in increased stress-strain on muscles during contraction. Mitochondrial and immunological responses continue for extended periods; this causes a chronic immunological response that perpetuates MD progression. This figure was created by Sanaz Haghani.

GENETIC MODELS

Most types of MD are believed to have genetic origin [26]. The genes implicated in MD have functions in numerous biological processes, thus obscuring a univocal characterization of MD pathogenesis. Although each form of MD is caused by different genes, MDs often share similar phenotypes. Finding genetic pathways shared by different MDs could allow for interventions at the downstream node. Furthermore, studying these pathways allow for a greater understanding of how these genes normally interact.

The genetic causes of observed phenomena can be inferred computationally through the reconstruction of gene regulatory networks (GRNs). The characterization of a GRN usually starts with experimental measures of many genes at at least two conditions or points in time. Gene regulatory networks (GRNs) can provide insight regarding subtleties in dis-

ease development; graph theoretic models like Boolean Networks [44], Bayesian graphs [18], and Petri Net [21] have emerged as important tools for understanding and reconstructing GRNs. To develop models of GRNs, researchers may employ database like OMIM [4] or computer programs like Snoopy [61] that use experimental genetic expression data to reconstruct such networks. GRNs can then be studied to find nodes that have more parent genes, are up-regulated or down-regulated by parent genes, affect more downstream genes, or express more often by different but related diseases like MDs. For time dependent data or complex processes, though, directed graphs may be impossible to construct. Instead continuous models like dynamic Bayesian networks [49] and temporal Bayesian classifiers [74] can be constructed. Although continuous models are unable to answer qualitative questions about the GRN, continuous models can estimate quantitative values. Lack of data hampers continuous models ability to explain large complex GRNs.

PUBLISHED GENETIC MODELS ABOUT MD

Relatively few GRNs have been constructed for MD; among the few successful studies are Grunwald et al. (2008) [21] and Tucker et al. (2006) [74].

Grunwald et al. (2008) [21] created a Petri net of the downstream gene affected by dystrophin (DMD and BMD). The model incorporated sixty-four places and eighty-eight transitions. The authors used Muaritus maps to analysis this model to elucidate the important nodes through knockout experiments. The knockout of the RAP2B-calcineurin pathway caused a 78% disturbance of the whole model. This – the authors concluded – made that pathway important for future study in DMD/BMD therapeutics.

Tucker et al. (2006) [74] constructed a continuous model of a GRN involving DMD, LGMD2C, and LGMD2E by using temporal Bayesian classifiers. The model was trained using mouse models (*mdx, bsg, gsg*) of each form of MD. The model predicts that genes *Dlk1*, *Dusp13*, and *Casq2* play a part in all three MDs and should be studied further as a possible influence of MD pathogenesis. The role of *Dlk1* and *Casq2* as shared pathways in MD has

been independently verified by statistical studies of gene expressions by Vinciotti et al. (2006) [79].

MODELING OTHER IMPLICATED GENETIC PATHWAYS

The task of elucidating gene regulatory networks is a vast area of research (see e.g. DREAM challenge 2008 [56]); given the correct data most of these model schemes could be used to study MD. A modern review on this subject can be found at Chai et al. (2014) [11]. Fig. 1.2 illustrates the extracellular and intracellular domains of a muscle cell, relating proteins embedded within and associated with the nucleus, the dystrophin-glycoprotein complex (DGC) and the extracellular matrix (ECM). The nuclear outer membrane (OM) is an integral part of the rough ER (Fig 1.2). Destabilized lamina release proteins that diffuse along the OM to the ER. To aid in future modeling, Table 1.1 includes the known single nucleotide polymorphisms (SNPs) that have been strongly implicated in MD and the genes affected. Publicly available databases can be used to investigate each SNP's related genes, proteins, and pathways (e.g. SNPedia [10], OMIM [4], and 1000 Genome Project [13]).

SNPs	Gene Target	MD
rs28928901	LMNA	EDMD, LGMD
rs104894466	PABPN1	OPMD
rs28937902 rs28937903 rs28937904 rs28937900 rs28937905 rs28937901	FKRP	LGMD
rs2296949	POMT1	LGMD
rs28933096	LAMA2	LAMA2 Congenital Type MD
rs28937597	MYOT	LGMD
rs1800278	DMD	DMD, BMD
rs28933693 rs28933694	SGCA	LGMD
rs28936383 rs28936384 rs28936385 rs28936386	SGCB	LGMD
rs1042917 rs2839110	COL6A2	UCMD
rs1131296 rs2270669	COL6A3	UCMD

Table 1.1: **SNPs Implicated in MD.** SNPs Implicated in different types of MD. SNP IDs correspond to SNPedia.com [10].

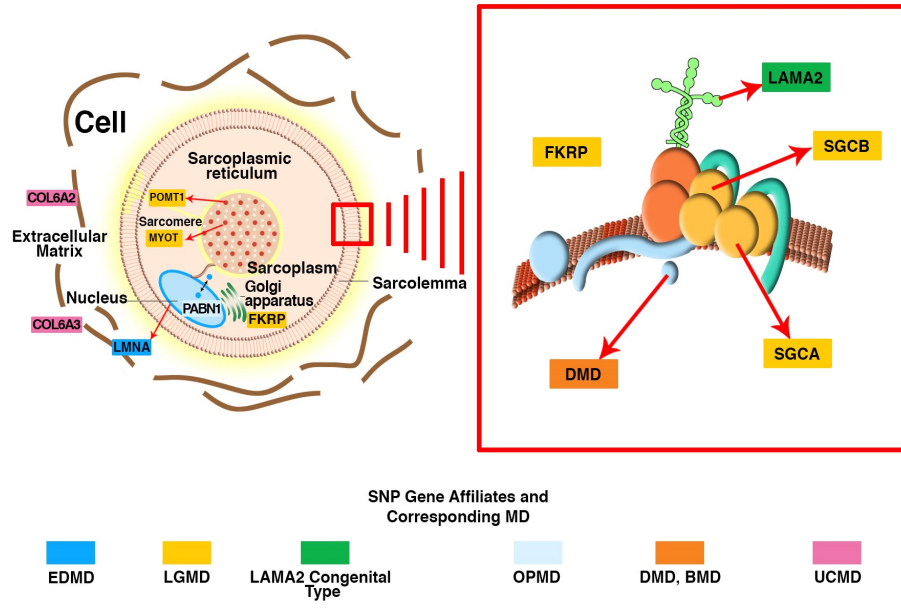


Figure 1.2: **Location of Genes Implicated in MD.** A diagram of cell structure proteins implicated in MD pathogenesis. The corresponding SNPs for each type of MD shown here are given in Table 1.1. The proteins affected by many types of MD have yet to be elucidated. This figure was created by Sanaz Haghani and Amanda N Cameron.

MUSCULAR MODELS

Although vulnerabilities caused by mutated genes, poorly regulated immune responses, and damaged mitochondria perpetuate MD, muscle wasting is the main phenotypical effect of MD. Damage to muscle fibers caused by contractions exposes muscle tissue to the effects of chronic immune activation and mitochondrial dysfunction. Fibrosis due to chronic immune activation reduces vulnerable muscle cell's contractual force and flexibility. Understanding these interactions by using mathematical models could offer a macroscopic view of MD progression.

One muscle cell contains thousands of sarcomeres interlaced and primed for contraction. A single sarcomere is made up of parallel thin and thick filaments called actin and myosin; these entwined filaments pull on each other during contraction [22, 32]. MD leaves mus-

cles vulnerable to unhealthy contraction which exacerbates damage in a cycle that proves catastrophic to muscle cells perpetuating MD progression [12, 17].

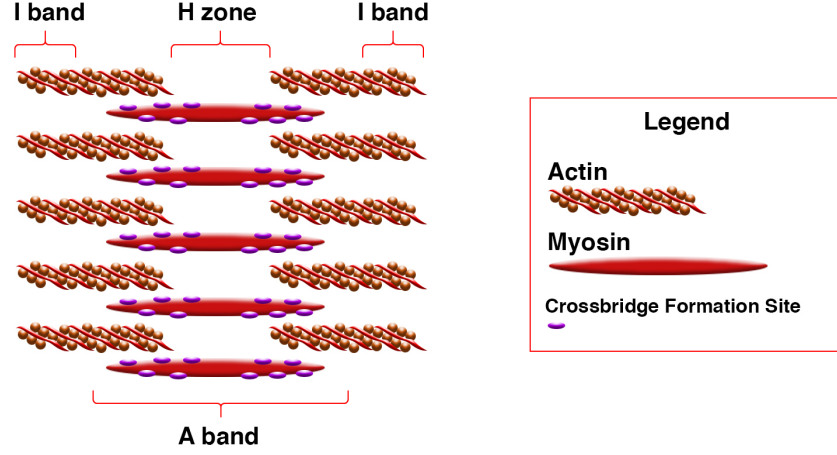


Figure 1.3: **Sarcomere Regional Classification.** Muscle contraction occurs when calcium ions allow crossbridge sites on myosin to bind with actin and slide actin into the H zone. This action called a powerstroke is followed by ATP binding to the crossbridge allowing the crossbridge to release the actin; Rebinding and powerstroke may then occur again until calcium ions depart the muscle [22, 32]. This figure was created by Sanaz Haghani and Amanda N Cameron.

PUBLISHED MUSCULAR MODELS ABOUT MD

Fibrotic tissue accumulation caused by chronic inflammation, contractures, and vulnerabilities in cell structure due to genetic mutation, complicate the process of modeling muscle tissue in MD. Of those attempted, Virgilio et al. (2015) [80] and Zöllner (2015) [84] created models describing the macro-scale complexities of fibrotic tissue and contractures. Lammerding et al. (2004) [41] considers cell deformation in the cytoskeleton due to lamina-deficiency.

Model of fibrosis: Virgilio et al. (2015) [80] created a simulation to study the effects of various pathologies including MD. The simulation used an agent based model to create fascicle geometry with the addition of fibrosis and fatty tissue infiltration followed by the use of the micromechanical model described by Sharafi and Blemker (2010) [64]. This model, using only *in silico* methods, agreed with the results that fibrosis aggravates the symptoms of DMD.

Model of contractures: MD patients can experience higher-degree of ankle plantarflexion due to contractures; “toe-walking” can make plantar flexor muscles such as the gastrocnemius, soleus and peroneus therapeutic targets. Although the model concerns with long term use of high heels, Zöllner’s model (2015) [84] could be used to describe toe-walking in MD. Due to removal of sarcomeres, fascicles shorten with long term toe walking causing less than optimal motion range and increased muscle stress. This increased stress coupled with DCG vulnerabilities accelerates fibrosis and adipose replacement.

Model of cytoskeleton: Nuclear lamina-deficiencies are linked with cytoskeletal defects in muscle cells [63, 69]. Among the mechanisms for these deficiencies are: (i) Destabilized DGC due to cytoskeletal compromise within the plasma membrane leads to the aggregation of mechanical stress in contracting muscle cells [41]. (ii) Histology in lamina-deficient diseases is commonly concomitant with maligned and internalized nuclei in muscle fibers [63, 69]. (iii) Chronically strained A/C deficient lamins result in cells experiencing higher rates of apoptosis [41, 57]. Intriguingly, regenerating muscle fibers also share high levels of internalized nuclei- the distinction between pathology and regeneration is unclear presently [63].

Lammerding et al. (2004) [41] modeled nuclear misalignment and cytoskeletal stress in lamin A/C-deficient mouse embryo fibroblasts. A/C lamins form compartments for splicing factors as well as RNA polymerase II transcription. Inhibition of A/C lamins suppresses RNA polymerase II-dependent transcription in mammalian cells, while its as a scaffold in nuclear compartments remain unknown. A sinusoidal force with amplitude 0.6 nanonewtons (nN) and with frequency 1 Hz, offset 0.6 nN, was applied through a magnetic trap. Cylindrical coordinates (r, θ) were used to measure bead displacement with the magnetic bead at the origin and $\theta = 0$ for the force direction. The equation represents the induced strain field described by the analytic cell mechanics model proposed by Bausch et al. (1998) [5] u_r is the radial component of the induced bead displacement as a function of the applied force, F , cell stiffness μ the characteristic cut of radius κ^{-1} , the distance from the magnetic bead

center r , and the polar angle θ . Thus,

$$u_r(r) = \frac{F}{2\pi\mu^*} \left(\cos(\theta) \frac{3(1-\sigma)}{4} K_0(\kappa_1 r) - \frac{K_1(\kappa r)}{\kappa r} + \sqrt{\frac{(1-\sigma)}{2}} \frac{K_1(\kappa_1 r)}{\kappa r} \right),$$

K_0 and K_1 are modified Bessel functions of respective order 0 and 1 with

$$K_1(r) = \kappa \left(\frac{(1-\sigma)}{2} \right)^{1/2}.$$

Letting for incompressibility and metallic bead contact radius of $2\mu m$, parameters μ_* and κ were computed by fitting Fig. 6 to the bead displacement.

In this model, the cytoskeleton was exposed to the same biaxial strain as the membrane; for each cell type, nuclear deformation increased approximately linearly with applied membrane strain. Both lamin A/C deficient cells result in decreased nuclear stiffness and altered nuclear mechanics. Under resting conditions, they found that the integrity of the nuclear envelope was maintained in A/C deficient cells. However, under pressure, the control cells maintained nuclei integrity far more than lamin-deficient cells, though both could be ruptured. Increased vulnerability of A/C deficient cells to mechanical stress comparatively was also concluded, with an increase in both necrotic and apoptotic cell fraction [41]. Necrosis mediated through nuclear rupture is not wholly attributed to vulnerability to mechanical stimulation in this model; only about 3-5% of A/C deficient nuclei ruptured.

MODELING OTHER IMPLICATED MUSCULAR PATHWAYS

Future muscle models into MD could follow several pathways including crossbridge modeling and whole tissue stress-strain simulation. Models of crossbridges combine the physiology of muscle contraction with ATP production in mitochondria. Stress-strain simulation allows for quantification of damage to tissue that activates the immune response.

Cross-bridge models: The crossbridge cycle allows for the ratcheting of muscles during contraction. To understand crossbridges, we must describe the regions of the sarcomere. The A band is made up of thick myosin filaments; throughout contraction its length remains constant centrally localized to the H zone. Actin filaments are laced with the protein

tropomyosin. At rest, tropomyosin covers the hinges that catch on actin. Alternatively, the I band is composed of thin actin filaments that alter its length between myosin filament pairs. Structures called Z discs fetter actin filaments at their opposite ends [22, 32]. In S1 regions, myosin edges are hinged and highly flexible, catching on actin and releasing in an indefinite binding cycle (Figure 1.3). Upon their release, myosin filaments perform a power stroke catalyzed by the hydrolysis of ATP. ATP is the means for crossbridge formation; it is the release of phosphate during ATP hydrolysis that contracts the S1 region [22, 32]. Calcium provides the means for binding whereas ATP drives contraction. Upon the release of calcium, the protein troponin pushes the tropomyosin to expose binding sites for actin. Upon exposure and a threshold level of ATP expression, the contraction cycle begins [42].

Smith et al. (2013) [66] described this procedure using a directed graph. The model uses twenty-five components in four domains to describe (mostly protein) interaction during a muscle contraction. The model is a micro scale representation of muscle contraction since it only incorporates one crossbridge. This model could be used to show how lack of ATP due to mitochondrial defects in MD could effect muscle contraction.

Huxley (1957) [33] proposed an early mathematical description of this power stroke process. Distance from binding site to crossbridge is taken as the independent variable while function $n(x, t)$ is the probability of a crossbridge being bound at position x at time t . This yields a conservation law of

$$\frac{\partial n}{\partial t}(x, t) - v(t) \frac{\partial n}{\partial x}(x, t) = (1 - n(x, t))f(x) - n(x, t)g(x).$$

The rate of energy release, ϕ , by ATP is

$$\phi = \rho\epsilon \int_{-\infty}^{\infty} (1 - n(x, t))f(x) dx.$$

Variable	Description	Variable	Description
x	is crossbridge position	t	is time
$n(x, t)$	is the probability of a crossbridge being bound	$v(t)$	is the velocity of actin to myosin
$f(x)$	is the binding rate of crossbridges	$g(x)$	is the unbinding rate of cross-bridges
ϕ	is rate of energy release	ϵ	is the energy released by a single crossbridge
ρ	is the total number of cross-bridges.		

This model captures the key physiological properties of crossbridges but fails to illuminate the biochemical interactions; Pate (1989) [52] proposed a model based on the Huxley model rectifying the biochemical interaction issue.

The weakening of cardiac muscles due to fibrotic/adipose tissue replacement and metabolic dysfunction is known as cardiomyopathy. This weakening can cause fatigue, swelling in extremities, arrhythmias, chest pain, and possibly heart failure. Cardiomyopathy is the end result of most moderate to severe forms of MD [14, 54]. Understanding how MD results in cardiomyopathy could save lives.

Tewari et al. (2016) [72] expanded on Huxley’s concepts with a model of cardiac muscle that connects metabolic and mechanistic crossbridge actions. Using five state variables, the model allows for experimenting with lack of ATP and Ca^{2+} in cardiac muscle contraction. Additionally, passive muscle action is modeled using spring dynamics. The model was able to fit several experimental data sets of cardiac contraction under different ATP and inorganic phosphate concentrations. The model does not account for three dimensional dynamics nor fibrotic/adipose tissue influences. An expansion of this model to include calcium has also been created [73].

Stress-strain models: Levels of healthy isometric force production can be maintained under the strain of intense activity. Rest commenced with the buffering of calcium levels in

the cytosol reduces mechanical stress to a point; inappropriate high or low levels of activity leave muscles vulnerable to damage. Furthermore, both types of activity are associated with increased levels of cytosolic calcium additionally linked with fiber damage and apoptosis [53].

A.V. Hill (1938) [24] created a Force-Velocity relationship model to understand isotonic and isometric muscle contractions; his work provides a basis for multibody, dynamic musculoskeletal modeling and simulation. Hill’s model relates rate of muscle contraction (shortening length), v , to the load p by

$$(p + a)v = b(p_0 - p)$$

for constants a and b given by experimental data and p_0 is the isometric force. Although Jewell and Wilkie (1958) [36] demonstrated that this model lost accuracy when exposed to sudden changes to muscle length, Hill’s model remains pivotal and is integrated in many other models.

Van der Linden et al. (1998) attempted whole tissue interaction simulations examining aponeurosis/muscle under stress, limited by computational power, they were restricted to 2D and simplified 3D models [76][75]. Johansson et al. (2000) [37] improved upon van Leeuwen-Kier’s (1997) [77] model of squid tentacles by separately modeling active muscle attributes with Hill’s force-velocity model and describing passive elements as a hyperelastic material. Unfortunately since they used parameter values based on van Leeuwen-Kier’s research and ANSYS, Johansson et al. failed to significantly improve upon the predictions made by van Leeuwen-Kier. Yucesoy et al. (2002) [83] introduced a similar model expanding on Van der Linden’s work. Like Johansson et al., Yucesoy et al. separated muscle into the extracellular matrix (passive) and myofiber (active); from that they created a linked fiber-matrix mesh that fuses a passive element and an active element. This “two domain” approach allowed a glimpse into the interaction of muscle fibers and the extracellular matrix. In an effort to understand the effects of geometries on muscle tissue, Sharafi and Blemker (2010) [64] devised a model where actual rabbit muscle biopsies were estimated with linear functions. They captured both the geometries of fibers (microscopic level) as well as the fascicles (macroscopic).

Since they were modeling muscle stress, Sharafi-Blemker only modeled the passive elements of muscle which were described as a hyperelastic, nearly incompressible material. In opposition to assumptions made by earlier modelists, Sharafi-Blemker discovered that fascicle display anisotropic characteristics.

MITOCHONDRIAL MODELS

Mitochondria are organelles inside a typical cell. Mitochondria produce ATP, which is the currency for sarcomere contraction. In a single cell, there can be hundreds of mitochondria; these organelles are responsible for far more than ATP synthesis. Mitochondria also regulate cell life-death cycle with implications to the immune system. Mitochondria synthesize protein encoded in mitochondrial DNA (mtDNA) in addition to dividing independently as needed inside the cell. mtDNA is vulnerable to mutations that perpetuate the mitochondrial cascade, with rates up to ten times higher than nuclear DNA mutation [39, 40].

Poorly regulated mitochondria have been implicated in several forms of MD. The distinct role of dystrophin in DMD and BMD's pathogenesis and progression remains unknown; despite devastating consequences in its absence, the dystrophin protein comprises a mere 0.002% of healthy skeletal muscle proteins. [27]. Mitochondrial Myopathies are a group of disorders closely related to – in some articles and web sites included as a form of – MD. An accumulation of defective mtDNA could be the cause of these myopathies [38].

PUBLISHED MITOCHONDRIAL MODELS ABOUT MD

Mitochondria's shape, number, and energy processing power are constantly in flux; mitochondrial dynamics alternate between fusion or fission. This fluctuation allows for the dispersal of mtDNA mutation. Tam et al. (2013) [70] and Taylor et al. (2003) [71] created stochastic models emulating these dynamics.

Tam et al. (2013) [70] produced a stochastic model examining rates of fusion-fission that would optimize mitochondrial function and minimize clonal expansion in neighboring

mitochondria. This model classifies fusion-fission events in mitochondria using the following criterion:

1. Fusion events feature nucleoid exchange between mitochondria; one is emptied out to the other and marked for degradation.
2. Fission sites appear close to the fusion event, regionally containing original nucleoid distributions from precursor mitochondria.
3. Healthy fission features low levels of exchange of nucleoids, with only mitochondrial matrix contents being mixed.
4. Larger and longer mitochondria have higher propensities for fission, and smaller ones are more likely to fuse.

The model predicts that protective nuclear retrograde signaling could rescue the mitochondrial cascade through the promotion of mitochondrial nucleoid replication propensity up to sixteen times the basal rate, increasing stochasticity by neutralizing clonal mutant aggregation. Benefits of nuclear retrograde signaling are limited by rates of fusion-fission. Within this simulation, rate of mitochondrial fusion-fission plays a significant role in clonal expansion. Slow exchanges of mtDNA result in homoplasmy, where interventionist retrograde signaling could compound the issue by increasing rate of nucleoid replication. Higher rates of fusion-fission result in a heteroplasmic steady state; increasing levels of mitochondria in cells mix nucleoids faster. These patterns persist regardless of mitochondrial presence in the cell or their replication parameters. A cytoskeletal, cellular model that considers mitochondrial movement independent of fusion-fission, as well as mitochondrial morphology in a differentiated cell context, is needed to further conclude potential therapeutic benefits of retrograde signaling [70].

Taylor et al. (2003) [71] also created a probabilistic model. Using this model, the authors were able to estimate mtDNA mutation rate as approximately $5 \cdot 10^{-5}$ mutations per genome per day. This rate guaranteed that clonal expansion in stem cells after 80 years matched

Propensity	Propensity equations
Upregulated propensity for nucleoid replication due to higher ratio of mutant mtDNA	$a_{R,del} = a_{R,0} \left(r_{max} \left(1 - \frac{R_W^m}{K_{retro}^m + R_W^m} \right) + 1 \right)$
Propensity for mitochondrial autophagy	$a_{D,mito} = k_D N_{Mito}$
Propensity for mitochondrial fission	$a_{fis,i} = V_{F,max} \frac{(W_i + M_i)^n}{K_F^n + (W_i + M_i)^n}$

Table 1.2: **Propensity Equations for Tam et al. (2013).** This table lists the equations used in Tam et al. (2013)[70] to update the number of mtDNA in the mitochondria where $r_{max} + 1$ – is the maximum copy number for amplification of mtDNA, M_{mito} – is the number of mitochondria in a cell at a given time k_D – the rate of autophagy, and $V_{F,max}$ – is the maximum propensity of fission.

known literature. The model provides a simpler example simulating similar results to Tam et al.

MODELING OTHER IMPLICATED MITOCHONDRIAL PATHWAYS

Future research into the effects of mitochondrial dysfunction could follow several paths including models of apoptosis and ATP productions. As discussed above, a newer model studying the effect of accumulations of deleterious mtDNA could be considered for both Mitochondrial Myopathy and DMD. As chronic inflammation has been implicated in several forms of MD, models that simulate apoptosis and necrosis could help explain how acute inflammation turns chronic. Understanding ATP production would also be useful since all muscle contractions require ATP.

Apoptosis models: Byproducts of mitochondrial metabolism include small amounts of electrons that leak from inner membrane complexes and attach themselves to oxygen, forming free radicals called reactive oxidative species (ROS). In a healthy immune response, free radicals such as superoxide and nitric oxide are produced by macrophages for destruction of foreign species. Small amounts of the free radical superoxide produced by the mitochondrion are neutralized by antioxidant enzymes such as superoxide dismutase. Mutated mtDNA leak more ROS in a degenerative mitochondrial cascade essentially poisoning vulnerable cells through ROS release [39, 40].

Intrinsic apoptosis results from a stressed cellular response. Severe ROS damage can result in cellular necrosis whereas the release of cytochrome c to the cytosol from the inner membrane of the mitochondria triggers intrinsic apoptosis. Depolarization in MOMP (mitochondrial outer membrane permeabilization) in stressed cells triggers the MOMP cascade. MOMP stress markers in survival-apoptotic dynamics feature members of the Bcl-2 protein family and BH-3 only proteins. [19, 59]. When triggered by MOMP, mitochondria release cytochrome c and Smac (second mitochondrial-derived activator of caspases) into the cell's cytosol. By binding with XIAPs (x-linked inhibitors of apoptosis), Smac allows for cytochrome c, Apaf-1, and ATP to combine into apoptosome and cleave procaspase-9 forming the initiator caspase-9; cleavage of procaspase-3 by caspase-9 then forms the executioner caspase-3 which in turn activates executioner caspase-6,7 and creates a positive feedback loop by cleaving more caspase-9. These executioners finalize the death of the cell [19, 31, 59]. Apoptosis will not occur if threshold levels of effector caspases are not reached [19, 31, 59].

Extrinsic apoptosis occurs when an extracellular self-destruct order is given. Stressed cells signal macrophages to engage with apoptotic cells through phagocytosis as a protective mechanism. Extracellular death ligands act as messengers of these orders by binding with FAS (CD95) death receptors. Subsequently, FAS receptors cluster allowing the binding with FADD (FAS-associated death domain). Recruited by FADD, multiple procaspase-8 compile

and mutually cleave forming caspase-8. This new protein cleaves pro-apoptotic, BH3-only protein Bid forming tBid (Truncated Bid). These interactions lead up to MOMP activation and subsequent cascade; the MOMP activation pathway bridges extrinsic and intrinsic apoptosis. Multidomain proteins are activated by apoptotic tBid activation, which can be inhibited due to protective Bcl-2 proteins [19, 59].

Fussenegger et al. (2000) [19] proposed a model simulating apoptosis to study caspase activation and inhibition. The model confirms experimental observations that Bcl-2 above a critical level effectively inhibits procaspase-9 activation but fails to adequately inhibit procaspase-8 activation, and suppression of FADD's binding to FAS/FASL complex blocks caspase-8 activation but has little effect on caspase-9 activation. The model assumes isotropic reactions with a well mixed single domain and omits proteins including Bid/tBid, reactions like caspase-8 cleaving of Bid, and bundles executioner caspases 3,6,7 into a single variable. Furthermore, intrinsic and extrinsic apoptosis were not distinguished. Since Fussenegger et al., several models have been proffered to redress omissions.

$$\begin{aligned}
\frac{d[RL]_T}{dt} &= b_L - \mu[RL]_T & \frac{d[C_{8a}]}{dt} &= 2r_{8zal} - \mu[C_{8a}], \\
\frac{d[R]_T}{dt} &= b_R - \mu[R]_T, & \frac{d[C_{9a}]}{dt} &= 2r_{9zal} - \mu[C_{9a}], \\
\frac{d[F]}{dt} &= b_F - 2r_F - \mu[F], & \frac{d[C_{Ez}]}{dt} &= b_{Ez} - \sum_{w=8}^9 r_{wEa} - \mu[C_{Ez}], \\
\frac{d[RL.F_2]_T}{dt} &= r_F - \mu[RL.F_2]_T, & \frac{d[C_{Ea}]}{dt} &= \sum_{w=8}^9 r_{wEa} - \mu[C_{Ea}] - r_{IAP}, \\
\frac{d[C_c]}{dt} &= r_c - r_{A1} - \mu[C_c], & \frac{d[B_2]}{dt} &= b_{B_2} - \mu[B_2], \\
\frac{d[Al]_T}{dt} &= b_{A1} - \mu[A1]_T, & \frac{d[B_x]}{dt} &= b_{B_x} - \mu[B_x], \\
\frac{d[A1.C_c]}{dt} &= r_{A1} - \mu[A1.C_c], & \frac{d[I_8]}{dt} &= b_{I_8} - \mu[I_8], \\
\frac{d[C_{8z}]}{dt} &= b_8 - 2r_{8zal} - \mu[C_{8z}], & \frac{d[I_9]}{dt} &= b_{I_9} - \mu[I_9], \\
\frac{d[C_{9z}]}{dt} &= b_9 - 2r_{9zal} - \mu[C_{9z}].
\end{aligned}$$

Variable	Description	Variable	Description
$[RL]$	Concentration of FAS-FAS ligand complex.	$[C_{8a}]$	Concentration of active caspase-8.
$[R]$	Concentration of FAS receptor.	$[C_{9a}]$	Concentration of active caspase-9.
$[F]$	Concentration of FADD protein.	$[C_{Ez}]$	Concentration of executioner pro-caspase.
$[RL.F_2]$	Concentration of FAS-FASL-FADD complex.	$[C_{Ea}]$	Concentration of active executioner caspase.
$[C_c]$	Concentration of cytosolic cytochrome c.	$[B_2]$	Concentration of Bcl-2.
$[A1]$	Concentration of Apaf-1 protein.	$[B_2]$	Concentration of Bcl- X_L .
$[A1.C_c]$	Concentration of Apaf-1-cytochrome c complex.	$[I_8]$	Concentration of FLIPs.
$[C_{8z}]$	Concentration of procaspase-8.	$[I_9]$	Concentration of ARC.
$[C_{9z}]$	Concentration of procaspase-9.		

Albeck et al. (2008) [1, 2] introduced a model concentrating on the extrinsic apoptosis death switch as well as MOMP interactions. The model represents both cytosol and mitochondria as two separate domains interacting after MOMP with parameters trained by live-cell imaging of HeLa cells. Similar to Fussenegger et al., Albeck et al. bundles many proteins with similar properties – such as caspase-8 and -10 are represented as a single variable C8 – to simplify the model and all reactions are isotropic. However, unlike the previous model, Albeck et al. incorporates a time delay mechanism to compensate for the delay of death ligand reception to MOMP as oppose to the quick death of the cell post-MOMP. Intriguingly, western blot fails to show enough XIAP pre-MOMP to properly inhibit caspase-3. Albeck et al. concluded that another protein/reaction must exist to account for this discrepancy. The model did confirm that MOMP occurs after proapoptotic Bcl-2 proteins reach

a certain level depended on the physiological state of the cell. An alternate stable state – partial cell death – is predicted by the model.

To eliminate the isotropic assumption, Huber et al. (2010) [31] combined previous models with one-dimensional diffusion PDEs. The typical mass reactions and kinetics are extended by a PDE where $v_n(x, t)$ is the chemical reactions. Although their goal was to investigate anisotropic reactions in MOMP, the reaction-diffusion equations remain applicable to wider studies

$$\frac{\partial C_n(x, t)}{\partial t} = \frac{\partial^2 C_n(x, t)}{\partial x^2} + v_n(x, t).$$

Variable	Description
$C_n(x, t)$	is concentration of a given protein (n=1,2,..,23).
$v_n(x, t)$	is chemical reactions given by usual Mass/Kinetic action.

Metabolism model: Smith et al. (2013) [66] created a directed graph model for ATP production. The model describes the interactions of fifty-nine genes, signals, and transcription factors in 4 domains. Indication of activation or inhibition is included in each interaction although no time scale is given. This network lays the groundwork for future modeling using ODEs and PDEs.

IMMUNOLOGICAL MODELS

Immunology describes the coordination of the immune system to redress threats of the pathogenic, viral, and parasitic kind in addition to the filtration and disposal of excessively damaged cells. Unfortunately, while lifesaving and essential for protection, immune system responses can include self-induced attacks in areas of the body that become so damaged they leave immune cells unable to recognize their healthy peers [15, 67].

The cells in the immune system are split into two categories – innate and adaptive. Adaptive immune cells have the ability to learn and recognize markers (called antigens) possessed by infections previously encountered by the immune system. Depending on the type of adaptive immune cell when an adaptive cell recognizes an antigen, the cell can attack the cell that presents the antigen (*CD8+* cytotoxic T cell), release antibodies (B cells), or regulate/signal other cells to respond (*CD4+* Helper T cells). Innate immune cells like macrophages, neutrophils, basophils, and eosinophils are unable to learn from previous infections. They do, however, recognize foreign/damaged cells and react by eating (phagocytosis) or signaling the cell to self-destruct (apoptosis). Antigens encountered by innate immune cells are presented to adaptive immune cells to check if the antigen has been confronted before by the immune system. Innate immune cells, also, possess many signals to activate other immune cells and signal for inflammation of local tissue. More information on the immune system can be found in a review article by Iwasaki et al. (2015) [34] or Immunology textbooks like Essential Immunology (2017) [16].

The innate immune system has been implicated in perpetuating multiple forms of MD [20, 81]. Vulnerabilities from missing proteins that stabilize the DCG promote a switch from acute inflammatory response to chronic response; the results of extended inflammation are adipose tissue replacement and muscle fibrosis [15, 43]. This has been confirmed with microscopic analysis of damaged muscle cells lacking dystrophin. Cellular analysis further indicates abnormal, elevated, levels of macrophages, helper and cytotoxic T cells in damaged tissues [67].

PUBLISHED IMMUNOLOGICAL MODELS ABOUT MD

Cyclic immunological activation purported by mechanical stress is associated with coexisting restorative and degenerative processes in muscle cells; immunological players act both as starters and finishers of apoptosis. *CD8+* (cytotoxic T-cells) initiate apoptosis in compromised cells, and the cellular remains of apoptosis are eaten by macrophages. Two models,

Dell'Acqua and Castiglione (2009) [15] and Jarrah et al. (2014) [35], have been proposed using a predator-prey system to study chronic immune activation by employing a log-normal distribution given by

$$\alpha(t) = \frac{h}{t\sigma\sqrt{2\pi}} e^{-\frac{(\ln(t)-m)^2}{2\sigma^2}} \quad (1.1)$$

to analyze initial (acute) muscle damage in time t . Both models utilize concentrations of the previously listed immune response helpers in addition to concentrations of healthy, damaged, and regenerating muscle cells.

Dell'Acqua and Castiglione (2009) [15] is generated by five ODEs in addition to a conservation law (Equations 1.2) describing the immune response of DMD in the mice model. They used COPASI's [28] optimization methods on experimental *mdx* mice data from Spencer et al. (2001)[67] and Wehling et al. (2001) [81] to find the best fit parameters. The set of equations used is

$$\begin{aligned} \frac{dM}{dt} &= b_m + k_1MD - d_M M, \\ \frac{dH}{dt} &= b_H + k_2MD - d_H H, \\ \frac{dC}{dt} &= k_2HD - d_C C, \\ \frac{dN}{dt} &= k_4R - k_5CN - \alpha(t)N, \\ \frac{dD}{dt} &= k_5CN - k_6MD - d_D D + \alpha(t)N, \\ 100 &= N + D + R. \end{aligned} \quad (1.2)$$

Variable	Description	Variable	Description
$\alpha(t)$	Given by equation 1.1.	N	Percentage of normal muscle fibers.
M	Concentration of macrophages.	D	Percentage of damaged muscle fibers.
H	Concentration of CD4+.	R	Percentage of regenerating muscle fibers.
C	Concentration of CD8+.		

Jarrah et al. (2014) [35] (Equations 1.3) refines Dell’Acqua-Castiglione’s model by adding an additional ODE which allows the conservation law to be implicit. The parameters of this model were derived from recent experimental data of *mdx* mice. Both models assume that the missing dystrophin in the muscle causes damaged muscle cells to initiate the immune response which contributes to their own damage until eventual apoptosis. The set of equations used is

$$\begin{aligned}
\frac{dM}{dt} &= b_m + k_1MD - d_M M, \\
\frac{dH}{dt} &= b_H + k_2MD - d_H H, \\
\frac{dC}{dt} &= b_C + k_2HD - d_C C, \\
\frac{dN}{dt} &= k_4R - k_5CN - \alpha(t)N, \\
\frac{dD}{dt} &= k_5CN - k_6MD - d_D D + \alpha(t)N, \\
\frac{dR}{dt} &= k_6MD + d_D D - k_4R.
\end{aligned} \tag{1.3}$$

The definition of variables is the same as Equation 1.2. Initial conditions have levels of cytotoxic T cell levels at 0; this changes when the impulse damage represented by equation 1.1 sets the system into motion. When $h = 0$, the impulse damage is negated and the system remains in a stable state; allowing $h > 0$, the model ensures that T helper cells draw cytotoxic T cells to the damaged region. Damage caused by the immune system reaches a

peak in weeks four through eight until the presence of the players wanes. By week twelve, the decreased presence of macrophages, CD4+ and CD8+ T cells (week fourteen) results in diminished levels of degeneration and restoration.

Both models display regions of bistability. Depending on the initial damaged caused and M_0 , the system collapses to healthy muscle stability or approaches a stability with heterogeneous mixtures of healthy and damaged muscle. This suggests that immune response to muscle damage could be a major contributor to DMD's pathophysiology [15, 35] which has been shown experimentally [67, 81].

Both Dell'Acqua-Castiglione and Jarrah et al. models are a broad overview of the processes involved and leave out several proteins, signal pathways, and reactions. Cytotoxic T cells act as the only non-impulse perpetrator of muscle damage in both models yet fail to elucidate on the mechanics on which cytotoxic T cells cause the damage. Extrinsic apoptosis caused by cytotoxic T cells has been proposed as a possible path [68].

These models indicate that the strength of the immune response and maintenance of the positive feedback system relies upon moving past these threshold points to enter another stability state. Driving the system into these recovery regions could prove to be a potential therapeutic target for redressing the role of the inflammatory response.

MODELING OTHER IMPLICATED IMMUNOLOGICAL PATHWAYS

The above models incorporate only cellular interactions in an chronic immune response; any multi-scale model would need to include molecular signals that are used in an acute and chronic immune responses to muscle damage. Veltman et al. (2017) and López-Caamal et al. (2012) [46] introduced models describing molecular signals.

Inflammasome Model: Inflammasome has been implicated in initiating inflammation after an injury or infection occurs by stimulating caspase-1 and activating IL-1 β . Both LGMD2B and DMD have shown upregulation of inflammasome [58].

A recently published model, Veltman et al. (2017) [78], describes inflammasome interactions by using an ODE model to study molecular signaling input to IL-1 β activation output. The model also includes inhibitors of inflammasome – IL-10 (signaled by IFN β) and IFN γ . The model illustrates that negative feedback by IFN γ has less effect on IL-1 β than the inhibition by IL-10/IFN β . This result matches experimental research. The model does not account for anisotropic dispersion nor cellular interactions.

AKT/mTOR Model: Another important avenue for research would be propagation of immune cell signals. López-Caamal et al. (2012) [46] created a model describing AKT/mTOR pathway activation by IGF-1. IGF-1 is an important protein released by M2 macrophages during muscle repair that promotes muscle growth, and AKT/mTOR has been implicated as the main signal pathway to promoting muscle growth. The model simulates a positive feedback and diffusion of AKT/mTOR signals once activated. The model also is used to speculate on consequences of signals from other sources like AMPK or pharmaceuticals.

AREAS OF FUTURE QUANTITATIVE RESEARCH

Characterizing pathology and pathogenesis of MD requires further study; future models could accelerate therapeutic discovery by testing potential pathways *in silico* as well as detecting new therapeutic pathways. There are areas for computational research that have not yet been explored mathematically. These pathways may be worthwhile exploring to better understand MD disease development and providing treatments that may delay onset or progression.

The creation of rAAV/AAV1 (Recombinant Adeno-associated virus, Adeno-associated virus 1) delivery system and CRISPR/Cas system indicates the possibility of a genetic cure for MD [45, 55]. Heller et al. (2015) [23] overexpressed the human $\alpha 7$ integrin Gene, ITGA7, using the AAV1 delivery system in *mdx* mice. ITGA7 is a skeletal muscle laminin receptor (Figure 1.2) whose overexpression does not cause an immune response in *mdx* mice. Protective benefits of the DGC were restored with ITGA7 overexpression; lifespans were also prolonged. Xu et al. (2015) [82] used CRISPR/Cas9 to remove mutated exon 23 with the

dystrophin genomic region to restore dystrophin expression in the DGC of *mdx* mice. Clinical trials are currently taking place for LGMD2D [48]. Mendell et al. (2012) [47] wrote a review outlining future work in gene therapy. Both systems require a relatively small number of injection sites. Population dispersion models and GRNs could help in the development and effective administration of both rAAV/AAV1 and CRISPR/Cas systems. Population dispersion and diffusion models could be used to predict the outcome from a series of injections to the spread of the corrected genes throughout the body. Potentially, these models could indicate the most effective injection sites. Beyond the correction of defective genes, both systems could target genes that promote muscle growth and regeneration [60]. GRNs may help in developing new therapies by finding pathways that both system could target and thereby accelerate the body's muscle regeneration. These types of treatments could be applicable to both MD patients and in sports medicine.

Myostatin – a TGF- β protein – has long been recognized as a possible therapeutic option for MD. Early murine testing for several MD phenotypes produced mixed results. For DMD (*mdx* mice) and LGMD2F (*scgd*^{-/-} mouse), Parsons et al. (2006) [51] concluded positive results for mice treated early in development before widespread necrosis occurred. Treating *sgcg*^{-/-} mice (modeling LGMD2C) resulted in positive muscle physiology including increase fiber size, muscle mass, and grip force in addition to reduce frequency of apoptosis; however, muscle histology remained unfazed signifying lack of pathology change [7]. A possible solution proposed by Rodino-Klapac (2009) used AAV1 to genetically edit Follistatin (the major myostatin inhibitor). A few experiments show that the new treatment has few side effects and shows similar improvements as other myostatin inhibitors in LGMD2A (Calpainopathy). Future research is needed to show if myostatin inhibition is a means to maintain pathophysiology in MD patients [60]. GRNs and physiological muscle models could be used to understand effective usage of myostatin. GRNs could discover new inhibitors and enzyme activators of myostatin; this may allow targeted genetic editing to regulate myostatin similar to Rodino-Klapac (2009) [60]. GRNs might also explain why these methods will work

with some types of MD but fail with other types. Physiological models may demonstrate the increase in fiber size, muscle mass, and grip force due to myostatin therapies.

A plethora of models could be used to describe cellular, molecular, and pharmaceutical interactions of the immune system. Precise molecular, mathematical models bridging arginine metabolism with oscillations in macrophage phenotypic expression could be used to model nitric oxide mediated cytotoxicity as well as fibrosis during satellite cell proliferation. Mechanical models for macrophage infiltration and molecular models for macrophage phenotype oscillation could also be useful to better characterize chronic immune system activation due to structural defects. Integration into musculoskeletal simulation may be useful to model the immunological role in MD pathogenesis and progression. Agent based models could be used to imitate immune cells.

MD disease progression also results in alterations to pathophysiology such as gait and muscle atrophy. Noninvasive studies with patients, especially children, could be critical to create a staged model for gait devolution and morphology. Quantifying degrees of eccentric contraction using musculoskeletal simulation could possibly explain selective degeneration in DMD [30].

DISCUSSION

With new developments in computational power and data availability, a growing amount of research is using a systems biology approach to understand pathogenesis and progression of disease. Effective and integrated *in vitro* and *in silico* models could inform biological phenomena, even without the need of a living subject. For instance, over the last few decades, collagen hydrogel with muscle derived cells (CHMDCs) have promised to revolutionize *in vitro* experiments and tissue engineering. For CHMDCs to reach the envisioned use, verification by use of mathematical simulations are needed. Recently while examining shape and design, Hodgson (2015) [25] used a combination finite elements and agent based analysis to illustrate the lines of principle strain and cell migration in CHMDCs confirming earlier *in*

vitro work by Smith et al. (2012) [65]. As MD is a rare disorder, the use of mathematical models could help elucidate the underlining mechanisms of the disease that might not be easily detectable given the limited subject pool.

Although genetic studies have implicated genes as the cause of many types of MD, relatively little is known about common pathways between these genes that may affect pathogenesis and create similar phenotypes; this necessitates the use of mathematical models describing GRNs. Common genes like *Dik1*, *Dusp13*, and *Casq2* [74] and their downstream pathways provide future prospects for therapeutic intervention.

Mitochondrial and immunological mechanisms further MD progression. Mathematical models of apoptosis and mitochondrial fission/fusion help to understand intracellular processes that directly affect cellular vitality and death. Tam et al. [70]’s mitochondrial fission/fusion model related mitochondrial health with nuclear mechanisms to rescue mutated mtDNA; unhealthy mitochondria can trigger intrinsic apoptosis with the release of cytochrome c. Fusseneger et al. [19] and Albeck et al. [1, 2] furthered the study of apoptotic mechanics by creating models to simulate the processes; both models agree with already published results. Extrinsic apoptosis can be signaled by immune cells. Immunological mathematical models display a larger view of cellular interactions that bridge the gap of molecular actions and tissue level muscle damage. Damage caused by stress and weakened cellular structure is repaired and debris removed by extrinsic apoptosis and phagocytosis.

Mathematical models of muscles under contractile stress are essential to understanding the long term development of most types of MD. Partial differential equations and agent based models of anisotropic strain from contraction display where cellular rupture and immune response will likely occur. Physical therapeutic and pharmaceutical interventions can be targeted to such areas of high stress to stymie MD progression. Expanding cellular models of the immune response and combining with molecular signals could create a more comprehensive view of muscle tissue regeneration and damage caused by chronic inflamma-

tion. Future research could incorporate multiple levels of models into a unified simulation to give a whole view of the progression of MD.

Future research that sheds light on MD disease dynamics, which is likely to occur through mathematical modeling, will provide the means to engage and perhaps ultimately bypass biological systems coordinating together to exacerbate degeneration.

CHAPTER 2

THE FRIND MODEL

2.1 INTRODUCTION TO MUSCLE REPAIR BY THE IMMUNE SYSTEM

Muscle degeneration in MD can be caused by replacement of healthy muscle fiber by fibrous connective [88] and adipose tissue [96]; tissue replacement could be caused by chronic inflammation and immune activation. This occurs since weaknesses in muscle cell structure caused by MD affect genes increase frequency and induce stronger activation of the immune system during muscle repair [97, 98, 99].

Macrophages, a type of innate immune cell, perform a significant role in tissue repair [85]. Macrophages phagocytose damaged muscle fibers [85] and promote the proliferation, differentiation, and binding of myoblasts into nascent myofibers which form new muscle tissue [95]. Macrophages accomplish these tasks by switching phenotypes (macrophage plasticity) throughout the repair process. While in an inflammatory phenotype, macrophages migrate to damaged tissue, perform phagocytosis, and promote myoblast proliferation. Macrophages follow this stage with anti-inflammatory phenotypes which phagocytose, halt inflammation, differentiate myoblasts, lay fibrous connective tissue which gives structure to muscle, and signal myotube migration/binding.

Although many mathematical models have been created to study the immune defense, few have been devised to quantify tissue regeneration by the immune system and chronic inflammation [91]. Dell’Acqua-Castiglione (2009) [87] and Jarrad et al. (2014) [92] both attempt to explain muscle degeneration and regeneration by the interactions of macrophages, T helper cells, and cytotoxic T cells.

Both previous models fail to elaborate on macrophage plasticity and the relation between acute and chronic inflammation. The previous models treated all macrophages as a single group that acts on all other cells the same. However, Arnold et al. (2007) [85] showed that macrophages have several phenotypes that interact with other cells differently. The previous models also investigated the long term effects a single acute, damage event. The weakening of skeletal muscle tissue in MD, though, occurs after continuous damage from everyday activity which causes chronic inflammation [100].

The purpose of this chapter is to present a mathematical model that simulates macrophage plasticity and its effects on muscle regeneration. A correct characterization of this dynamics could allow a greater understanding of the causes of chronic inflammation, and muscle degeneration in MD patients.

2.2 THE FRIND MODEL

2.2.1 CONCEPT OF THE FRIND MODEL

To understand the interactions needed for muscle repair, we could create a model that incorporates 3 groups of cells: muscle tissue cells (in percentage of tissue), myocytes (in number of cells per mm^3), and macrophages (in number of cells per mm^3). The interactions of these cells in a mathematical model should ideally stay in equilibrium until any damage to muscle occurs.

Following damage to normal muscle tissue (N), damage tissue (D) increases. Macrophages phagocytose this tissue and leave the area ready for regeneration (R). Transforming Growth Factor Beta, $TGF-\beta$, which is released by M_2 macrophages promotes the formation of fibrous tissue (F) [85, 93]. This tissue is the foundation upon which myotubes bind to form new healthy muscle tissue (N) [95]. In the model, the F , R , N , and D are given as percents of overall tissue as this is assumed to be conserved.

A subpopulation of satellite cells called myocytes form the core of new muscles. Pro-inflammatory signals from M_1 macrophages activate $MyoD$ in resident satellite cells

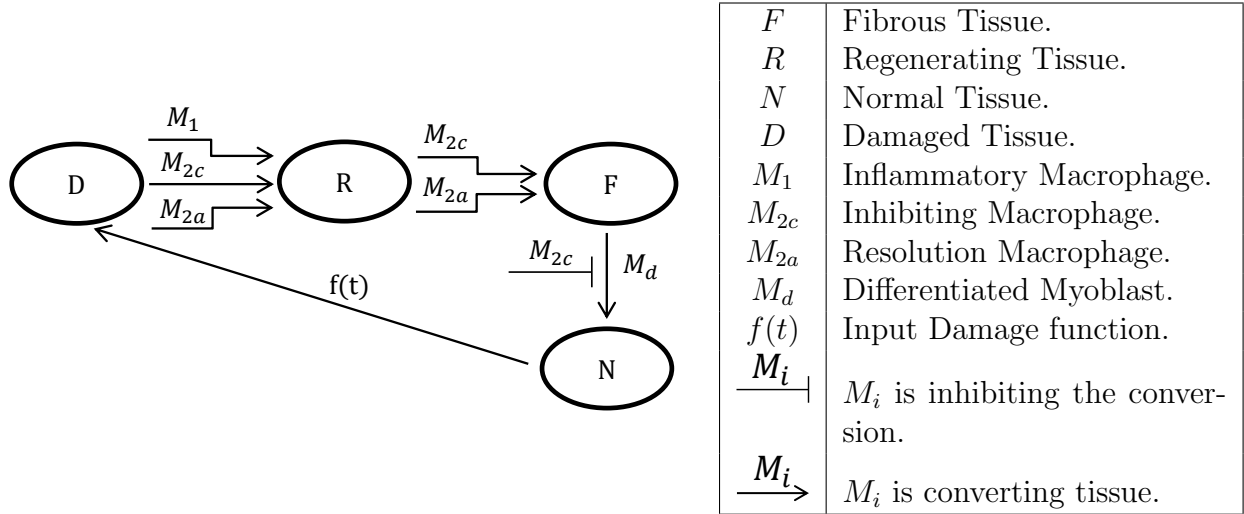


Figure 2.1: **Muscle Tissue Repair.** This figure shows the process of muscle repair. Phagocytosis of damaged tissue (D) by macrophages gives regenerating tissue (R). Fibrous connective tissue (F) is laid by some types of macrophages over the regenerating tissue. Fibrous tissue binds with differentiate myoblasts to form normal muscle tissue (N).

becoming myoblasts allowing for proliferation. The model simplifies this by combining the resident satellite cells and myoblasts into M_b . When exposed to anti-inflammatory signals by M_{2a} , myoblasts lose the expression of Pax and differentiate. These differentiated myoblasts later form into myotubes. The model combines the differentiated myoblasts and myotubes as a single variable, M_d . M_1 macrophage are known to signal M_d myoblasts to prematurely apoptose [95].

For purposes of this model, macrophage populations have been split into four phenotypes derived from *in vitro* studies: M_1 , M_{2c} , M_{2a} , and M_3 . Although *in vivo* studies have shown few macrophages display the exact qualities of these phenotypes, they can be shown to have certain genes – corresponding to those *in vitro* phenotypes – upregulated [94].

Pro-inflammatory macrophage (M_1) population increases directly to the damage dealt by injury. M_1 macrophages release cytokines TNF_α and IL-1 which further allows M_1 infiltration and recruitment of phenotypeless macrophages (M_3). These cytokines also promote pro-

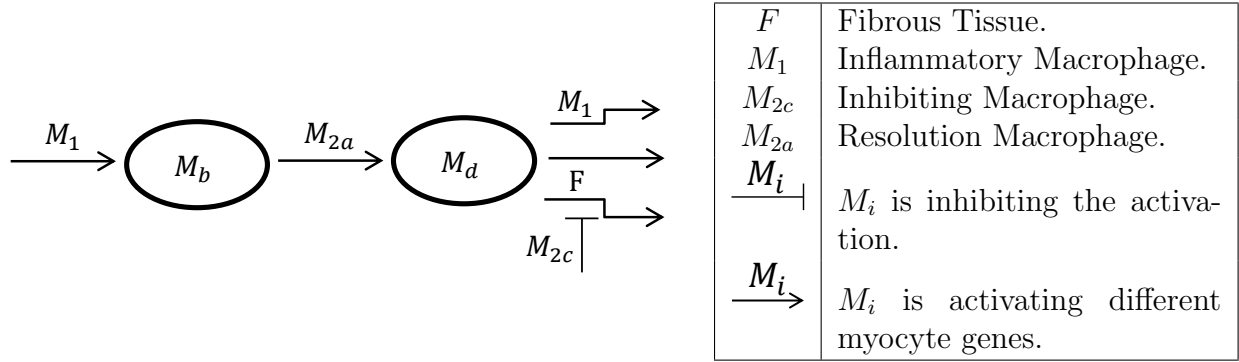


Figure 2.2: **Formation of Nascent Myofibers.** This figure shows the creation of nascent myofibers. Pro-inflammatory macrophages promote activation and proliferation of myoblasts (M_b). Exposure to resolution macrophages promotes differentiation of myoblasts (M_d) which eventually bind together into myofibers.

inflammatory genes in resident macrophages forcing them into a M_3 phenotypeless state. TGF- β is released when macrophages phagocytose damaged tissue and apoptotic immune cells; this promotes a shift in macrophages to an anti-inflammatory phenotype [85].

Anti-inflammatory macrophages (M_2) fall into two phenotypes: the deactivating macrophages (M_{2c}) and resolving macrophages (M_{2a}). Increased exposure to TGF- β and certain glucocorticoids promote M_{2c} phenotype genes in M_1 macrophages. IL-10 which is released by M_{2c} macrophages inhibits the effects of IL-1 and suppresses M_1 macrophage's inflammatory abilities effectively becoming phenotypeless. Further exposure by M_{2c} to TGF- β promotes a shift to the M_{2a} phenotype. M_{2a} macrophages release cytokines IL-4 and IL-13 which accelerates M_{2c} phenotype change and promote myoblasts differentiation. [101].

Phenotypeless macrophages (M_3) are used in this model to represent any macrophage which has a phenotype not sufficiently polarized or is suppressed by another macrophage. The M_3 macrophages will act only as a feedback loop for M_1 and M_{2a} .

Damage to muscle tissue is controlled by a time-dependent function $f(t)$ which can be designed to simulate different types of muscle injury. For acute injury, $f(t)$ could be set as a Gaussian normal or a lognormal function (see equation 1.1) as described by Jarrad et al.

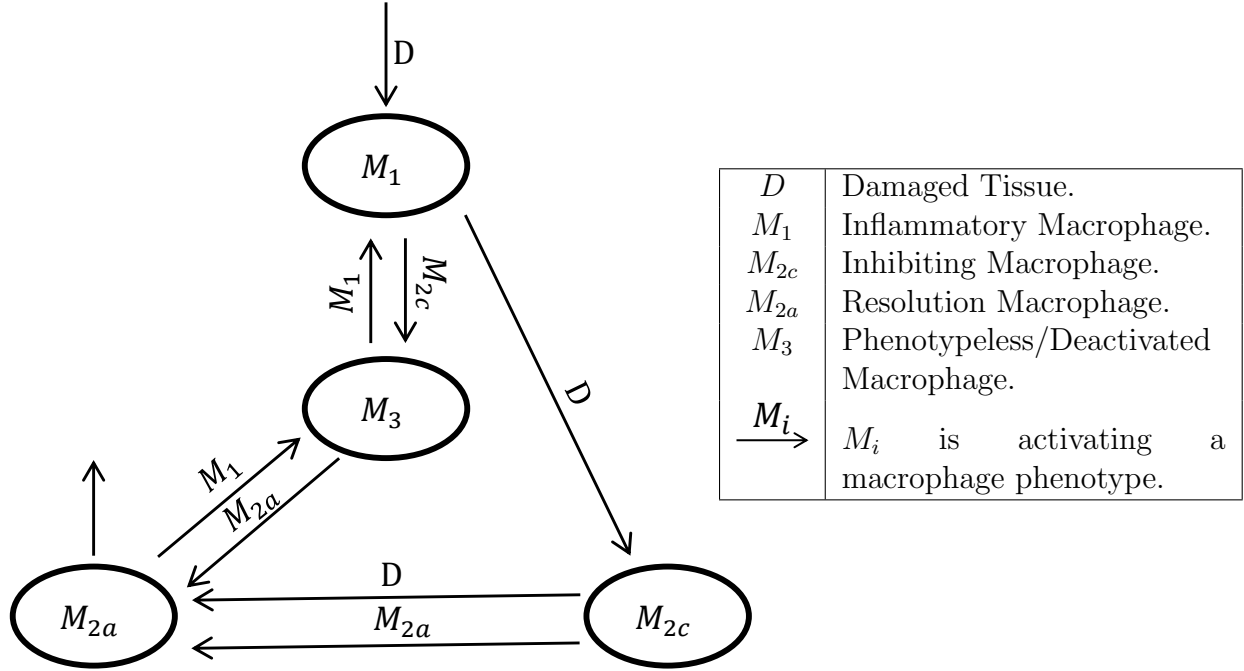


Figure 2.3: **Macrophage Interactions.** This figure describes the interaction of different type of macrophages during muscle repair. Inflammatory signals from resident macrophages are released after tissue is damaged prompting invasion by pro-inflammatory macrophages (M_1). Phagocytosis of damaged tissue by pro-inflammatory macrophages promotes inhibiting macrophage phenotype (M_{2c}) which starts deactivating (M_3) pro-inflammatory macrophages. Resolution macrophages (M_{2a}) appear after further phagocytosis of damaged tissue by inhibiting macrophages. Conflicting signals by pro- and anti-inflammatory macrophages force some macrophages into a phenotypeless state (M_3).

(2014) [92]. For smaller but periodic injury, $f(t)$ could be set with a random amplitude and period $\sin^2$ function.

We will call this model the FRiND (Fibrous, Regenerating, inflammation, Normal, Damaged) model.

2.2.2 EQUATIONS

The FRiND model uses the law of generalized mass action (GMA) to convert the diagrams in Section 2.2.1 into ordinary differential equations (ODEs). GMA states that the rate of

reaction/interaction is directly and/or indirectly proportional to the product of the numbers (or masses) of objections. For each interaction, a kinetic parameter is incorporated and is estimated using data. In the FRiND model, activation and deactivation will be modeled using direct proportions; inhibition of an interaction will be modeled using an indirect proportion. An extra term $-g(t)M_1$ has been added for predictions of Diphtheria toxin in Section 4.3; we assume $g(t) = 0$ in every other section.

FRiND Model

$$\dot{F} = d_1 M_{2c} R + d_2 M_{2a} R - \frac{d_6 M_d F}{M_{2c} - M_{2c}(0) + 1}, \quad (2.1)$$

$$\dot{R} = d_3 M_1 D - d_1 M_{2c} R + d_4 M_{2a} D + d_5 M_{2c} D - d_2 M_{2a} R, \quad (2.2)$$

$$\dot{N} = \frac{d_6 M_d F}{M_{2c} - M_{2c}(0) + 1} - f(t)N, \quad (2.3)$$

$$\dot{D} = f(t)N - d_3 M_1 D - d_4 M_{2a} D - d_5 M_{2c} D, \quad (2.4)$$

$$\dot{M}_1 = k_1 D^2 - k_2 M_1 D - k_3 M_1 M_{2c} + k_4 M_1 M_3 - g(t)M_1, \quad (2.5)$$

$$\dot{M}_{2c} = k_2 M_1 D - k_5 (M_{2c} - M_{2c}(0)) D - k_8 (M_{2c} - M_{2c}(0)) M_{2a}, \quad (2.6)$$

$$\begin{aligned} \dot{M}_{2a} = & k_5 (M_{2c} - M_{2c}(0)) D + k_6 M_{2a} M_3 - k_7 M_1 M_{2a}, \\ & + k_8 (M_{2c} - M_{2c}(0)) M_{2a} - k_9 (M_{2a} - M_{2a}(0)), \end{aligned} \quad (2.7)$$

$$\dot{M}_3 = k_3 M_1 M_{2c} + k_7 M_1 M_{2a} - k_4 M_1 M_3 - k_6 M_{2a} M_3, \quad (2.8)$$

$$\dot{M}_b = a_1 M_1 M_b - a_2 (M_b - M_b(0)) M_{2a}, \quad (2.9)$$

$$\dot{M}_d = a_2 (M_b - M_b(0)) M_{2a} - a_4 M_d - a_5 M_1 M_d - \frac{a_3 M_d F}{M_{2c} - M_{2c}(0) + 1}. \quad (2.10)$$

<i>Time</i>	<i>F</i>	<i>R</i>	<i>N</i>	<i>D</i>	<i>M</i> ₁	<i>M</i> _{2c}	<i>M</i> _{2a}	<i>M</i> ₃	<i>M</i> _b	<i>M</i> _d
2	0	45	0	55	5,000	3,700	1300	2,500	1,000	0
4	70	25	0	1	1,000	500	4,500	1,500	1,412	1,427
7	5	2	95	0	250	200	1,800	375	500	100
14	0	0	100	0	0	200	200	0	100	0
24	0	0	100	0	0	200	200	0	100	0

Table 2.1: **Data Used to Estimate Parameters.**

This table gives the data used to estimate the parameters for the FRiND model. The data was gleamed from Mounier et al. (2013)[93] and Ogawa et al. (2015)[95].

Variable	Description	Variable	Description
<i>F</i>	Fibrous Tissue.	<i>M</i> ₁	Macrophage 1 (Inflammatory).
<i>R</i>	Regenerating Tissue.	<i>M</i> _{2c}	Macrophage 2c (Deactivating).
<i>N</i>	Normal Tissue.	<i>M</i> _{2a}	Macrophage 2a (Resolving).
<i>D</i>	Damaged Tissue.	<i>M</i> ₃	No Phenotype Macrophage.
<i>M</i> _b	Myoblasts.	<i>M</i> _d	Differentiated Myotubes.

2.2.3 ESTIMATING THE MODEL PARAMETERS

The FRiND Model has 19 parameters plus any parameters needed for the initial damage function.

Data for estimating the parameters comes from Mounier et al. (2013)[93] and Ogawa et al. (2015)[95] (see table 2.1); both experiments used cardiotoxin (CTX) as a source of damage to mouse muscle. The damage by CTX is modeled using a lognormal distribution:

$$f(t) = \frac{h}{\sigma\sqrt{2\pi}(t+1)} \exp\left(-\frac{1}{2\sigma^2}(\ln(t+1) - m)^2\right).$$

All data points were taken from the control mice data in both papers.

<i>Parameter Name</i>	<i>EstimatedParameter</i>	<i>Units</i>	<i>Parameter Name</i>	<i>EstimatedParameter</i>	<i>Units</i>
a_1	0.0003420387898	$\frac{mm^3}{cells \cdot day}$	k_1	0.9167670584	$\frac{mm^3}{cells \cdot day}$
a_2	0.00027805269	$\frac{mm^3}{cells \cdot day}$	k_2	0.007512675612	$\frac{mm^3}{cells \cdot day}$
a_3	5.926671787	$\frac{1}{day}$	k_3	0.01113163887	$\frac{mm^3}{cells \cdot day}$
a_4	0.1685466672	$\frac{1}{day}$	k_4	0.01332585023	$\frac{mm^3}{cells \cdot day}$
a_5	0.0004735032862	$\frac{mm^3}{cells \cdot day}$	k_5	0.002183270219	$\frac{mm^3}{cells \cdot day}$
d_1	0.0001	$\frac{mm^3}{cells \cdot day}$	k_6	0.001135187023	$\frac{mm^3}{cells \cdot day}$
d_2	0.000199043595	$\frac{mm^3}{cells \cdot day}$	k_7	0.0005064492007	$\frac{mm^3}{cells \cdot day}$
d_3	0.00001041101141	$\frac{mm^3}{cells \cdot day}$	k_8	0.0002272581636	$\frac{mm^3}{cells \cdot day}$
d_4	0.0005912309411	$\frac{mm^3}{cells \cdot day}$	k_9	0.6179192106	$\frac{1}{day}$
d_5	0.000103662336	$\frac{mm^3}{cells \cdot day}$	h	9.466706714	$\frac{1}{day}$
d_6	0.310343	$\frac{1}{day}$	m	0.2	$\ln(days)$
σ	0.01				

Table 2.2: **Estimated Parameters for the FRiND Model.**

This table shows the estimated parameters for the FRiND model using an acute lognormal damage with data from 2.1.

A standard genetic algorithm [86] and pattern search [89] were used to train parameters to the data on both COPASI [90] and MATLAB (The MathWorks, Inc., Natick, MA, USA).

2.3 DISCUSSION

Mitigating the effects of MD begins with understanding MD's pathology and pathogenesis. Since all forms of MD are rare, future therapies could be discovered and tested by mathematically modeling.

Previous models of MD pathology have relied on simple dynamics of macrophage interaction by assuming all macrophages were pro-inflammatory (M_1). Macrophage's ability to switch phenotype provides the body a flexible repair system with cells that both can clear debris and repair the tissue. Without this interaction previous models depended on linear mass action dynamics to simulate the evolution of tissue from damaged to normal; this simplification disallows the possibility of studying acceleration and deceleration of tissue repair depending on therapy treatments targeting macrophage numbers and types.

The FRiND model uses a more modern approach by incorporating macrophage plasticity. M_1 macrophages infiltrate the damaged tissue prompted by dentritic and resident macrophages signals. M_1 macrophages phagocytose damaged tissue converting it to a regenerating state. Phagocytosis of damaged tissue activates genes promoting a M_{2c} phenotype; these macrophages start suppressing M_1 macrophage cytokines. Further phagocytosis by macrophages promote a shift to M_{2a} macrophages. Both M_{2c} and M_{2a} macrophages lay fibrous tissue where damaged tissue was. This allows for binding of nascent myotubes into normal tissue which were proliferated by M_1 macrophages and differentiated by M_{2a} macrophages.

Incorporating macrophage plasticity allows for the model to accelerate and decelerate tissue repair depending on both the number and type of macrophages. In Section 4.3, we will show that is ability can predict the outcome of an experiment from Arnold et al. (2007) [85].

The use of a damage function $f(t)$, also, allows a broadening of the modeling to the effects of acute and chronic inflammation. Using different input damage functions gives the opportunity to discuss the possibility that mechanical damage from compromised cell

structure – caused by MD affected genes – gives rise to chronic inflammation and fibrous tissue replacement. This will be shown using the parameters from Table 2.2 in Chapter 4.

A mathematical analysis of the FRiND model with unknown parameters must occur before a proper discussion of results using a specific parameter set. In Chapter 3, we will discuss steady states, stability, and identifiability of the parameter sets.

CHAPTER 3

MATHEMATICAL ANALYSIS OF THE FRiND MODEL

Mathematical models like the FRiND model represent a real phenomenon with mathematical symbols and rules. For a model to aspire to confidently make predictions, though, the model has to be mathematical sound. We will begin by studying the potential biological significant steady states of the FRiND model; this is followed by stability of each steady state. A discussion on identifiability of the FRiND model finishes the chapter.

3.1 STEADY STATES OF THE FRiND MODEL

A steady state is a substrate vector where the system will remain constant over time. This occurs when the substrate vector $\tilde{x}$ (called a fixed point) gives $\frac{dx}{dt} = 0$ for all x . For a large dynamical system, finding a fixed point analytically can be difficult [104].

In the FRiND model, several simplifying assumptions can be made. **(1)** Since the change in substrate amount is given in the model, parameters may be assumed to be always positive and nonzero. Furthermore, **(2)** steady state and initial conditions of the substrate must be nonnegative to make biological sense. **(3)** We will, though, insist that $M_{2a}(0) > 0$ and $M_b(0) > 0$ (the initial conditions of M_{2a} macrophages and M_b myocytes) as this will allow resident macrophages to signal inflammation when damage occurs and satellite cells to proliferate. Finally, **(4)** we want to see how the system responds after contractual damage has dissipated or any diphtheria toxins have left; this would require $\lim_{t \rightarrow \infty} f(t) = 0$ and $\lim_{t \rightarrow \infty} g(t) = 0$.

Although the initial conditions for substrates depends on the situation being modeled, the muscle cells has a built in conservation law $\frac{dF}{dt} + \frac{dR}{dt} + \frac{dN}{dt} + \frac{dD}{dt} = 0$. Since muscle cells are in percentage of muscle tissue, we will have that $F(t) + R(t) + N(t) + D(t) = 100$.

Lemma 3.1.1. *Assume the FRiND model follows the above assumptions, then $\tilde{D} = 0$, $\tilde{R} = 0$, $\tilde{M}_{2c} = M_{2c}(0)$, and $\tilde{M}_{2a} = M_{2a}(0)$.*

Proof. By the definition of steady states, we have

$$0 = d_1 \tilde{M}_{2c} \tilde{R} + d_2 \tilde{M}_{2a} \tilde{R} - \frac{d_6 \tilde{M}_d \tilde{F}}{\tilde{M}_{2c} - M_{2c}(0) + 1}, \quad (3.1)$$

$$0 = d_3 \tilde{M}_1 \tilde{D} - d_1 \tilde{M}_{2c} \tilde{R} + d_4 \tilde{M}_{2a} \tilde{D} + d_5 \tilde{M}_{2c} \tilde{D} - d_2 \tilde{M}_{2a} \tilde{R}, \quad (3.2)$$

$$0 = \frac{d_6 \tilde{M}_d \tilde{F}}{\tilde{M}_{2c} - M_{2c}(0) + 1} - f(t) \tilde{N}, \quad (3.3)$$

$$0 = f(t) \tilde{N} - d_3 \tilde{M}_1 \tilde{D} - d_4 \tilde{M}_{2a} \tilde{D} - d_5 \tilde{M}_{2c} \tilde{D}, \quad (3.4)$$

$$0 = k_1 \tilde{D}^2 - k_2 \tilde{M}_1 \tilde{D} - k_3 \tilde{M}_1 \tilde{M}_{2c} + k_4 \tilde{M}_1 \tilde{M}_3 - g(t) \tilde{M}_1, \quad (3.5)$$

$$0 = k_2 \tilde{M}_1 \tilde{D} - k_5 \left(\tilde{M}_{2c} - M_{2c}(0) \right) \tilde{D} - k_8 \left(\tilde{M}_{2c} - M_{2c}(0) \right) \tilde{M}_{2a}, \quad (3.6)$$

$$0 = k_5 \left(\tilde{M}_{2c} - M_{2c}(0) \right) \tilde{D} + k_6 \tilde{M}_{2a} \tilde{M}_3 - k_7 \tilde{M}_1 \tilde{M}_{2a} + k_8 \left(\tilde{M}_{2c} - M_{2c}(0) \right) \tilde{M}_{2a} - k_9 \left(\tilde{M}_{2a} - M_{2a}(0) \right), \quad (3.7)$$

$$0 = k_3 \tilde{M}_1 \tilde{M}_{2c} + k_7 \tilde{M}_1 \tilde{M}_{2a} - k_4 \tilde{M}_1 \tilde{M}_3 - k_6 \tilde{M}_{2a} \tilde{M}_3, \quad (3.8)$$

$$0 = a_1 \tilde{M}_1 \tilde{M}_b - a_2 \left(\tilde{M}_b - M_b(0) \right) \tilde{M}_{2a}, \quad (3.9)$$

$$0 = a_2 \left(\tilde{M}_b - M_b(0) \right) \tilde{M}_{2a} - a_4 \tilde{M}_d - a_5 \tilde{M}_1 \tilde{M}_d - \frac{a_3 \tilde{M}_d \tilde{F}}{\tilde{M}_{2c} - M_{2c}(0) + 1}. \quad (3.10)$$

Notice that the sum of equations 3.5 through 3.8 and $\lim_{t \rightarrow 0} g(t) = 0$ gives us

$$k_1 \tilde{D}^2 - k_9 \left(\tilde{M}_{2a} - M_{2a}(0) \right) = 0.$$

Thus,

$$k_9 \left(\tilde{M}_{2a} - M_{2a}(0) \right) = k_1 \tilde{D}^2 \geq 0.$$

Therefore, $\tilde{M}_{2a} \geq M_{2a}(0) > 0$.

Since $\lim_{t \rightarrow \infty} f(t) = 0$, then from equation 3.4 we have

$$0 = -d_3 \tilde{M}_1 \tilde{D} - d_4 \tilde{M}_{2a} \tilde{D} - d_5 \tilde{M}_{2c} \tilde{D} = \left(-d_3 \underbrace{\tilde{M}_1}_{\geq 0} - d_4 \underbrace{\tilde{M}_{2a}}_{> 0} - d_5 \underbrace{\tilde{M}_{2c}}_{\geq 0} \right) \tilde{D}.$$

Thus $\tilde{D} = 0$ by assumption **(2)**. Using this fact and the same argument as above in equation 3.2, we have that $\tilde{R} = 0$. Combining equation 3.6 and $\tilde{D} = 0$ gives us

$$0 = k_2 \tilde{M}_1 \cdot 0 - k_5 \left(\tilde{M}_{2c} - M_{2c}(0) \right) \cdot 0 - k_8 \left(\tilde{M}_{2c} - M_{2c}(0) \right) \underbrace{\tilde{M}_{2a}}_{>0}.$$

Hence $\tilde{M}_{2c} = M_{2c}(0)$. Furthermore, if we add equations 3.5 through 3.8 we have

$$k_1 D^2 - k_9 \left(\tilde{M}_{2a} - M_{2a}(0) \right) = 0.$$

This yields $\tilde{M}_{2a} = M_{2a}(0)$. □

Notice that with these four steady state values fixed, we reach a choice when considering equation 3.3.

Theorem 3.1.2. *Assume the FRiND model follows the above assumptions, then the model will have the two possible steady states.*

Proof. From equation 3.3 and $\tilde{M}_{2c} = M_{2c}(0)$, we have that either $\tilde{F} = 0$ or $\tilde{M}_d = 0$.

Let $\tilde{F} = 0$, then $\tilde{N} = 100$ since $F(t) + R(t) + N(t) + D(t) = 100$. From equation 3.5 and $\tilde{D} = 0$, we have

$$0 = k_1 \tilde{D}^2 - k_2 \tilde{M}_1 \tilde{D} - k_3 \tilde{M}_1 \tilde{M}_{2c} + k_4 \tilde{M}_1 \tilde{M}_3 = \left(-k_3 \tilde{M}_{2c} + k_4 \tilde{M}_3 \right) \tilde{M}_1.$$

This gives us two possible cases **Case 1:** $\tilde{M}_1 = 0$ and **Case 2:** $-k_3 \tilde{M}_{2c} + k_4 \tilde{M}_3 = 0$.

Case 1: Let $\tilde{M}_1 = 0$, then equation 3.8 gives us $\tilde{M}_3 = 0$. From equation 3.9, we have

$$0 = a_1 \tilde{M}_b \cdot 0 - a_2 \left(\tilde{M}_b - M_b(0) \right) \underbrace{\tilde{M}_{2a}}_{>0}.$$

Hence $\tilde{M}_b = M_b(0)$. From equation 3.10, we have

$$\begin{aligned} 0 &= a_2 (M_b(0) - M_b(0)) \tilde{M}_{2a} - a_4 \tilde{M}_d - a_5 \tilde{M}_1 \tilde{M}_d - \frac{a_3 \tilde{M}_d \cdot 0}{\tilde{M}_{2c} - M_{2c}(0) + 1} \\ &= \left(-a_4 \tilde{M}_d - a_5 \tilde{M}_1 \right) \tilde{M}_d \end{aligned}$$

We can conclude that $\tilde{M}_d = 0$. Therefore, we have that our fixed point as

$$\tilde{x}_1 = \left[0, 0, 100, 0, 0, M_{2a}(0), M_{2c}(0), 0, M_b(0), 0\right].$$

Case 2: Let $-k_3\tilde{M}_{2c} + k_4\tilde{M}_3 = 0$, then equation 3.8 and $\tilde{M}_{2c} = M_{2c}(0)$ gives us

$$M_3 = \frac{k_3 M_{2c}(0)}{k_4}. \quad (3.11)$$

We also have from equation 3.8 that $k_7\tilde{M}_1\tilde{M}_{2a} - k_6\tilde{M}_{2a}\tilde{M}_3 = 0$. Thus, using $\tilde{M}_{2a} = M_{2a}(0)$ and 3.11, we have

$$\tilde{M}_1 = \frac{k_6\tilde{M}_{2a}\tilde{M}_3}{k_7\tilde{M}_{2a}} = \frac{k_6 \overbrace{M_{2a}(0)}^{>0} \tilde{M}_3}{k_7 \underbrace{M_{2a}(0)}_{>0}} = \frac{k_3 k_6 M_{2c}(0)}{k_4 k_7}.$$

Turning to equation 3.9, we have that

$$\tilde{M}_b \left(a_2 \tilde{M}_{2a} - a_1 \tilde{M}_1 \right) = a_2 M_b(0) \tilde{M}_{2a}.$$

Combining with our early results we have

$$\begin{aligned} \tilde{M}_b &= \frac{a_2 M_b(0) \tilde{M}_{2a}}{a_2 \tilde{M}_{2a} - a_1 \tilde{M}_1} \\ &= \frac{a_2 k_4 k_7 M_b(0) M_{2a}(0)}{a_2 k_4 k_7 M_{2a}(0) - a_1 k_3 k_6 M_{2c}(0)} \\ &= M_b(0) + \frac{a_1 k_3 k_6 M_{2c}(0) M_b(0)}{a_2 k_4 k_7 M_{2a}(0) - a_1 k_3 k_6 M_{2c}(0)}. \end{aligned}$$

Combining this result with equation 3.10 we have

$$\begin{aligned} 0 &= a_2 \left(M_b(0) + \frac{a_1 k_3 k_6 M_{2c}(0) M_b(0)}{a_2 k_4 k_7 M_{2a}(0) - a_1 k_3 k_6 M_{2c}(0)} - M_b(0) \right) \tilde{M}_{2a} \\ &\quad - a_4 \tilde{M}_d - a_5 \tilde{M}_1 \tilde{M}_d - \frac{a_3 \tilde{M}_d \cdot 0}{\tilde{M}_{2c} - M_{2c}(0) + 1}. \end{aligned}$$

Notice that by assumption **(3)**, we have that $M_{2a}(0) > 0$. Thus $\tilde{M}_b$ will always exist. Solving for $\tilde{M}_d$ and simplifying, we have

$$\left(a_4 + a_5 \tilde{M}_1 \right) \tilde{M}_d = \frac{a_1 a_2 k_3 k_6 M_{2c}(0) M_{2a}(0) M_b(0)}{a_2 k_4 k_7 M_{2a}(0) - a_1 k_3 k_6 M_{2c}(0)}.$$

Hence:

$$\begin{aligned}\tilde{M}_d &= \frac{a_1 a_2 k_3 k_6 M_{2c}(0) M_{2a}(0) M_b(0)}{a_2 k_4 k_7 M_{2a}(0) - a_1 k_3 k_6 M_{2c}(0)} \cdot \frac{1}{a_4 + a_5 \tilde{M}_1} \\ &= \frac{a_1 a_2 k_3 k_4 k_6 k_7 M_{2c}(0) M_{2a}(0) M_b(0)}{\left(a_2 k_4 k_7 M_{2a}(0) - a_1 k_3 k_6 M_{2c}(0)\right) \left(a_4 k_4 k_7 + a_5 k_3 k_6 M_{2c}(0)\right)}.\end{aligned}$$

Therefore, we have that our fixed point as

$$\tilde{x}_2 = \begin{bmatrix} 0, \\ 0, \\ 100, \\ 0, \\ \frac{k_3 k_6 M_{2c}(0)}{k_4 k_7}, \\ M_{2a}(0), \\ M_{2c}(0), \\ \frac{k_3 M_{2c}(0)}{k_4}, \\ \frac{a_2 k_4 k_7 M_b(0) M_{2a}(0)}{a_2 k_4 k_7 M_{2a}(0) - a_1 k_3 k_6 M_{2c}(0)}, \\ \frac{a_1 a_2 k_3 k_4 k_6 k_7 M_{2c}(0) M_{2a}(0) M_b(0)}{\left(a_2 k_4 k_7 M_{2a}(0) - a_1 k_3 k_6 M_{2c}(0)\right) \left(a_4 k_4 k_7 + a_5 k_3 k_6 M_{2c}(0)\right)} \end{bmatrix}.$$

Let $\tilde{M}_d = 0$, then equation 3.10 yields $\tilde{M}_b = M_b(0)$. Using this fact in equation 3.9, we have that $\tilde{M}_1 = 0$. From equation 3.8, reduces to $\tilde{M}_3 = 0$. However, $\tilde{F}$ and $\tilde{N}$ are free variables. Thus we can say that $\tilde{N} = 100 - \tilde{F}$. Therefore, we have that our fixed point as

$$\tilde{x}_1 = \left[\tilde{F}, 0, 100 - \tilde{F}, 0, 0, M_{2c}(0), M_{2a}(0), 0, M_b(0), 0 \right].$$

Notice that the first fixed point is a special case of this one.

Therefore, the FRiND model has only two potential steady states. □

A few observations of the steady states should be made. The first steady state will always exist as each component of the fixed point are positive, and the second steady state is equal to the first when $M_{2c}(0) = 0$. In the second steady state, $\tilde{M}_1 = \frac{k_3 k_6 M_{2c}(0)}{k_4 k_7}$ and $\tilde{M}_3 = \frac{k_3 M_{2c}(0)}{k_4}$ are always nonnegative. However, $\tilde{M}_b$ and $\tilde{M}_d$ could be negative which violates assumption (2). In fact, this occurs when using the parameters found in Table 2.2. The FRiND model may have either one or two steady states.

Theorem 3.1.3. *If $a_1 k_3 k_6 M_{2c}(0) < a_2 k_4 k_7 M_{2a}(0)$, then $\tilde{M}_b$ and $\tilde{M}_d$ are nonnegative and the second steady state exists.*

Proof. Let $a_1 k_3 k_6 M_{2c}(0) < a_2 k_4 k_7 M_{2a}(0)$. Notice that $a_2 k_4 k_7 M_b(0) M_{2a}(0)$ is nonnegative. Thus $\tilde{M}_b$ will be nonnegative if the denominator is positive. Since $a_1 k_3 k_6 M_{2c}(0) < a_2 k_4 k_7 M_{2a}(0)$, then we know that $a_2 k_4 k_7 M_{2a}(0) - a_1 k_3 k_6 M_{2c}(0) > 0$. Therefore, $\tilde{M}_b$ exists and is nonnegative. Furthermore, we have that

$$\tilde{M}_d = \frac{\overbrace{a_1 a_2 k_3 k_4 k_6 k_7 M_{2c}(0) M_{2a}(0) M_b(0)}^{\geq 0}}{\left(\underbrace{a_2 k_4 k_7 M_{2a}(0) - a_1 k_3 k_6 M_{2c}(0)}_{>0} \right) \left(\underbrace{a_4 k_4 k_7 + a_5 k_3 k_6 M_{2c}(0)}_{>0} \right)}.$$

Hence, $\tilde{M}_d$ exists and is nonnegative.

Therefore, all components of the second fixed point exist and are nonnegative. The FRiND model will have a second steady state. $\square$

The free variable $\tilde{F}$ shows that $M_d(t)$ dictates whether fibrous tissue will remain after regeneration is completed. If too much F still exists when differentiated myoblasts' numbers dwindle, fibrous tissue replacement of normal tissue will occur in the muscle tissue. This fibrous tissue replacement caused by mechanical effects could be seen as approximating muscular dystrophy pathology.

3.2 STABILITY ANALYSIS OF THE STEADY STATES

A steady state can be called stable, unstable, or saddle depending on the system's reaction to small perturbations to the fixed point. The system will return to a steady state after

perturbations when a fixed point is stable whereas the system will repel if the fixed point is unstable. With a saddle fixed point, the system will repel from the point unless on specific vectors [104].

The sufficient conditions for stability is given by eigenvalues of the Jacobian evaluated at the fixed points. A stable steady state will have only negative eigenvalues. An unstable state will have only positive eigenvalues, and a saddle steady state will have a mix of positive and negative eigenvalues [104].

The Jacobian of the FRiND model is found by forming a matrix of partial derivatives of each ODE in terms of each cell type. Thus the Jacobian of the FRiND model is

$$J = [J_1 \ J_2 \ J_3 \ J_4 \ J_5 \ J_6 \ J_7 \ J_8 \ J_9 \ J_{10}],$$

where

$$J_1 = \begin{bmatrix} -\frac{d_6 M_d}{M_{2c} - M_{2c}(0) + 1} \\ 0 \\ \frac{d_6 M_d}{M_{2c} - M_{2c}(0) + 1} \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -\frac{a_3 M_d}{M_{2c} - M_{2c}(0) + 1} \end{bmatrix}, \quad J_2 = \begin{bmatrix} d_1 M_{2c} + d_2 M_{2a} \\ -d_1 M_{2c} - d_2 M_{2a} \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix},$$

$$\begin{aligned}
J_3 &= \begin{bmatrix} 0 \\ 0 \\ -f(t) \\ f(t) \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \\
J_4 &= \begin{bmatrix} 0 \\ d_3 M_1 + d_4 M_{2a} + d_5 M_{2c} \\ 0 \\ -d_3 M_1 - d_4 M_{2a} - d_5 M_{2c} \\ 2k_1 D - k_2 M_1 \\ k_2 M_1 - k_5 (M_{2c} - M_{2c}(0)) \\ k_5 (M_{2c} - M_{2c}(0)) \\ 0 \\ 0 \\ 0 \end{bmatrix}, \\
J_5 &= \begin{bmatrix} 0 \\ d_3 D \\ 0 \\ -d_3 D \\ -k_2 D - k_3 M_{2c} + k_4 M_3 \\ k_2 D \\ -k_7 M_{2a} \\ k_3 M_{2c} + k_7 M_{2a} - k_4 M_3 \\ a_1 M_b \\ -a_5 M_d \end{bmatrix}, \\
J_6 &= \begin{bmatrix} d_1 R + \frac{d_6 M_d F}{(M_{2c} - M_{2c}(0) + 1)^2} \\ d_5 D - d_1 R \\ -\frac{d_6 M_d F}{(M_{2c} - M_{2c}(0) + 1)^2} \\ -d_5 D \\ -k_3 M_1 \\ -k_5 D - k_8 M_{2a} \\ k_5 D + k_8 M_{2a} \\ k_3 M_1 \\ 0 \\ -\frac{a_3 M_d F}{(M_{2c} - M_{2c}(0) + 1)^2} \end{bmatrix},
\end{aligned}$$

$$\begin{aligned}
J_7 &= \begin{bmatrix} d_2 R \\ d_4 D - d_2 R \\ 0 \\ -d_4 D \\ 0 \\ -k_8 (M_{2c} - M_{2c}(0)) \\ k_6 M_3 - k_7 M_1 + k_8 (M_{2c} - M_{2c}(0)) - k_9 \\ k_7 M_1 - k_6 M_3 \\ -a_2 (M_b - M_b(0)) \\ a_2 (M_b - M_b(0)) \end{bmatrix}, & J_8 &= \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ k_4 M_1 \\ 0 \\ k_6 M_{2a} \\ -k_4 M_1 - k_6 M_{2a} \\ 0 \\ 0 \end{bmatrix}, \\
J_9 &= \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ a_1 M_1 \\ a_2 M_{2a} \end{bmatrix}, & J_{10} &= \begin{bmatrix} -\frac{d_6 F}{M_{2c} - M_{2c}(0) + 1} \\ 0 \\ \frac{d_6 F}{M_{2c} - M_{2c}(0) + 1} \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -\frac{a_3 F}{M_{2c} - M_{2c}(0) + 1} - a_4 - a_5 M_1 \end{bmatrix}.
\end{aligned}$$

The Jacobian evaluated at $\tilde{x}_1$ is

$$J(\tilde{x}_1) = [S_1 \ S_2 \ S_3 \ S_4 \ S_5 \ S_6 \ S_7 \ S_8 \ S_9 \ S_{10}], \quad (3.12)$$

where

$$\begin{aligned}
S_1 &= \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \\
S_2 &= \begin{bmatrix} M_{2a}(0)d_2 + M_{2c}(0)d_1 \\ -M_{2a}(0)d_2 - M_{2c}(0)d_1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \\
S_3 &= \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \\
S_4 &= \begin{bmatrix} 0 \\ M_{2a}(0)d_4 + M_{2c}(0)d_5 \\ 0 \\ -M_{2a}(0)d_4 - M_{2c}(0)d_5 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix},
\end{aligned}$$

$$S_5 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ -M_{2c}(0)k_3 \\ 0 \\ -k_7M_{2a}(0) \\ k_7M_{2a}(0) + M_{2c}(0)k_3 \\ a_1M_b(0) \\ 0 \end{bmatrix},$$

$$S_7 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -k_9 \\ 0 \\ -a_2(M_b(0) - 100) \\ a_2(M_b(0) - 100) \end{bmatrix},$$

$$S_6 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -M_{2a}(0)k_8 \\ M_{2a}(0)k_8 \\ 0 \\ 0 \\ 0 \end{bmatrix},$$

$$S_8 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ M_{2a}(0)k_6 \\ -M_{2a}(0)k_6 \\ 0 \\ 0 \end{bmatrix},$$

$$S_9 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ a_2 M_{2a}(0) \end{bmatrix}, \quad S_{10} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -a_4 - a_5 \end{bmatrix}.$$

The eigenvalues of equation 3.12 are

$$\lambda_1 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ -k_9 \\ -M_{2a}(0)k_8 \\ -k_6 M_{2a}(0) \\ -k_3 M_{2c}(0) \\ -d_4 M_{2a}(0) - d_5 M_{2c}(0) \\ -d_2 M_{2a}(0) - d_1 M_{2c}(0) \\ -a_4 - a_5 \end{bmatrix}.$$

Since all parameters and initial conditions are non-negative, then all the eigenvalues are non-positive. Thus $\tilde{x}_1$ is a stable steady state.

The Jacobian evaluated at $\tilde{x}_2$ is

$$J(\tilde{x}_2) = [S_1 \ S_2 \ S_3 \ S_4 \ S_5 \ S_6 \ S_7 \ S_8 \ S_9 \ S_{10}], \quad (3.13)$$

where

$$S_1 = \begin{bmatrix} -\frac{d_6 M_{2a}(0) M_b(0) M_{2c}(0) a_1 a_2 k_3 k_4 k_6 k_7}{M_{2a}(0) M_{2c}(0) a_2 a_5 k_3 k_4 k_6 k_7 - M_{2c}(0)^2 a_1 a_5 k_3^2 k_6^2 + M_{2a}(0) a_2 a_4 k_4^2 k_7^2 - M_{2c}(0) a_1 a_4 k_3 k_4 k_6 k_7} \\ 0 \\ \frac{d_6 M_{2a}(0) M_b(0) M_{2c}(0) a_1 a_2 k_3 k_4 k_6 k_7}{M_{2a}(0) M_{2c}(0) a_2 a_5 k_3 k_4 k_6 k_7 - M_{2c}(0)^2 a_1 a_5 k_3^2 k_6^2 + M_{2a}(0) a_2 a_4 k_4^2 k_7^2 - M_{2c}(0) a_1 a_4 k_3 k_4 k_6 k_7} \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -\frac{a_3 M_{2a}(0) M_b(0) M_{2c}(0) a_1 a_2 k_3 k_4 k_6 k_7}{M_{2a}(0) M_{2c}(0) a_2 a_5 k_3 k_4 k_6 k_7 - M_{2c}(0)^2 a_1 a_5 k_3^2 k_6^2 + M_{2a}(0) a_2 a_4 k_4^2 k_7^2 - M_{2c}(0) a_1 a_4 k_3 k_4 k_6 k_7} \end{bmatrix},$$

$$S_2 = \begin{bmatrix} M_{2a}(0) d_2 + M_{2c}(0) d_1 \\ -M_{2a}(0) d_2 - M_{2c}(0) d_1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, S_3 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, S_4 = \begin{bmatrix} 0 \\ \frac{M_{2c}(0) k_3 k_6 d_3}{k_4 k_7} + M_{2a}(0) d_4 + M_{2c}(0) d_5 \\ 0 \\ -\frac{M_{2c}(0) k_3 k_6 d_3}{k_4 k_7} - M_{2a}(0) d_4 - M_{2c}(0) d_5 \\ -\frac{M_{2c}(0) k_3 k_6 k_2}{k_4 k_7} \\ \frac{M_{2c}(0) k_3 k_6 k_2}{k_4 k_7} \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix},$$

$$S_5 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -k_7 M_{2a}(0) \\ k_7 M_{2a}(0) \\ \frac{a_1 k_7 k_4 a_2 M_b(0) M_{2a}(0)}{M_{2a}(0) a_2 k_4 k_7 - M_{2c}(0) a_1 k_3 k_6} \\ -\frac{a_5 M_{2a}(0) M_b(0) M_{2c}(0) a_1 a_2 k_3 k_4 k_6 k_7}{M_{2a}(0) M_{2c}(0) a_2 a_5 k_3 k_4 k_6 k_7 - M_{2c}(0)^2 a_1 a_5 k_3^2 k_6^2 + M_{2a}(0) a_2 a_4 k_4^2 k_7^2 - M_{2c}(0) a_1 a_4 k_3 k_4 k_6 k_7} \end{bmatrix},$$

$$S_6 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ -\frac{k_3^2 M_{2c}(0) k_6}{k_4 k_7} \\ -M_{2a}(0) k_8 \\ M_{2a}(0) k_8 \\ \frac{k_3^2 M_{2c}(0) k_6}{k_4 k_7} \\ 0 \\ 0 \end{bmatrix},$$

$$S_7 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -k_9 \\ 0 \\ -a_2 \left(\frac{k_7 k_4 a_2 M_b(0) M_{2a}(0)}{M_{2a}(0) a_2 k_4 k_7 - M_{2c}(0) a_1 k_3 k_6} - M_b(0) \right) \\ a_2 \left(\frac{k_7 k_4 a_2 M_b(0) M_{2a}(0)}{M_{2a}(0) a_2 k_4 k_7 - M_{2c}(0) a_1 k_3 k_6} - M_b(0) \right) \end{bmatrix},$$

$$\begin{aligned}
S_8 &= \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ \frac{M_{2c}(0)k_3k_6}{k_7} \\ 0 \\ k_6M_{2a}(0) \\ -\frac{M_{2c}(0)k_3k_6}{k_7} - k_6M_{2a}(0) \\ 0 \\ 0 \end{bmatrix}, & S_9 &= \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ \frac{M_{2c}(0)a_1k_3k_6}{k_4k_7} \\ M_{2a}(0)a_2 \end{bmatrix}, \\
S_{10} &= \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -a_4 - a_5 - \frac{M_{2c}(0)k_3k_6}{k_4k_7} \end{bmatrix}.
\end{aligned}$$

The eigenvalues of equation 3.13 are

$$\lambda_2 = \begin{bmatrix} 0 \\ -k_9 \\ -M_{2a}(0)k_8 \\ -1/2 \frac{M_{2a}(0)k_6k_7 + M_{2c}(0)k_3k_6 - \sqrt{M_{2a}(0)^2k_6^2k_7^2 + 2M_{2a}(0)M_{2c}(0)k_3k_6^2k_7 + 4k_7^2M_{2a}(0)M_{2c}(0)k_3k_6 + M_{2c}(0)^2k_3^2k_6^2}}{k_7} \\ -1/2 \frac{M_{2a}(0)k_6k_7 + M_{2c}(0)k_3k_6 + \sqrt{M_{2a}(0)^2k_6^2k_7^2 + 2M_{2a}(0)M_{2c}(0)k_3k_6^2k_7 + 4k_7^2M_{2a}(0)M_{2c}(0)k_3k_6 + M_{2c}(0)^2k_3^2k_6^2}}{k_7} \\ - \frac{M_{2a}(0)d_4k_4k_7 + M_{2c}(0)d_3k_3k_6 + M_{2c}(0)d_5k_4k_7}{k_4k_7} \\ -M_{2a}(0)d_2 - M_{2c}(0)d_1 \\ - \frac{M_{2c}(0)k_3k_6 + a_4k_4k_7 + a_5k_4k_7}{k_4k_7} \\ \frac{M_{2c}(0)a_1k_3k_6}{k_4k_7} \\ - \frac{M_{2a}(0)M_b(0)M_{2c}(0)a_1a_2d_6k_3k_4k_6k_7}{M_{2a}(0)M_{2c}(0)a_2a_5k_3k_4k_6k_7 - M_{2c}(0)^2a_1a_5k_3^2k_6^2 + M_{2a}(0)a_2a_4k_4^2k_7^2 - M_{2c}(0)a_1a_4k_3k_4k_6k_7} \end{bmatrix}.$$

Notice that there exists at least one positive eigenvalue and one negative eigenvalue. Thus when the second steady state $\tilde{x}_2$ exists, it is a saddle node.

3.3 IDENTIFIABILITY OF THE FRiND MODEL.

The question could arise at this point whether the FRiND model's parameters can be estimated by any appropriate dataset. In mathematics, this concept is called identifiability. Models which are unidentifiable can be reduced by eliminating parameters; this helps to estimate parameters faster and creates a uniqueness to parameter estimation.

Let

$$\dot{x} = FRiND(x(t, p), t, u, p).$$

Variable	Description
x	is a vector of the substrates of the FRiND model.
t	is time.
u	is the control functions (ie the damage function or diphtheria control function).
p	is any parameter set (a subset of the parameter space).

A model is said to be globally identifiable if and only if $x(t, p^*) = x(t, p)$ implies that $p^* = p$. A model is said to be locally identifiable if and only if $x(t, p^*) = x(t, p)$ inside a small neighborhood around p^* implies that $p^* = p$. Otherwise, a model is said to be unidentifiable when $x(t, p^*) = x(t, p)$ for infinite choices of p [102, 103].

A useful test for identifiability is the Fisher Information Matrix (FIM). If the determinant of the FIM is zero (analytically calculated) or close to zero (numerically calculated), then the model is unidentifiable. We can calculate the FIM by first computing the sensitivity matrix (SM)

$$SM = \begin{bmatrix} \frac{\partial x_1}{\partial p_1}(t_1) & \frac{\partial x_1}{\partial p_2}(t_1) & \cdots & \frac{\partial x_1}{\partial p_n}(t_1) \\ \frac{\partial x_1}{\partial p_1}(t_2) & \frac{\partial x_1}{\partial p_2}(t_2) & \cdots & \frac{\partial x_1}{\partial p_n}(t_2) \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\partial x_1}{\partial p_1}(t_m) & \frac{\partial x_1}{\partial p_2}(t_m) & \cdots & \frac{\partial x_1}{\partial p_n}(t_m) \\ \frac{\partial x_2}{\partial p_1}(t_1) & \frac{\partial x_2}{\partial p_2}(t_1) & \cdots & \frac{\partial x_2}{\partial p_n}(t_1) \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\partial x_{10}}{\partial p_1}(t_m) & \frac{\partial x_{10}}{\partial p_2}(t_m) & \cdots & \frac{\partial x_{10}}{\partial p_n}(t_m) \end{bmatrix}.$$

FIM and SM are connected by [102, 103]

$$FIM = SM^T \cdot SM. \quad (3.14)$$

To compute SM, we need to estimate $\frac{\partial x_k}{\partial p_j}(t_i)$ for each i, j, k . For this, we can use a central-difference equation

$$\frac{\partial x_k}{\partial p_j}(t_i) \approx \frac{x_k(t_i, p + h \cdot e_j) - x_k(t_i, p - h \cdot e_j)}{2h}$$

where h is a small step and e_j is a unit vector in the direction of p_j in the parameter space.

We can now calculate SM and FIM. We start off by calculating SM (see supplementary file). After that we use equation 3.14 to calculate the FIM

$$FIM = \begin{bmatrix} 7.50E+14 & -2.09E+14 & -1.98E+09 & -2.80E+10 & -3.26E+13 & -2.67E+12 & -4.22E+12 & 2.01E+14 & -2.17E+12 & 5.05E+13 & \dots \\ -2.09E+14 & 9.15E+13 & 5.27E+08 & 6.26E+09 & 4.98E+12 & -2.67E+11 & 2.42E+11 & -1.80E+14 & -2.46E+13 & -9.88E+13 & \dots \\ -1.98E+09 & 5.27E+08 & 1.53E+04 & 1.84E+05 & 1.56E+08 & -4.23E+06 & 1.11E+07 & 3.01E+08 & 4.09E+08 & 9.06E+08 & \dots \\ -2.80E+10 & 6.26E+09 & 1.84E+05 & 2.37E+06 & 2.18E+09 & 1.06E+07 & 1.56E+08 & 5.88E+09 & 4.87E+09 & 1.14E+10 & \dots \\ -3.26E+13 & 4.98E+12 & 1.56E+08 & 2.18E+09 & 2.50E+12 & 1.92E+11 & 3.19E+11 & 9.60E+12 & 4.43E+12 & 1.11E+13 & \dots \\ -2.67E+12 & -2.67E+11 & -4.23E+06 & 1.06E+07 & 1.92E+11 & 8.96E+10 & 8.36E+10 & 1.55E+12 & -1.64E+10 & 2.19E+11 & \dots \\ -4.22E+12 & 2.42E+11 & 1.11E+07 & 1.56E+08 & 3.19E+11 & 8.36E+10 & 1.18E+11 & 1.56E+12 & 4.52E+11 & 1.10E+12 & \dots \\ 2.01E+14 & -1.80E+14 & 3.01E+08 & 5.88E+09 & 9.60E+12 & 1.55E+12 & 1.56E+12 & 7.89E+15 & 3.57E+15 & 9.44E+15 & \dots \\ -2.17E+12 & -2.46E+13 & 4.09E+08 & 4.87E+09 & 4.43E+12 & -1.64E+10 & 4.52E+11 & 3.57E+15 & 2.10E+15 & 5.28E+15 & \dots \\ 5.05E+13 & -9.88E+13 & 9.06E+08 & 1.14E+10 & 1.11E+13 & 2.19E+11 & 1.10E+12 & 9.44E+15 & 5.28E+15 & 1.39E+16 & \dots \\ 3.44E+10 & -1.10E+10 & -3.13E+05 & -3.68E+06 & -2.75E+09 & 2.22E+08 & -7.15E+07 & -3.03E+09 & -8.10E+09 & -1.76E+10 & \dots \\ 3.97E+10 & -7.96E+10 & 1.83E+06 & 1.91E+07 & 1.26E+10 & -1.84E+09 & 6.59E+08 & 1.61E+13 & 1.02E+13 & 2.53E+13 & \dots \\ -1.92E+13 & 3.20E+13 & -5.18E+08 & -5.63E+09 & -4.07E+12 & 4.11E+11 & -2.53E+11 & -4.90E+15 & -3.02E+15 & -7.60E+15 & \dots \\ -1.09E+12 & 1.82E+13 & -3.88E+08 & -4.30E+09 & -3.31E+12 & 2.39E+11 & -2.49E+11 & -3.34E+15 & -2.06E+15 & -5.15E+15 & \dots \\ 1.51E+12 & -1.48E+13 & 3.10E+08 & 3.44E+09 & 2.64E+12 & -1.92E+11 & 2.01E+11 & 2.68E+15 & 1.65E+15 & 4.11E+15 & \dots \\ -1.71E+13 & -1.69E+13 & 5.14E+08 & 5.85E+09 & 4.76E+12 & -2.25E+11 & 3.99E+11 & 4.09E+15 & 2.52E+15 & 6.32E+15 & \dots \\ -9.36E+14 & 1.49E+15 & -3.29E+09 & -3.43E+10 & -2.94E+13 & 9.06E+11 & -4.60E+12 & -4.78E+16 & -2.78E+16 & -8.95E+16 & \dots \\ 1.95E+15 & -2.80E+15 & 2.90E+09 & 2.60E+10 & 2.49E+13 & 4.61E+10 & 7.04E+12 & 7.11E+16 & 4.10E+16 & 1.33E+17 & \dots \\ 2.26E+17 & -3.14E+17 & -1.08E+11 & -1.62E+12 & -8.75E+14 & 2.06E+14 & 3.06E+14 & 4.72E+18 & 2.64E+18 & 8.81E+18 & \dots \\ 9.06E+10 & -1.32E+10 & -7.67E+05 & -8.67E+06 & -7.04E+09 & 3.38E+08 & -6.15E+08 & -4.25E+12 & -2.79E+12 & -6.88E+12 & \dots \\ -2.10E+07 & 1.39E+07 & 7.19E+01 & 6.16E+02 & 1.18E+05 & -1.64E+05 & -4.57E+04 & 2.10E+08 & 2.18E+08 & 5.09E+08 & \dots \end{bmatrix}$$

$$\begin{bmatrix} 3.44E+10 & 3.97E+10 & -1.92E+13 & -1.09E+12 & 1.51E+12 & -1.71E+13 & -9.36E+14 & 1.95E+15 & 2.26E+17 & 9.06E+10 & -2.10E+07 \\ -1.10E+10 & -7.96E+10 & 3.20E+13 & 1.82E+13 & -1.48E+13 & -1.69E+13 & 1.49E+15 & -2.80E+15 & -3.14E+17 & -1.32E+10 & 1.39E+07 \\ -3.13E+05 & 1.83E+06 & -5.18E+08 & -3.88E+08 & 3.10E+08 & 5.14E+08 & -3.29E+09 & 2.90E+09 & -1.08E+11 & -7.67E+05 & 7.19E+01 \\ -3.68E+06 & 1.91E+07 & -5.63E+09 & -4.30E+09 & 3.44E+09 & 5.85E+09 & -3.43E+10 & 2.60E+10 & -1.62E+12 & -8.67E+06 & 6.16E+02 \\ -2.75E+09 & 1.26E+10 & -4.07E+12 & -3.31E+12 & 2.64E+12 & 4.76E+12 & -2.94E+13 & 2.49E+13 & -8.75E+14 & -7.04E+09 & 1.18E+05 \\ 2.22E+08 & -1.84E+09 & 4.11E+11 & 2.39E+11 & -1.92E+11 & -2.25E+11 & 9.06E+11 & 4.61E+10 & 2.06E+14 & 3.38E+08 & -1.64E+05 \\ -7.15E+07 & 6.59E+08 & -2.53E+11 & -2.49E+11 & 2.01E+11 & 3.99E+11 & -4.60E+12 & 7.04E+12 & 3.06E+14 & -6.15E+08 & -4.57E+04 \\ -3.03E+09 & 1.61E+13 & -4.90E+15 & -3.34E+15 & 2.68E+15 & 4.09E+15 & -4.78E+16 & 7.11E+16 & 4.72E+18 & -4.25E+12 & 2.10E+08 \\ -8.10E+09 & 1.02E+13 & -3.02E+15 & -2.06E+15 & 1.65E+15 & 2.52E+15 & -2.78E+16 & 4.10E+16 & 2.64E+18 & -2.79E+12 & 2.18E+08 \\ -1.76E+10 & 2.53E+13 & -7.60E+15 & -5.15E+15 & 4.11E+15 & 6.32E+15 & -8.95E+16 & 1.33E+17 & 8.81E+18 & -6.88E+12 & 5.09E+08 \\ 6.70E+06 & -3.94E+07 & 1.10E+10 & 8.09E+09 & -6.48E+09 & -1.06E+10 & 6.49E+10 & -5.33E+10 & 2.78E+12 & 1.58E+07 & -1.72E+03 \\ -3.94E+07 & 5.06E+10 & -1.48E+13 & -1.01E+13 & 8.12E+12 & 1.24E+13 & -1.33E+14 & 1.96E+14 & 1.24E+16 & -1.39E+10 & 1.16E+06 \\ 1.10E+10 & -1.48E+13 & 4.38E+15 & 2.99E+15 & -2.39E+15 & -3.66E+15 & 4.16E+16 & -6.17E+16 & -4.06E+18 & 4.07E+12 & -3.30E+08 \\ 8.09E+09 & -1.01E+13 & 2.99E+15 & 2.04E+15 & -1.63E+15 & -2.49E+15 & 2.74E+16 & -4.05E+16 & -2.61E+18 & 2.78E+12 & -2.26E+08 \\ -6.48E+09 & 8.12E+12 & -2.39E+15 & -1.63E+15 & 1.31E+15 & 2.00E+15 & -2.17E+16 & 3.21E+16 & 2.05E+18 & -2.23E+12 & 1.81E+08 \\ -1.06E+10 & 1.24E+13 & -3.66E+15 & -2.49E+15 & 2.00E+15 & 3.05E+15 & -3.40E+16 & 5.03E+16 & 3.27E+18 & -3.41E+12 & 2.78E+08 \\ 6.49E+10 & -1.33E+14 & 4.16E+16 & 2.74E+16 & -2.17E+16 & -3.40E+16 & 2.49E+18 & -4.23E+18 & -4.12E+20 & 3.62E+13 & -2.94E+09 \\ -5.33E+10 & 1.96E+14 & -6.17E+16 & -4.05E+16 & 3.21E+16 & 5.03E+16 & -4.23E+18 & 7.43E+18 & 7.77E+20 & -5.33E+13 & 4.30E+09 \\ 2.78E+12 & 1.24E+16 & -4.06E+18 & -2.61E+18 & 2.05E+18 & 3.27E+18 & -4.12E+20 & 7.77E+20 & 9.23E+22 & -3.36E+15 & 2.67E+11 \\ 1.58E+07 & -1.39E+10 & 4.07E+12 & 2.78E+12 & -2.23E+12 & -3.41E+12 & 3.62E+13 & -5.33E+13 & -3.36E+15 & 3.87E+09 & -3.29E+05 \\ -1.72E+03 & 1.16E+06 & -3.30E+08 & -2.26E+08 & 1.81E+08 & 2.78E+08 & -2.94E+09 & 4.30E+09 & 2.67E+11 & -3.29E+05 & 4.31E+01 \end{bmatrix}.$$

(3.15)

The Determinant of equation 3.15 is $3.6063 \cdot 10^{225}$. As the determinant is large, the parameters of the FRiND model are at least locally identifiable [102, 103].

3.4 DISCUSSION

In preparation for the results and predictions to be made in the next chapter, a mathematical analysis of the FRiND model was necessary. The parameter set and several initial conditions were non-fixed under a few biological significant assumptions.

This chapter proves that the FRiND model has two potential steady states. The first steady state (x_1) always exists and represents a family of related fixed points which depends on the steady state of $\tilde{F}$. An existence criterion for the second steady state (x_2) was introduced; this criterion only depended on the estimated parameters and initial conditions.

The Jacobian for the FRiND model was derived and used to show stability of x_1 and x_2 . x_1 was shown to always be stable while x_2 when it exists is a saddle node. This implies that the FRiND model is a saddle node bifurcation.

The chapter finishes by showing that all the parameters in the FRiND model are identifiable. We calculated the sensitivity matrix then used it to form the Fisher information matrix (FIM). The determinant of the FIM was quite large which signifies that the parameters are identifiable.

CHAPTER 4

RESULTS

One of the goals of the FRiND model is to use a damage function ($f(t)$) to study the effects of damage to muscle tissue from chronic immune activation; specifically, the FRiND model allows exploration into how the acute immune response becomes a chronic immune response which causes muscle to be replaced by fibrous tissue.

To accomplish this goal, the model must be fairly accurate on the data used to estimate the model parameters (Section 2.2.3) which were obtained from an acute damage event. This will be followed by simulations of chronic muscle damage. All time-course graphs were created using Adams-Bashforth-Moulton Predictor-Corrector method [106] on MATLAB and verified using COPASI (which uses LSODA) [109].

4.1 ACUTE MUSCLE DAMAGE

As mentioned in Chapter 2, parameters were estimated using data from literature [112, 113] (Table 2.1). The parameter values are reported in Table 2.2. The damage function

$$f(t) = \frac{h}{\sigma\sqrt{2\pi}(t+1)} \exp\left(-\frac{1}{2\sigma^2}(\ln(t+1) - m)^2\right) \quad (4.1)$$

was chosen to simulate damage from Cardiotoxin (CTX) and to emulate previous models [107, 110].

Muscle tissue predictions followed closely to that given by Mounier et al. (2013)[112]. From Figure 4.1A, normal tissue experiences a steep drop as muscle is exposed to CTX; normal tissue is completely compromised within 24 hours. Damage peaks within the first 24 hours followed by complete phagocytosis over the next 3 days. The model gives regenerating

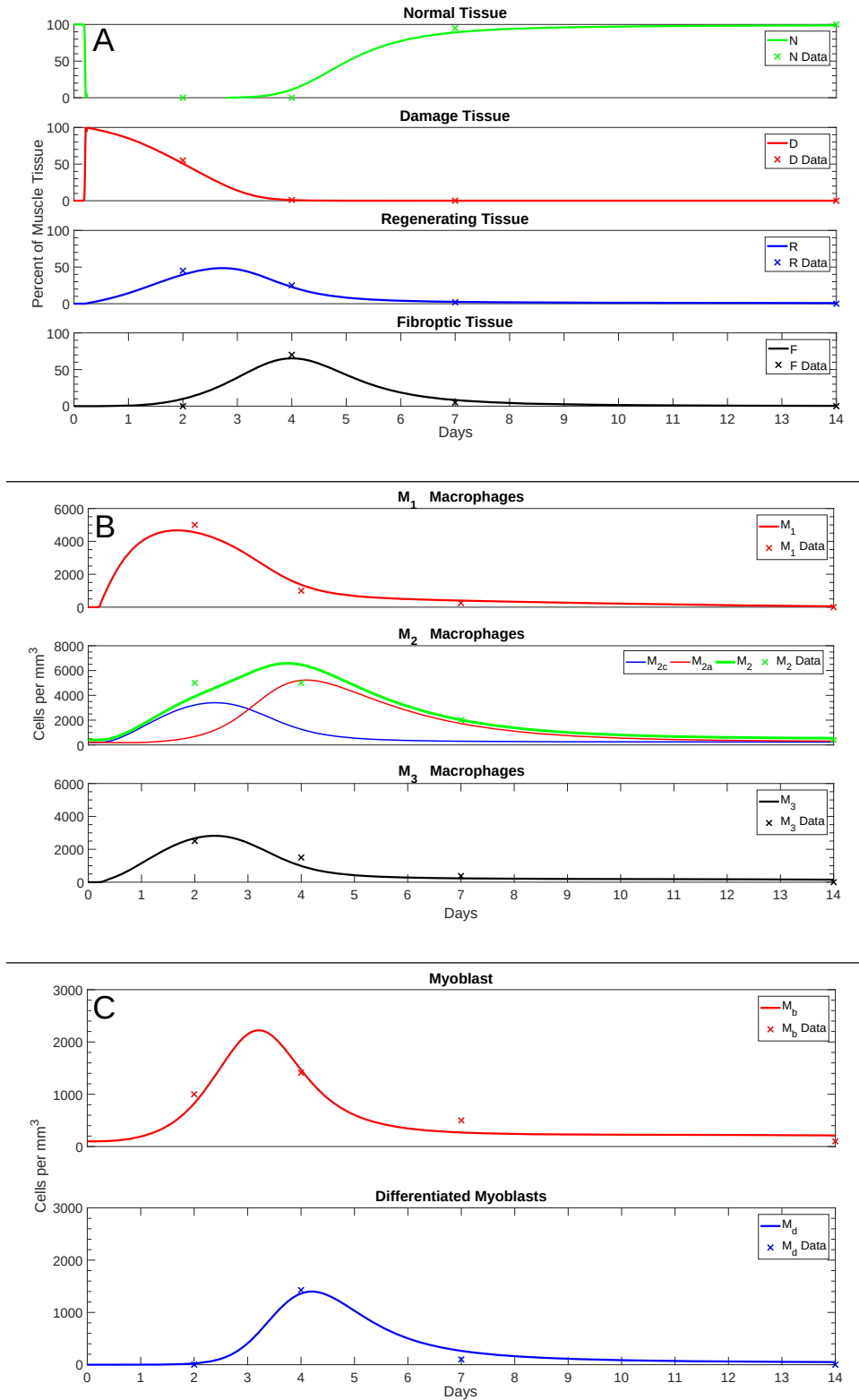


Figure 4.1: **Time Course of FRiND Model.**

This figure shows the time course of the A) muscle tissue equations, B) macrophage equations, and C) myocytes equations. Actual data in Table 2.1 is given as x's on the graphs.

tissue at about 40% at day 2 which is slightly less than Phagocytosed myofibers given in the literature [112]. However, the model's values for regenerating tissue at day 4 and 7 are closer. Fibrous tissue occurs earlier and peaks slightly lower than reported by literature [112]. Recovery of new normal tissue does occur within the reported period. The model also closely follows the trends in tissue data reported by Arnold et al. (2007) [105] which used a notexin.

Macrophage predictions followed the general characteristic of the data given in Mounier et al. (2013)[112]. From Fig. 4.1B M_1 macrophages peak between day 1 and day 2 as reported by [105, 112]. However the number of M_1 macrophages is less than expected. M_2 macrophages are noted to peak between day 2 and 4 as expected by [105, 112]; the numbers do, however, understate day 2 and overshoot day 4 numbers. M_3 macrophage predictions closely follow data from Mounier et al. [112].

Myocyte predictions closely follow the expected cell numbers before day 7 (Fig. 4.1C). After day 7, myoblast numbers fall short of expected numbers and differentiated myoblast numbers overshoot reported cell numbers [113].

4.2 CHRONIC MUSCLE DAMAGE

The archetypal pathology of MD is the replacement of healthy muscle tissue with fibrous (scar) tissue caused by severe acute damage to muscle from normal daily activity [108]. We would like to use the FRiND model to predict this condition by only changing the input damage function. This would show that repeat severe acute damage events cause the chronic immune response and replacement of muscle tissue.

A choice for this daily damage could be $f(t) = a \sin(bt + c)$ for some choice of parameters a, b, c . However, to avoid negative damage we should use $f(t) = a \sin^2(bt + c)$. If we assume that damage peaks around midday for a mouse, then $b = \pi$ and $c = 0$ would give daily periodic damage (with severity given by a random number a) peaking at midday. To allow a steady state to occur, a time for cessation of damage must be given (see Theorem 3.1.2).

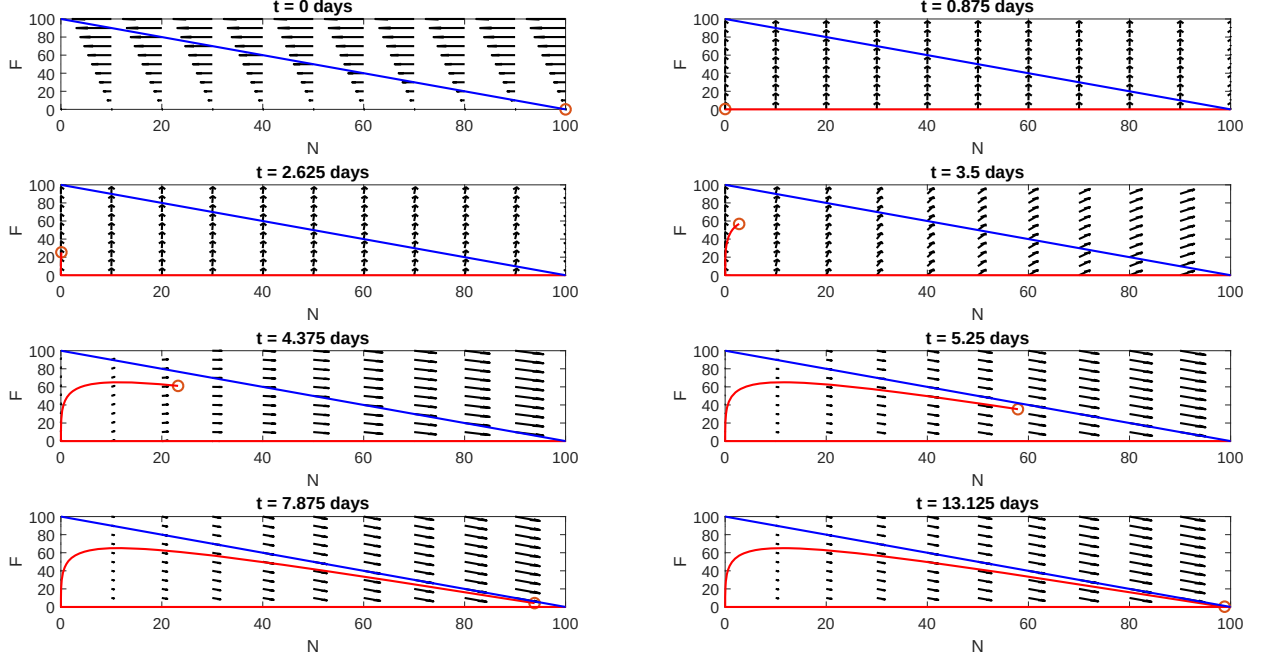


Figure 4.2: **Phase Portrait of $N \times F$ plane.**

This figure shows the time course in the $N \times F$ phase plane where vector arrows show direction of flow and the red circle gives position of $(N(t), F(t))$. The blue line $F = N - 100$ acts as a barrier where the red circle cannot cross since $N(t) + F(t) = 100$.

The following results are again given using Adams-Bashforth-Moulton Predictor-Corrector method on MATLAB and $f(t) = a \sin^2(\pi t)$ with severity a being randomly chosen for each day.

Figure 4.3 shows that over a period of several weeks normal tissue is repaired with some replacement by fibrous tissue. M_1 macrophages are nullified by M_{2c} macrophages and prevented from encouraging proliferation of more myoblasts. This causes a lack of differentiate myoblasts to bind with fibrous tissue into normal tissue; a buildup of fibrous tissue follows. Normal tissue is partially replaced by fibrous tissue around 16 weeks. A partial replacement by fibrous tissue matches studies of gastrocnemius muscle in $mdx/utrn^{-/-}$ mice [111].

The phase portrait of the FRiND model (Figure 4.4) when $f(t) = a \sin^2(\pi t)$ emphasizes the cyclic nature of the process. Fibrous and normal tissue interchange constantly

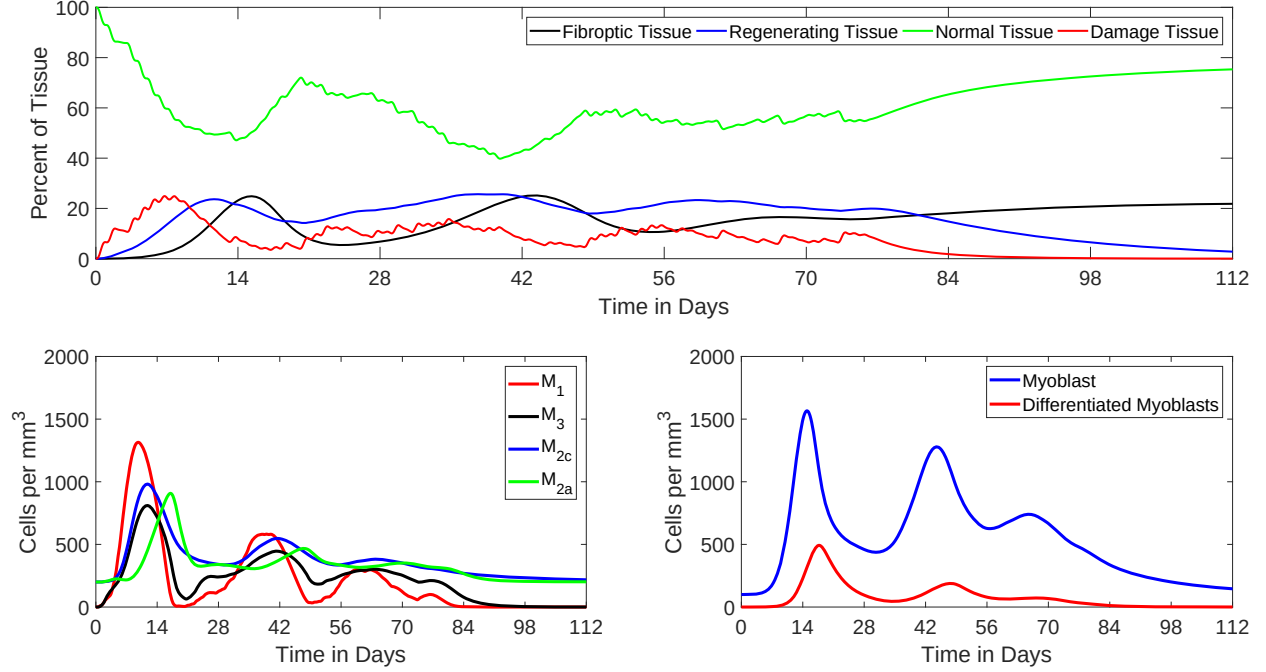


Figure 4.3: **Non-fading periodic damage.** These figures show the time course for the FRiND model with $f(t) = a \sin^2(\pi t)$ with a randomly selected each day. *A* shows the results for muscle tissue. *B* shows the results for macrophages. *C* shows the time course of myocytes.

creating jagged ellipses throughout the first 11 weeks. Following the depletion of differentiated myoblasts, the phase portrait shows that the cyclic nature ends and spirals toward the $F(t) = N(t) - 100$ line which is a push towards a steady state with elevated fibrous tissue and loss of normal tissue (both damage and regenerating tissue steady state to zero) by the 16th week.

A similar function to consider is $f(t) = \frac{a \sin^2(\pi t)}{t+1}$; this function allows for weakening of a patient's strength over time as muscle is replaced by fibrous tissue. Using this damage function, we can create results (Figure 4.5 and 4.6) with similar properties as the earlier non-fading damage function and fibrous tissue steady stating around 7% which closely aligns with *mdx* mice studies [114, 115].

Further studies of MD can be gleaned from the FRiND model without changing f .

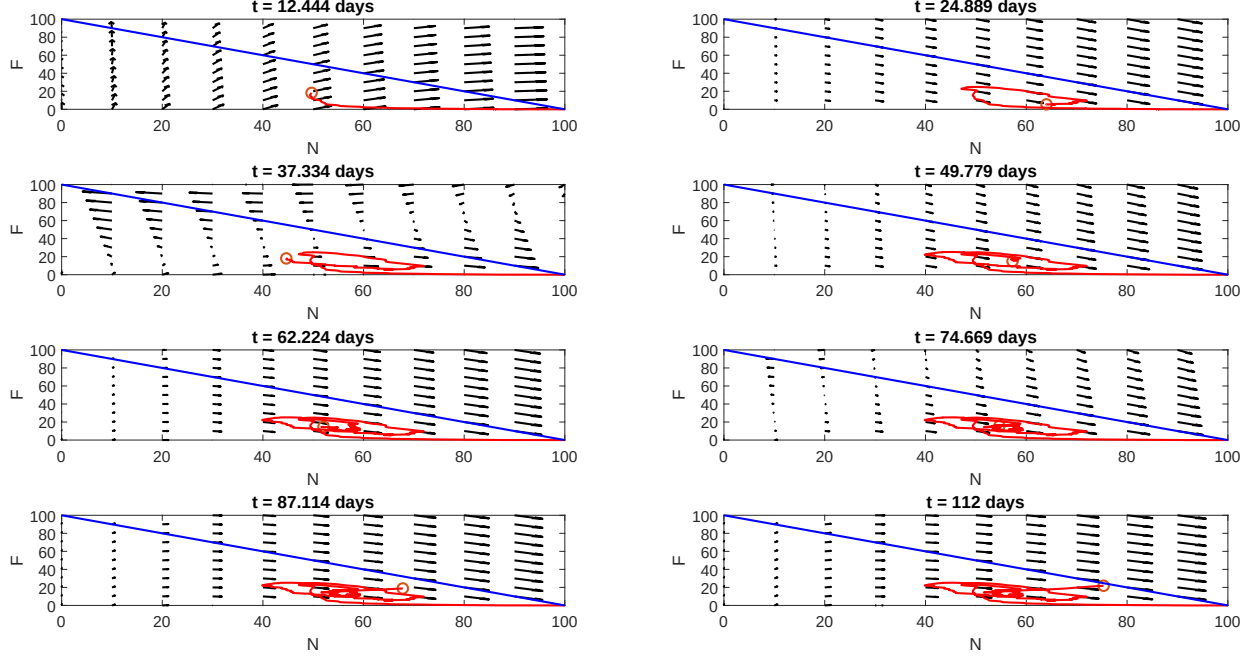


Figure 4.4: **Phase portrait of $N \times F$ plane for non-fading periodic damage.**

This figure shows the time course in the $N \times F$ phase plane where vector arrows show direction of flow and the red circle gives position of $(N(t), F(t))$. The blue line $F(t) = N(t) - 100$ acts as a barrier where the red circle cannot cross since $N(t) + F(t) \leq 100$.

4.3 INFLAMMATORY MACROPHAGE DEPLETION BY DIPHTHERIA

The FRiND model can be further modify by changing or adding parameters.

Arnold et al. (2007) [105] performed several experiments studying the actions of macrophages on muscle regeneration. The group noticed when M_1 macrophages are depleted within the first day after damage by exposure to diphtheria toxin (DT) that necrotic (damaged) tissue removal is delayed significantly. Furthermore, the regeneration process resumes after M_1 macrophages were allowed to return and the steady state lacked significant replacement of normal tissue by fibrous tissue.

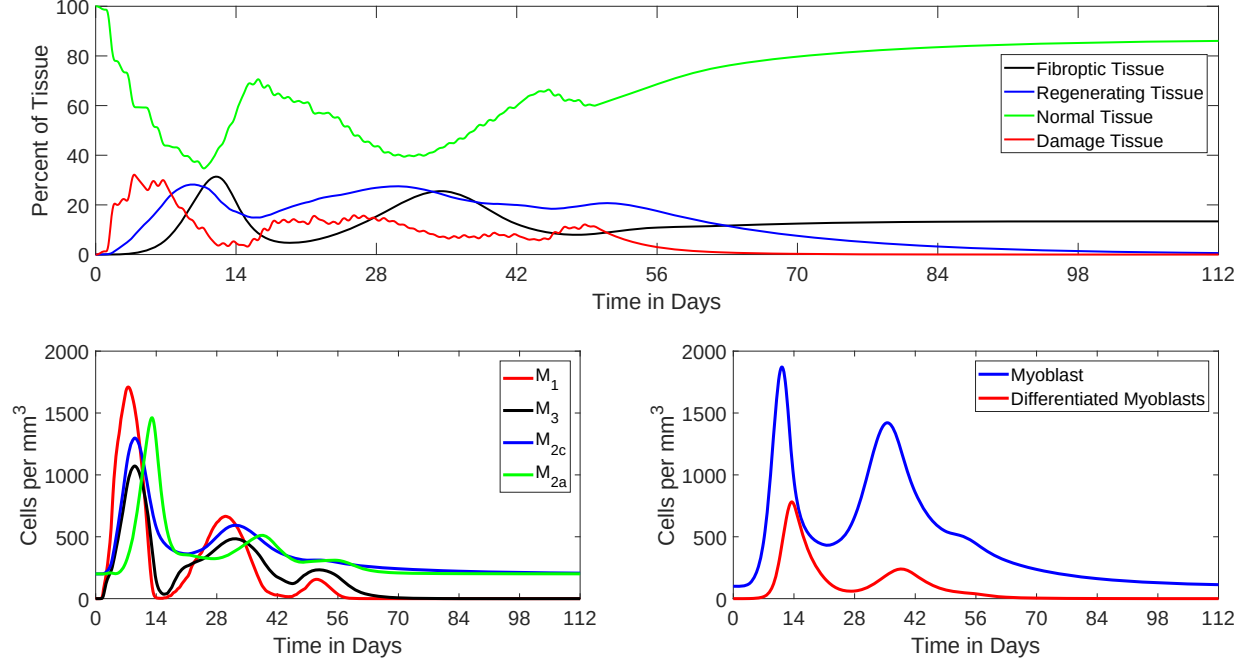


Figure 4.5: **Terminating Periodic Damage.** These figures show the time course for the FRiND model with $f(t) = \frac{a \sin^2(\pi t)}{t+1}$. *A* shows the results for muscle tissue. *B* shows the results for macrophages. *C* shows the time course of myocytes.

To simulate this, the FRiND model can be modified by subtracting $g(t)M_1$ from the M_1 macrophage equation. Thus,

$$\dot{M}_1 = k_1 D^2 - k_2 M_1 D - k_3 M_1 M_{2c} + k_4 M_1 M_3 - g(t) M_1,$$

where

$$g(t) = \begin{cases} 50 & \text{for } 0.3 < t < 2 \\ 0 & \text{for otherwise} \end{cases}.$$

Fig. 4.7 shows the results of this adapted FRiND model. M_1 macrophages are depleted shortly after diphtheria toxin is administered and rebounds around day 2 when $k(t) = 0$. While M_1 macrophages are depleted, only a small amount of necrotic tissue is removed mainly by M_2 macrophages already in the tissue. The repair process resumes after DT subsides and

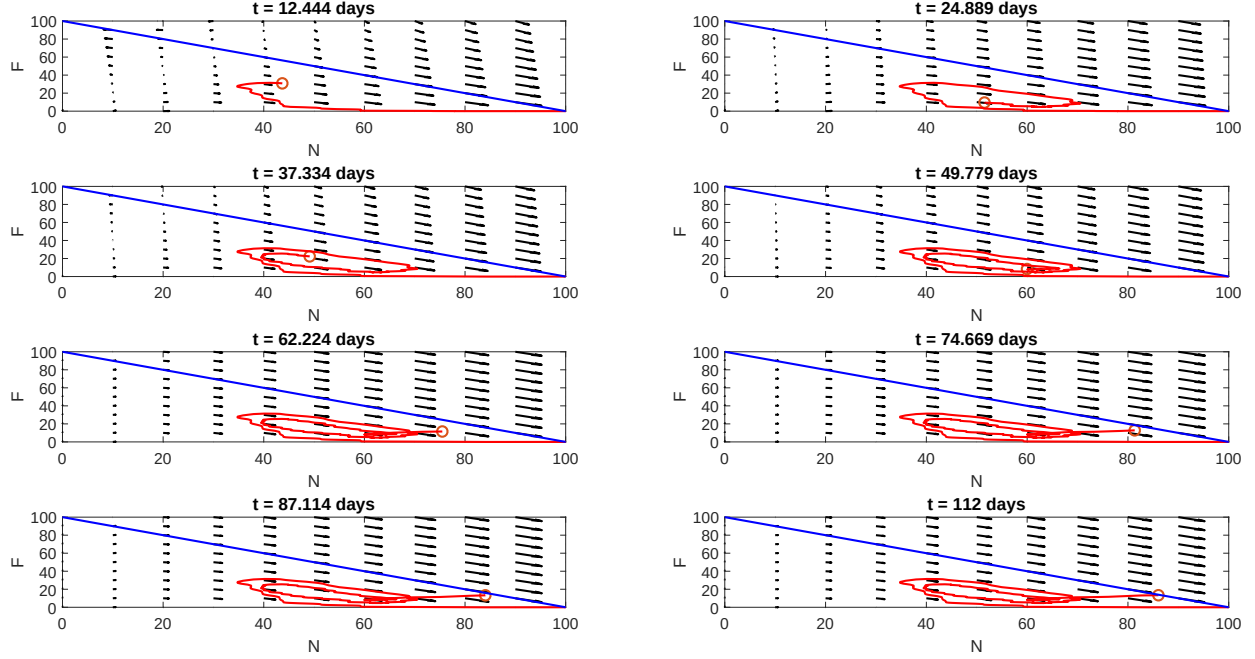


Figure 4.6: **Phase Portrait of $N \times F$ plane for fading periodic damage.**

This figure shows the time course in the $N \times F$ phase plane where vector arrows show direction of flow and the red circle gives position of $(N(t), F(t))$. The blue line $F(t) = N(t) - 100$ acts as a barrier where the red circle cannot cross since $N(t) + F(t) = 100$.

M_1 macrophage numbers rebounds with full recovery by 14 days. The steady state of the system also shows that no significant fibrosis remains by 14 days. This matches the results given by Arnold et al. (2007) [105].

4.4 DISCUSSION

We have illustrated in this chapter the predictive power of modeling immune cell interactions in muscle tissue repair; the FRiND model predicted the test data and several other experiments.

Setting $f(t)$ as in equation 4.1, the FRiND model reproduced results closely matching the original data from Mounier et al. (2013) [112] and Ogawa et al. (2015) [113]. Several

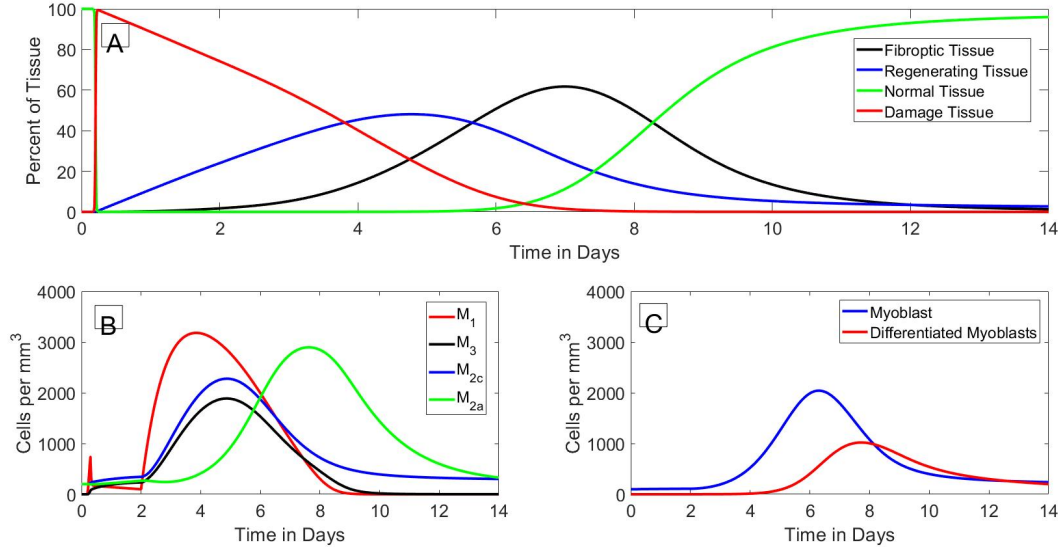


Figure 4.7: **Diphtheria Treated Mice.** These figures show the time course for the FRiND model with M_1 macrophages depleted by DT. The results are consistent with Arnold et al. (2007) [105]. *A* shows the results for muscle tissue. *B* shows the results for macrophages. *C* shows the time course of myocytes.

intermediate points were slightly off from the test data especially M_2 macrophage numbers. However, steady state results with the given parameters replicated the literature outcomes.

Furthermore, the FRiND model reproduced MD like pathology by introducing repeated daily, severe acute damage events. This could be used to validate that in some forms of MD the structural defects caused by dysfunctional genes is sufficient to cause MD's observed pathology. Section 4.2 reproduces results found in Lu et al. (2014) [111], Sun et al. (2015) [115], and Salimena et al. (2000) [114]. Using $f(t) = a \sin^2(\pi t)$ where a is random, we produced tissue repair with fibrous tissue replacing around 20% of normal tissue which concludes results similar to $mdx/utrn^{-/-}$ studies [111]. Using $f(t) = \frac{a \sin^2(\pi t)}{t+1}$ where a is still random, we predicted around 10% fibrous tissue replacement which is only slightly off the 7% mentioned in literature [114, 115].

Although the FRiND model was created to understand MD pathology, the model can still be used for understanding other experiments. Setting $g(t) = 50$, the FRiND model showed that early inhibition of M_1 macrophages slowed debris removal and muscle tissue repair. When $g(t)$ switched off, the M_1 numbers rebounded and soon the repair process returned to normal without fibrous replacement. This confirmed results from Arnold et al. (2007) [105].

CHAPTER 5

DISCUSSION

All forms of MD are rare [125], and the future of MD research might rely on mathematical modeling when few subjects are available. However, few mathematical models have been created to quantify the experiments about MD [120].

In Chapter 1, an exhaustive review of mathematical models concerned with MD was presented. Models dealing with several aspects of MD pathogenesis and pathology were discussed including genetic, physiological, mitochondrial, and immunological factors. Additional models were given that could be adapted for the study of MD. The review highlights the need for further research into models of MD especially in the repair of muscle tissue by the immune system.

Although most forms of MD are genetic disorders, chronic inflammation caused by repeated severe acute immune responses has been implicated in several forms of MD [119, 128, 129, 131, 132]. The two previous models of MD [118, 121] attempted to simulate research of inflammation during the lifetime of *mdx* mice [129, 132]. Both models used the law of mass action with three immune cells: CD8+ and CD4+ T cells and pro-inflammatory macrophages. They used a single acute damage event to start a perturbation that estimated chronic inflammation. The models were also bistable; either the normal tissue completely returned or a little damage remained permanently.

However, the previous models miss several key aspects of tissue repair. Treating macrophages as a homogeneous group reduces the predictive power of those models. Macrophages display several phenotypes that perform different tasks during muscle repair some of which play an important role in laying fibrous connective tissue and migrating

nascent myotubes to form new muscle; previous models handled this by using linear mass action dynamics for tissue repair after damaged tissue has been eaten by pro-inflammatory macrophages. In Chapter 2 we introduced the FRiND model to rectify these issues.

The FRiND model offers many avenues for future usage by integrating macrophage plasticity and nonlinear mass action dynamics. The ability to adapt to experiments with different criteria for subject injury and to study different pharmaceutical outcomes by changing parameters in the system could be useful in exploring biological outcomes in muscle regeneration.

Depending on the parameters, the FRiND model has at most two steady states of which one always exists and is stable (see Chapter 3). The other steady state exists under certain conditions and will always be a saddle node. When the second state exists, the model can be pushed into regions where the system explodes and substrate values approach infinity.

In Chapter 3, we also proved that the determinant of the Fisher information matrix (FIM) is large. This indicates that the FRiND model is very sensitive to parameter values. When estimating parameters from given data, the optimal solution will at least be locally identifiable.

While proving results in Chapter 3, we assume $\lim_{t \rightarrow 0} f(t) = 0$; however, the steady state (as a member of a family of steady states) depends on the intermediate values of $f(t)$. This provides flexibility to explore several types of functions depending on the injury caused to muscle tissue.

The first function introduced,

$$f(t) = \frac{h}{\sigma\sqrt{2\pi}(t+1)} \exp\left(-\frac{1}{2\sigma^2}(\ln(t+1) - m)^2\right),$$

simulates damage caused by CTX administered shortly after $t = 0$. The function is used to estimate all other parameters in the FRiND model with data from literature [124, 126]. The model showed that the pro-inflammatory macrophage numbers peak around the 2nd day and decline over a couple of days. Anti-inflammatory macrophages numbers (both M_{2c} and M_{2a}) rise to a peak around day 4 and completely steady state around day 14. The time

course results using these parameters closely matched the original data with steady state values matching by 28 days as the literature requires [116, 124, 126]. We then used these parameters to study chronic inflammation.

To simulate chronic inflammation two functions,

$$f_1(t) = a \sin^2(\pi t) \quad \text{and} \quad f_2(t) = \frac{a \sin^2(\pi t)}{t + 1}$$

were used where a is a random number chosen each day. In both cases, normal muscle tissue is replaced by fibrous tissue after steady state occurs. This replacement is more pronounced in f_1 since consistent damage proliferates myoblasts (M_b) during the pro-inflammatory peaks which quickly differentiate (M_d) during the valleys. This allows repeated recovery of normal muscle. However, the pro-inflammatory peaks also signal apoptosis of some differentiated myoblasts depleting the reserve necessary for sustained recovery of normal muscle. A replacement by fibrous tissue of around 20% of overall tissue approximates results of $mdx/utrn^{-/-}$ (double knockout of dystrophin and utrophin) mice which is a new mouse model for studies of DMD [123]. The same reasoning applies to f_2 , though, the reduction of damage over time allows for less fibrous tissue replacement (around 10%). This matches studies of mdx mice [127, 130]. Even though $mdx/utrn^{-/-}$ mice also lose strength over time, using f_1 to model this study is reasonable since the cell structure is severely compromised which allows consistent damage even with less strength. The FRiND model is not restricted to studies of MD.

Allowing

$$g(t) = \begin{cases} 50 & \text{if } 0.3 < t < 2 \\ 0 & \text{otherwise} \end{cases},$$

we can study direct removal of M_1 macrophages by diphtheria toxin (DT) during the early stages of muscle tissue repair. Shortly after DT is administered ($t = 0.3$ days) pro-inflammatory macrophages are completely depleted. Without the early infiltration of macrophages the repair process is stymied with few of any type of macrophages (except the anti-inflammatory macrophages already located in the muscle); this delays the repair

process as shown by tissue peaks translated to the right by several days. Pro-inflammatory macrophages return to normal numbers after DT concentration diminishes; the rest of tissue repair returns to normal with little if any normal tissue replaced by fibrous tissue. The results of delayed repair with a lack of fibrous tissue replacement mimics results reported in literature [116]. In a similar way to this example, we could study any experimental or pharmaceutical effects on immune cells modeled by the FRiND model.

The FRiND model does include some simplifications. The model does not include several immune and non-immune cells which have been shown to be significant in tissue repair including neutrophils [116], T cells [117, 131], fibro/adipogenic progenitors [122], and tissue cells under inflammation. With more data, many of these cells could be incorporated into the FRiND model. Inclusion of these cells and many others could allow further elucidation into the repair process.

The FRiND model also simplifies spatial dynamics by following the law of generalized mass action which assumes interactions are isotropic. Future research could expand this system into an anisotropic model using partial differential equations (PDEs). This could also allow three dimensional study of muscle regeneration. Another direction could be to include stochastic differential equations to differentiate early repair when cells are first infiltrating and later stages when cells are nearly isotropic.

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APPENDIX A

ABM.M

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% Adams-Bashforth-Moulton Method for Solving a System of ODEs with Initial
% Conditions.
% Method can be found in Numerical Mathematics and Computing, Sixth Edition
% By Cheney and Kincaid
% Code written by Matthew T. Houston
% This method is used in the LSODA method in COPASI.
function w = ABM(ODE,tspan,h,IC)

% Inputs:
% ODE is a function handle for the system of ODEs.
% tspan is a two element array giving starting and ending time.
% h is the fixed time step.
% IC are the initial conditions for the system of ODEs.

% Outputs:
% w is an array that estimates the time course of the solution to the ODE
% system

% Setting time Constraints
t_min = tspan(1);
t_max = tspan(2);

% Setting the number of time steps
n = (t_max-t_min)/h;

% Setting the initial state of the time course
w(:,1) = IC;
t = t_min;

% Estimatin the first four time steps with a Runge-Kutta order 4 method.
for i = 2:4
    x = w(:,i-1);
    K1 = h*feval(ODE,t,x);
    K2 = h*feval(ODE,t+0.5*h,x+.5*K1);
```

```

    K3 = h*feval(ODE,t+0.5*h,x+.5*K2);
    K4 = h*feval(ODE,t+h,x+K3);
    w(:,i) = x + (1/6)*(K1 + 2*K2 + 2*K3 + K4);
    t = t + h;
end

% Predictor-Corrector using the four previous time steps.
for i = 5:n+1
    x = w(:,i-1);
    predictor = x + (h/24)*(55*feval(ODE,t,x) - 59*feval(ODE,t-h,w(:,i-2)) + 37*feval(ODE,t-2*h,w(:,i-3)) - 9*feval(ODE,t-3*h,w(:,i-4)));
    w(:,i) = x + (h/24)*(9*feval(ODE,t+h,predictor) + 19*feval(ODE,t,x) - 5*feval(ODE,t-h,w(:,i-1)) + feval(ODE,t-2*h,w(:,i-2)));
    t = t+h;
end

end

```

APPENDIX B

FIGURES_4_1.M

```
% Code used to create Figures 4.2
%  $f(t) = (h/\sigma\sqrt{2\pi})(t+1)\exp((-1/(2\sigma^2))(\ln(t+1)-m)^2)$ 
% Creates one figure for Acute Damage function:
% Figure 1: Time course of model's substrates vs the real data.
% By Matthew T. Houston

% Set the limits of time calculation
t_min=0; t_max = 14;
h=.005;

% Converts provided data table into friendly variables.
% Time
Time = Combined51(:,1);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Muscle Tissue %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Fibrous Tissue
Fi = Combined51(:,2);
% Regenerating Tissue
Re = Combined51(:,3);
% Normal Tissue
No = Combined51(:,4);
% Damaged Tissue
Da = Combined51(:,5);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Myocytes %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Myoblast
Myob = Combined51(:,8);

% Differentiated Myocytes
Myod = Combined51(:,9);
```

```

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Macrophages %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%   Pro-Inflammatory Macrophages
Macro1 = Combined51(:,6);

%   Anti-Inflammatory Macrophage 2c
Macro2c = Combined51(:,10);

%   Anti-Inflammatory Macrophage 2a
Macro2a = Combined51(:,11);

%   Phenotypeless Macrophage
Macro3 = Combined51(:,7);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Initial Conditions %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
IC = [0 0 100 0 0 200 200 0 100 0];

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Setting Model Parameters %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
para = zeros(1,21);

%   a1 =
para(1) = 0.0003420387898;

%   a2 =
para(2) = 0.00027805269;

%   a3 =
para(3) = 5.926671787;

%   a4 =
para(4) = 0.1685466672;

%   a5 =
para(5) = 0.0004735032862;

%   d1 =
para(6) = 0.0001;

%   d2 =
para(7) = 0.000199043595;

%   d3 =
para(8) = 1.041101141e-05;

```

```
%      d4 =  
para(9) = 0.0005912309411;
```

```
%      d5 =  
para(10) = 0.000103662336;
```

```
%      d6 =  
para(11) = 0.310343;
```

```
%      k1 =  
para(12) = 0.9167670584;
```

```
%      k2 =  
para(13) = 0.007512675612;
```

```
%      k3 =  
para(14) = 0.01113163887;
```

```
%      k4 =  
para(15) = 0.01332585023;
```

```
%      k5 =  
para(16) = 0.002183270219;
```

```
%      k6 =  
para(17) = 0.001135187023;
```

```
%      k7 =  
para(18) = 0.0005064492007;
```

```
%      k8 =  
para(19) = 0.0002272581636;
```

```
%      k9 =  
para(20) = 0.6179192106;
```

```
%      h =  
%      Different from step size.  
para(21) = 9.466706714;
```

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Solving the ODE %%%%%%%%%  
%      Calls the function ABM by Matthew T. Houston.
```

```
% ABM requires:
% ABM(ODE handle, [start time,end time],time step,initial conditions)
w = ABM(@(t,w) DynSys(t,w,para,IC),[t_min,t_max],h,IC);

% ABM outputs a matrix of estimated solution points.
% w = (F(t); R(t); N(t); D(t); M1(t); M2c(t); M2a(t); M3(t); Mb(t); Md(t))

% Covertng w matrix into friendly variables
F=w(1,:);R=w(2,:); N=w(3,:); D=w(4,:);
M1=w(5,:); M2c=w(6,:); M2a=w(7,:); M3=w(8,:);
Mb=w(9,:); Md = w(10,:);

% Creating the time variable based on [start time,end time] used in ABM
t = t_min:h:t_max;
```

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Graphs of Tissue %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Figure 4.1 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

```
figure(1)

subplot(4,1,4);
h = plot(t,F,'k',Time,Fi,'xk');
set(h,'linewidth',3);
axis([t_min t_max 0 100])
legend('Model','Data');
% xlabel('Time in Days');
% ylabel('Percent of Tissue');
title('Fibrotic Tissue');

subplot(4,1,3)
h = plot(t,R,'b',Time,Re,'xb');
set(h,'linewidth',3);
axis([t_min t_max 0 100])
% legend('Model','Data');
% xlabel('Time in Days');
% ylabel('Percent of Tissue');
title('Regenerating Tissue');

subplot(4,1,1)
```

```

h = plot(t,N,'g',Time,No,'xg');
set(h,'linewidth',3);
axis([t_min t_max 0 100])
% legend('Model','Data');
% xlabel('Time in Days');
% ylabel('Percent of Tissue');
title('Normal Tissue');

```

```

subplot(4,1,2)
h = plot(t,D,'r',Time,Da,'xr');
set(h,'linewidth',3);
axis([t_min t_max 0 100])
% legend('Model','Data');
% xlabel('Time in Days');
% ylabel('Percent of Tissue');
title('Damage Tissue');

```

```

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Graphs of Myocytes %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Figure 4.1 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

```

```

figure(2)
subplot(2,1,1)
h = plot(t,Mb,'r',Time,Myob,'xr');
set(h,'linewidth',3);
axis([t_min t_max 0 5000])
%legend('Model','Data');
%xlabel('Time in Days');
%ylabel('Cells per mm^3');
title('Myoblast');

```

```

subplot(2,1,2)
h = plot(t,Md,'b',Time,Myod,'xb');
set(h,'linewidth',3);
axis([t_min t_max 0 5000])
%legend('Model','Data');
%xlabel('Time in Days');
%ylabel('Cells per mm^3');
title('Differentiated Myoblasts');

```

```

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Graphs of Macrophages %%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Figure 4.1 %%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

```

```
figure(3)
```

```

subplot(3,1,1)
h = plot(t,M1,'r',Time,Macro1,'xr');
set(h,'linewidth',3);
axis([t_min t_max 0 7000])
%legend('Model','Data');
%xlabel('Time in Days');
%ylabel('Cells per mm^3');
title('M_1');

```

```

subplot(3,1,3)
h = plot(t,M3,'k',Time,Macro3,'xk');
set(h,'linewidth',3);
axis([t_min t_max 0 7000])
%legend('Model','Data');
%xlabel('Time in Days');
%ylabel('Cells per mm^3');
title('M_3');

```

```

subplot(3,1,2)
plot(t,M2c,'b','linewidth',1);
hold on

```

```
plot(t,M2a,'r','linewidth',1);
```

```

h = plot(t,M2c+M2a,'g',Time,Macro2c+Macro2a,'xg');
set(h,'linewidth',3);

```

```

axis([t_min t_max 0 7000])
legend('M_{2c}','M_{2a}','M_2','M_2 data');
%xlabel('Time in Days');
%ylabel('Cells per mm^3');
title('M_2');

```

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

```
function f = inputDamage(t,h)
```

```

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Lognormal Damage %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% This is used to simulate CTX damage to muscle.
% sigma is the standard deviation of damage over time.
% m is the log(time) at which average damage occurs.
% h modifies peak damage.

sigma = .01;
m = .2;

f = (h./((t+1).*sigma.*sqrt(2*pi))).*exp(-1*(log(t+1)-m).^2/(2*sigma^2));

end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

function k = diphth(t)
% Not used in this script. Thus, the function is set to zero.
k = 0;

end

function dwdt = DynSys(t,w,para,IC)

%    Assign friendly names to depended variables.
F = w(1);
R = w(2);
N = w(3);
D = w(4);
M1 = w(5);
M2c = w(6);
M2a = w(7);
M3 = w(8);
Mb = w(9);
Md = w(10);

%    Assign friendly names to parameters.
a1 = para(1); a2 = para(2); a3 = para(3); a4 = para(4);
a5 = para(5); d1 = para(6); d2 = para(7); d3 = para(8);
d4 = para(9); d5 = para(10); d6 = para(11); k1 = para(12);

```

```

k2 = para(13); k3 = para(14); k4 = para(15); k5 = para(16);
k6 = para(17); k7 = para(18); k8 = para(19); k9 = para(20);
h = para(21);

Mc0 = IC(6);
Ma0 = IC(7);
Mb0 = IC(9);

% Calls to a user defined function that can be set for different damage
% outputs.
f = inputDamage(t,h);

% Calls to a user defined function that can be set for diptheria's inhibiting M_1
% macrophage recruitment.
g = diphth(t);

% The FRiND model ODE system
dF_dt = d1*M2c*R - d6*F*Md/(M2c - Mc0 + 1) + d2*M2a*R;
dR_dt = d3*M1*D - d1*M2c*R + d4*M2a*D + d5*M2c*D - d2*M2a*R;
dN_dt = d6*F*Md/(M2c - Mc0 + 1) - f*N;
dD_dt = f*N - d3*M1*D - d4*M2a*D - d5*M2c*D;

dM1_dt = k1*D^2 - k2*M1*D - k3*M1*M2c + k4*M1*M3 - g*M1;
dM2c_dt = k2*M1*D - k5*(M2c - Mc0)*D - k8*(M2c - Mc0)*M2a; % M_2c(0) = 200
dM2a_dt = k5*(M2c - Mc0)*D + k6*M2a*M3 + k8*(M2c - Mc0)*M2a - k7*M1*M2a - k9*(M2a -
dM3_dt = k3*M1*M2c + k7*M1*M2a - k4*M1*M3 - k6*M2a*M3;

dMb_dt = a1*M1*Mb - a2*(Mb - Mb0)*M2a; % M_b(0) = 100
dMd_dt = a2*(Mb - Mb0)*M2a - (a3*Md*F)/(M2c - Mc0 + 1) - a4*Md - a5*M1*Md;

% Assemble the vector with derivatives
dwdt=[dF_dt; dR_dt; dN_dt; dD_dt; dM1_dt; dM2c_dt; dM2a_dt; dM3_dt; dMb_dt; dMd_dt];
end

```

APPENDIX C

FIGURES_4.2.M

```
% Code used to create Figures 4.2
% f(t) = (h/sigma*sqrt(2*pi)*(t+1))*exp((-1/(2*sigma^2))*(ln(t+1)-m)^2)
% Creates one figure for Acute Damage function:
% Figure 1: Phase Portrait of the Normal x Fibrous plane over different time points.
% By Matthew T. Houston

% Set the number of days for the time course.
    t_min=0; t_max = 14;

% Step size of ABM solver
    h=.001;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Initial Conditions %%%%%%%%%%%%%
    IC = [0 0 100 0 0 200 200 0 100 0];

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Setting Model Parameters %%%%%%%%%
para = zeros(1,21);

%      a1 =
para(1) = 0.0003420387898;

%      a2 =
para(2) = 0.00027805269;

%      a3 =
para(3) = 5.926671787;

%      a4 =
para(4) = 0.1685466672;

%      a5 =
```

```

para(5) = 0.0004735032862;

%      d1 =
para(6) = 0.0001;

%      d2 =
para(7) = 0.000199043595;

%      d3 =
para(8) = 1.041101141e-05;

%      d4 =
para(9) = 0.0005912309411;

%      d5 =
para(10) = 0.000103662336;

%      d6 =
para(11) = 0.310343;

%      k1 =
para(12) = 0.9167670584;

%      k2 =
para(13) = 0.007512675612;

%      k3 =
para(14) = 0.01113163887;

%      k4 =
para(15) = 0.01332585023;

%      k5 =
para(16) = 0.002183270219;

%      k6 =
para(17) = 0.001135187023;

%      k7 =
para(18) = 0.0005064492007;

%      k8 =
para(19) = 0.0002272581636;

%      k9 =

```

```

para(20) = 0.6179192106;

% h =
% Different from step size.
para(21) = 9.466706714;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Plot Results %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Calls the function ABM by Matthew T. Houston.
% ABM requires:
% ABM(ODE handle, [start time,end time],time step,initial conditions)
w = ABM(@(t,w) DynSys(t,w,para,IC),[t_min,t_max],h,IC);

% ABM outputs a matrix of estimated solution points.
% w = (F(t); R(t); N(t); D(t); M1(t); M2c(t); M2a(t); M3(t); Mb(t); Md(t))

% Coverting w matrix into friendly variables
F=w(1,:);R=w(2,:); N=w(3,:); D=w(4,:);
M1=w(5,:); M2c=w(6,:); M2a=w(7,:); M3=w(8,:);
Mb=w(9,:); Md = w(10,:);

% Creating the time variable based on [start time,end time] used in ABM
t = t_min:h:t_max;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Phase Portrait of N x F %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Figure 4.2 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
figure(1)

n = size(t,2);
sub = 1; % Current subgraph
subb = 1; % Used to give only certain plots
nsub = 4; % Sqraure root of the number of plots created

F = 0:10:100; % Set Fibrous Tissue sampling
N = 0:10:100; % Set Normal Tissue sampling

prime = zeros(2,size(F,2)*size(N,2));
x = zeros(1,size(F,2)^2);
y = zeros(1,size(F,2)^2);

```

```

% Creates evenly timed phase portraits.
% FNSys is the FRiND model projected onto the Nx $F$  plane.
% Wprime uses feval to find the projected derivatives at a given time.
for i=1:ceil((n-1)/(nsub*nsub)):n-1

    for j = 1:size(N,2)
        for k=1:size(N,2)
            prime(:,(j-1)*size(N,2)+k) = Wprime(@(t,w) FNSys(t,w,para,IC),t(i),[F(j),N(k)]);
            x((j-1)*size(N,2)+k) = N(j);
            y((j-1)*size(N,2)+k) = F(k);
        end
    end

    subplot(4,2,subb)

    % Creates the arrows in the phase portrait.
    quiver(x,y,prime(2,:),prime(1,:),.6,'k','linewidth',2,'MarkerSize',100);

    title(['t = ',num2str(t(i)),' days']);
    xlabel('N')
    ylabel('F')
    axis([0 100 0 100])
    xticks(0:20:100);
    yticks(0:20:100);
    set(gca,'FontSize',15);

    hold on

    % Plots the red circle giving the point (N(t),F(t)).
    plot(w(3,i),w(1,i),'o','linewidth',2,'MarkerSize',10);
    plot(w(3,1:i),w(1,1:i),'r','linewidth',2);

    % Plots the N+F=100 constraint.
    plot(N,100-N,'b','linewidth',2);

    subb = subb + 1;
end

sub = sub+1;

end

```

```

function f = inputDamage(t,h)

    %%%%%%%%%%%%%%% Lognormal Damage %%%%%%%%%%%%%%%
    % This is used to simulate CTX damage to muscle.
    % sigma is the standard deviation of damage over time.
    % m is the log(time) at which average damage occurs.
    % h modifies peak damage.

    sigma = .01;
    m = .2;

    f = (h./((t+1).*sigma.*sqrt(2*pi))).*exp(-1*(log(t+1)-m).^2/(2*sigma^2));

end

function p = Wprime(ODE,t,w)
% This is used to calculate the velocity of the FRiND model at a given time point.
% Required for phase portrait graphs.
p = feval(ODE,t,w);

end

function k = dipth(t)
% Not used in this script. Thus, the function is set to zero.
k = 0;

end

function dwdt = FNSys(t,w,para,IC)
% A paired down version of DynSys. This allows calculation of Normal and
% Fibrous velocities which are needed for phase portraits.

% Assign friendly names to depended variables.
F = w(1);
N = w(2);
R = w(3);
M2c = w(4);
M2a = w(5);
Md = w(6);

```

```

%    Assign friendly names to parameters.
d1 = para(6);
d2 = para(7);
d6 = para(11);
h = para(21);

Mc0 = IC(6);

%    Calls to a user defined function that can be set for different damage
%    outputs.
f = inputDamage(t,h);

%    The Normal and Fibrous ODE system.
dF_dt = d1*M2c*R - d6*F*Md/(M2c - Mc0 + 1) + d2*M2a*R;
dN_dt = d6*F*Md/(M2c - Mc0 + 1) - f*N;

% Assemble the vector with derivatives
dwdt=[dF_dt; dN_dt];
end

function dwdt = DynSys(t,w,para,IC)

%    Assign friendly names to depended variables.
F = w(1);
R = w(2);
N = w(3);
D = w(4);
M1 = w(5);
M2c = w(6);
M2a = w(7);
M3 = w(8);
Mb = w(9);
Md = w(10);

%    Assign friendly names to parameters.
a1 = para(1); a2 = para(2); a3 = para(3); a4 = para(4);
a5 = para(5); d1 = para(6); d2 = para(7); d3 = para(8);
d4 = para(9); d5 = para(10); d6 = para(11); k1 = para(12);
k2 = para(13); k3 = para(14); k4 = para(15); k5 = para(16);
k6 = para(17); k7 = para(18); k8 = para(19); k9 = para(20);
h = para(21);

Mc0 = IC(6);
Ma0 = IC(7);

```

```

Mb0 = IC(9);

% Calls to a user defined function that can be set for different damage
% outputs.
f = inputDamage(t,h);

% Calls to a user defined function that can be set for diptheria's inhibiting M_1
% macrophage recruitment.
g = dipth(t);

% The FRiND model ODE system
dF_dt = d1*M2c*R - d6*F*Md/(M2c - Mc0 + 1) + d2*M2a*R;
dR_dt = d3*M1*D - d1*M2c*R + d4*M2a*D + d5*M2c*D - d2*M2a*R;
dN_dt = d6*F*Md/(M2c - Mc0 + 1) - f*N;
dD_dt = f*N - d3*M1*D - d4*M2a*D - d5*M2c*D;

dM1_dt = k1*D^2 - k2*M1*D - k3*M1*M2c + k4*M1*M3 - g*M1;
dM2c_dt = k2*M1*D - k5*(M2c - Mc0)*D - k8*(M2c - Mc0)*M2a;
dM2a_dt = k5*(M2c - Mc0)*D + k6*M2a*M3 + k8*(M2c - Mc0)*M2a - k7*M1*M2a - k9*(M2a -
dM3_dt = k3*M1*M2c + k7*M1*M2a - k4*M1*M3 - k6*M2a*M3;

dMb_dt = a1*M1*Mb - a2*(Mb - Mb0)*M2a;
dMd_dt = a2*(Mb - Mb0)*M2a - (a3*Md*F)/(M2c - Mc0 + 1) - a4*Md - a5*M1*Md;

% Assemble the vector with derivatives
dwdt=[dF_dt; dR_dt; dN_dt; dD_dt; dM1_dt; dM2c_dt; dM2a_dt; dM3_dt; dMb_dt; dMd_dt];
end

```

APPENDIX D

FIGURES_4_3_AND_4_4.M

```
% Code used to create Figures 4.3 and 4.4
% f(t) = a*sin^2(pi*t)
% Creates two figures for Non-Fading Damage function:
% Figure 1: Time course for all substrates.
% Figure 2: Phase Portrait of the Normal x Fibrous plane over different time points.
% By Matthew T. Houston

% Set the number of days for the time course.
    t_min=0; t_max = 112;

% Step size of ABM solver
    h=.001;

% Creates a matrix of random amplitude for daily damage.
    global severity
    severity = .2*rand(1,t_max - t_min + 1);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Initial Conditions %%%%%%%%%%%%%
    IC = [0 0 100 0 0 200 200 0 100 0];

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Setting Model Parameters %%%%%%%%%
    para = zeros(1,21);

%      a1 =
para(1) = 0.0003420387898;

%      a2 =
para(2) = 0.00027805269;

%      a3 =
para(3) = 5.926671787;
```

```

%      a4 =
para(4) = 0.1685466672;

%      a5 =
para(5) = 0.0004735032862;

%      d1 =
para(6) = 0.0001;

%      d2 =
para(7) = 0.000199043595;

%      d3 =
para(8) = 1.041101141e-05;

%      d4 =
para(9) = 0.0005912309411;

%      d5 =
para(10) = 0.000103662336;

%      d6 =
para(11) = 0.310343;

%      k1 =
para(12) = 0.9167670584;

%      k2 =
para(13) = 0.007512675612;

%      k3 =
para(14) = 0.01113163887;

%      k4 =
para(15) = 0.01332585023;

%      k5 =
para(16) = 0.002183270219;

%      k6 =
para(17) = 0.001135187023;

%      k7 =
para(18) = 0.0005064492007;

```

```

%      k8 =
para(19) = 0.0002272581636;

%      k9 =
para(20) = 0.6179192106;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Plot Results %%%%%%%%%%
%   Calls the function ABM by Matthew T. Houston.
%   ABM requires:
%   ABM(ODE handle, [start time,end time],time step,initial conditions)
w = ABM(@(t,w) DynSys(t,w,para,IC),[t_min,t_max],h,IC);

%   ABM outputs a matrix of estimated solution points.
%   w = (F(t); R(t); N(t); D(t); M1(t); M2c(t); M2a(t); M3(t); Mb(t); Md(t))

%   Coverting w matrix into friendly variables
F=w(1,:);R=w(2,:); N=w(3,:); D=w(4,:);
M1=w(5,:); M2c=w(6,:); M2a=w(7,:); M3=w(8,:);
Mb=w(9,:); Md = w(10,:);

%   Creating the time variable based on [start time,end time] used in ABM
t = t_min:h:t_max;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Time Course of the FRiND Model %%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Figure 4.3 %%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

figure(1)

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Muscle Tissue %%%%%%%%%%
subplot(2,2,[1,2]);

h = plot(t,F,'k');
set(h,'linewidth',2);
axis([t_min t_max 0 100])

hold on

h = plot(t,R,'b');
set(h,'linewidth',2);

```

```

h = plot(t,N,'g');
set(h,'linewidth',2);

h = plot(t,D,'r');
set(h,'linewidth',2);

xlabel('Time in Days');
ylabel('Percent of Tissue');
xticks(t_min:14:t_max);
yticks(0:20:100);
set(gca,'FontSize',20);

legend('Fibrotic Tissue','Regenerating Tissue','Normal Tissue','Damage Tissue')

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Macrophages %%%%%%%%%
subplot(2,2,3);

h = plot(t,M1,'r');
set(h,'linewidth',3);

hold on

h = plot(t,M3,'k');
set(h,'linewidth',3);

plot(t,M2c,'b','linewidth',3);
hold on

plot(t,M2a,'g','linewidth',3);

axis([t_min t_max 0 7000])
legend('M_1','M_3','M_{2c}','M_{2a}');
xlabel('Time in Days');
ylabel('Cells per mm^3');
axis([t_min t_max 0 2000])
xticks(t_min:14:t_max);
yticks(0:500:2000);
set(gca,'FontSize',20);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Myocytes %%%%%%%%%
subplot(2,2,4);

```

```

h = plot(t,Mb,'b');
set(h,'linewidth',3);

hold on

h = plot(t,Md,'r');
set(h,'linewidth',3);

xlabel('Time in Days');
ylabel('Cells per mm^3');
axis([t_min t_max 0 2000])
xticks(t_min:14:t_max);
yticks(0:500:2000);
set(gca,'FontSize',20);

legend('Myoblast','Differentiated Myoblasts');
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Phase Portrait of N x F %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Figure 4.4 %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

figure(2)

n = size(t,2);
sub = 1; % Current subgraph
nsub = 3; % Square root of the number of plots created

F = 0:10:100; % Set Fibrous Tissue sampling
N = 0:10:100; % Set Normal Tissue sampling

prime = zeros(2,size(F,2)*size(N,2));
x = zeros(1,size(F,2)^2);
y = zeros(1,size(F,2)^2);

% Creates evenly timed phase portraits.
% FNSys is the FRiND model projected onto the NxF plane.
% Wprime uses feval to find the projected derivatives at a given time.
for i=ceil((n-1)/(nsub*nsub)):ceil((n-1)/(nsub*nsub)):(n-1)

```

```

    if sub == (nsub*nsub-1)
        i = n;
    end

    for j = 1:size(N,2)
        for k=1:size(N,2)
            prime(:,(j-1)*size(N,2)+k) = Wprime(@(t,w) FNSys(t,w,para,IC),t(i),[F(j),N(k)]);
            x((j-1)*size(N,2)+k) = N(j);
            y((j-1)*size(N,2)+k) = F(k);
        end
    end

    subplot(4,2,sub)

    %    sub is used to populate the 3x3 subplots.

    %    Creates the arrows in the phase portrait.
    quiver(x,y,prime(2,:),prime(1,:),.6,'k','linewidth',2,'MarkerSize',100);

    %    Creates all the titles and labels for each subgraph.
    title(['t = ',num2str(t(i)),' days']);
    xlabel('N')
    ylabel('F')
    axis([0 100 0 100])
    xticks(0:20:100);
    yticks(0:20:100);
    set(gca,'FontSize',15);

    hold on

    %    Plots the red circle giving the point (N(t),F(t)).
    plot(w(3,i),w(1,i),'o','linewidth',2,'MarkerSize',10);
    plot(w(3,1:i),w(1,1:i),'r','linewidth',2);

    %    Plots the N+F=100 constraint.
    plot(N,100-N,'b','linewidth',2);

    sub = sub+1;

end

function f = inputDamage(t)

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Non-Fading Periodic Damage %%%%%%%%%%

```

```

% This is used to simulate damage to muscle due to normal daily activity.
% Severity is a matrix of peak damage for each day. In the dissertation
% this is referred to as 'a.'

% Matrix of random amplitude (peak) for daily damage.
global severity

day = findDay(t);

if(day<77)
    f = severity(day+1)*sin(pi*t).^2;
else
    f=0;
end

end

function p = Wprime(ODE,t,w)
% This is used to calculate the velocity of the FRiND model at a given time point.
% Required for phase portrait graphs.
p = feval(ODE,t,w);

end

function k = dipth(t)
% Not used in this script. Thus, the function is set to zero.
k = 0;

end

function day = findDay(t)
% Finds current day depending on given t.
% Needed for periodic damage functions to know which element of the
% severity matrix to use.
day = 0;

    while t > day + 1
        day = day + 1;

    end

end

function dwdt = FNSys(t,w,para,IC)

```

```
% A paired down version of DynSys. This allows calculation of Normal and
% Fibrous velocities which are needed for phase portraits.
```

```
% Assign friendly names to depended variables.
```

```
F = w(1);
N = w(2);
R = w(3);
M2c = w(4);
M2a = w(5);
Md = w(6);
```

```
% Assign friendly names to parameters.
```

```
d1 = para(6);
d2 = para(7);
d6 = para(11);
```

```
Mc0 = IC(6);
```

```
% Calls to a user defined function that can be set for different damage
% outputs.
```

```
f = inputDamage(t);
```

```
% The Normal and Fibrous ODE system.
```

```
dF_dt = d1*M2c*R - d6*F*Md/(M2c - Mc0 + 1) + d2*M2a*R;
dN_dt = d6*F*Md/(M2c - Mc0 + 1) - f*N;
```

```
% Assemble the vector with derivatives
```

```
dwdt=[dF_dt; dN_dt];
```

```
end
```

```
function dwdt = DynSys(t,w,para,IC)
```

```
% Assign friendly names to depended variables.
```

```
F = w(1);
R = w(2);
N = w(3);
D = w(4);
M1 = w(5);
M2c = w(6);
M2a = w(7);
M3 = w(8);
Mb = w(9);
Md = w(10);
```

```

%      Assign friendly names to parameters.
a1 = para(1); a2 = para(2); a3 = para(3); a4 = para(4);
a5 = para(5); d1 = para(6); d2 = para(7); d3 = para(8);
d4 = para(9); d5 = para(10); d6 = para(11); k1 = para(12);
k2 = para(13); k3 = para(14); k4 = para(15); k5 = para(16);
k6 = para(17); k7 = para(18); k8 = para(19); k9 = para(20);

Mc0 = IC(6);
Ma0 = IC(7);
Mb0 = IC(9);

%      Calls to a user defined function that can be set for different damage
%      outputs.
f = inputDamage(t);

%      Calls to a user defined function that can be set for diptheria's inhibiting M_1
%      macrophage recruitment.
g = diphth(t);

%      The FRiND model ODE system
dF_dt = d1*M2c*R - d6*F*Md/(M2c - Mc0 + 1) + d2*M2a*R;
dR_dt = d3*M1*D - d1*M2c*R + d4*M2a*D + d5*M2c*D - d2*M2a*R;
dN_dt = d6*F*Md/(M2c - Mc0 + 1) - f*N;
dD_dt = f*N - d3*M1*D - d4*M2a*D - d5*M2c*D;

dM1_dt = k1*D^2 - k2*M1*D - k3*M1*M2c + k4*M1*M3 - g*M1;
dM2c_dt = k2*M1*D - k5*(M2c - Mc0)*D - k8*(M2c - Mc0)*M2a;
dM2a_dt = k5*(M2c - Mc0)*D + k6*M2a*M3 + k8*(M2c - Mc0)*M2a - k7*M1*M2a - k9*(M2a -
dM3_dt = k3*M1*M2c + k7*M1*M2a - k4*M1*M3 - k6*M2a*M3;

dMb_dt = a1*M1*Mb - a2*(Mb - Mb0)*M2a;
dMd_dt = a2*(Mb - Mb0)*M2a - (a3*Md*F)/(M2c - Mc0 + 1) - a4*Md - a5*M1*Md;

% Assemble the vector with derivatives
dwdt=[dF_dt; dR_dt; dN_dt; dD_dt; dM1_dt; dM2c_dt; dM2a_dt; dM3_dt; dMb_dt; dMd_dt];
end

```

APPENDIX E

FIGURES_4_5_AND_4_6.M

```
% Code used to create Figures 4.5 and 4.6
%  $f(t) = a \sin^2(\pi t)/(t+1)$ 
% Creates two figures for Fading Damage function:
% Figure 1: Time course for all substrates.
% Figure 2: Phase Portrait of the Normal x Fibrous plane over different time points.
% By Matthew T. Houston

% Set the number of days for the time course.
t_min=0; t_max = 112;

% Step size of ABM solver
h=.001;

% Creates a matrix of random amplitude for daily damage.
global severity
severity = rand(1,t_max - t_min + 1);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Initial Conditions %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
IC = [0 0 100 0 0 200 200 0 100 0];

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Setting Model Parameters %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
para = zeros(1,21);

% a1 =
para(1) = 0.0003420387898;

% a2 =
para(2) = 0.00027805269;

% a3 =
para(3) = 5.926671787;

% a4 =
```

```

para(4) = 0.1685466672;

%      a5 =
para(5) = 0.0004735032862;

%      d1 =
para(6) = 0.0001;

%      d2 =
para(7) = 0.000199043595;

%      d3 =
para(8) = 1.041101141e-05;

%      d4 =
para(9) = 0.0005912309411;

%      d5 =
para(10) = 0.000103662336;

%      d6 =
para(11) = 0.310343;

%      k1 =
para(12) = 0.9167670584;

%      k2 =
para(13) = 0.007512675612;

%      k3 =
para(14) = 0.01113163887;

%      k4 =
para(15) = 0.01332585023;

%      k5 =
para(16) = 0.002183270219;

%      k6 =
para(17) = 0.001135187023;

%      k7 =
para(18) = 0.0005064492007;

%      k8 =

```

```

para(19) = 0.0002272581636;

%      k9 =
para(20) = 0.6179192106;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Plot Results %%%%%%%%%%
%      Calls the function ABM by Matthew T. Houston.
%      ABM requires:
%      ABM(ODE handle, [start time,end time],time step,initial conditions)
w = ABM(@(t,w) DynSys(t,w,para,IC),[t_min,t_max],h,IC);

%      ABM outputs a matrix of estimated solution points.
%      w = (F(t); R(t); N(t); D(t); M1(t); M2c(t); M2a(t); M3(t); Mb(t); Md(t))

%      Covertng w matrix into friendly variables
F=w(1,:);R=w(2,:); N=w(3,:); D=w(4,:);
M1=w(5,:); M2c=w(6,:); M2a=w(7,:); M3=w(8,:);
Mb=w(9,:); Md = w(10,:);

%      Creating the time variable based on [start time,end time] used in ABM
t = t_min:h:t_max;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Time Course of the FRiND Model %%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Figure 4.5 %%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

figure(1)

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Muscle Tissue %%%%%%%%%%
subplot(2,2,[1,2]);

h = plot(t,F,'k');
set(h,'linewidth',2);
axis([t_min t_max 0 100])

hold on

h = plot(t,R,'b');
set(h,'linewidth',2);

```

```

h = plot(t,N,'g');
set(h,'linewidth',2);

h = plot(t,D,'r');
set(h,'linewidth',2);

xlabel('Time in Days');
ylabel('Percent of Tissue');
xticks(t_min:14:t_max);
yticks(0:20:100);
set(gca,'FontSize',20);

legend('Fibrotic Tissue','Regenerating Tissue','Normal Tissue','Damage Tissue')

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Macrophages %%%%%%%%%
subplot(2,2,3);

h = plot(t,M1,'r');
set(h,'linewidth',3);

hold on

h = plot(t,M3,'k');
set(h,'linewidth',3);

plot(t,M2c,'b','linewidth',3);
hold on

plot(t,M2a,'g','linewidth',3);

axis([t_min t_max 0 7000])
legend('M_1','M_3','M_{2c}','M_{2a}');
xlabel('Time in Days');
ylabel('Cells per mm^3');
axis([t_min t_max 0 2000])
xticks(t_min:14:t_max);
yticks(0:500:2000);
set(gca,'FontSize',20);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Myocytes %%%%%%%%%
subplot(2,2,4);

```

```

h = plot(t,Mb,'b');
set(h,'linewidth',3);

hold on

h = plot(t,Md,'r');
set(h,'linewidth',3);

xlabel('Time in Days');
ylabel('Cells per mm^3');
axis([t_min t_max 0 2000])
xticks(t_min:14:t_max);
yticks(0:500:2000);
set(gca,'FontSize',20);

legend('Myoblast','Differentiated Myoblasts');
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Phase Portrait of N x F %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Figure 4.6 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

figure(2)

n = size(t,2);
sub = 1; % Current subgraph
nsub = 3; % Square root of the number of plots created

F = 0:10:100; % Set Fibrous Tissue sampling
N = 0:10:100; % Set Normal Tissue sampling

prime = zeros(2,size(F,2)*size(N,2));
x = zeros(1,size(F,2)^2);
y = zeros(1,size(F,2)^2);

% Creates evenly timed phase portraits.
% FNSys is the FRiND model projected onto the NxF plane.
% Wprime uses feval to find the projected derivatives at a given time.
for i=ceil((n-1)/(nsub*nsub)):ceil((n-1)/(nsub*nsub)):(n-1)

```

```

    if sub == (nsub*nsub-1)
        i = n;
    end

    for j = 1:size(N,2)
        for k=1:size(N,2)
            prime(:,(j-1)*size(N,2)+k) = Wprime(@(t,w) FNSys(t,w,para,IC),t(i),[F(j),N(k)]);
            x((j-1)*size(N,2)+k) = N(j);
            y((j-1)*size(N,2)+k) = F(k);
        end
    end

    subplot(4,2,sub)

    %    sub is used to populate the 3x3 subplots.

    %    Creates the arrows in the phase portrait.
    quiver(x,y,prime(2,:),prime(1,:),.6,'k','linewidth',2,'MarkerSize',100);

    %    Creates all the titles and labels for each subgraph.
    title(['t = ',num2str(t(i)),' days']);
    xlabel('N')
    ylabel('F')
    axis([0 100 0 100])
    xticks(0:20:100);
    yticks(0:20:100);
    set(gca,'FontSize',15);

    hold on

    %    Plots the red circle giving the point (N(t),F(t)).
    plot(w(3,i),w(1,i),'o','linewidth',2,'MarkerSize',10);
    plot(w(3,1:i),w(1,1:i),'r','linewidth',2);

    %    Plots the N+F=100 constraint.
    plot(N,100-N,'b','linewidth',2);

    sub = sub+1;

end

function f = inputDamage(t)

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Fading Periodic Damage %%%%%%%%%
% This is used to simulate damage to muscle due to normal daily activity.

```

```

% Severity is a matrix of peak damage for each day. In the dissertation
% this is referred to as 'a.'

% Matrix of random amplitude (peak) for daily damage.
global severity

day = findDay(t);

if(day < 50)
    f = severity(day+1)*sin(pi*t).^2 ./ (t+1).^(.5);
else
    f=0;
end

end

function p = Wprime(ODE,t,w)
% This is used to calculate the velocity of the FRiND model at a given time point.
% Required for phase portrait graphs.
p = feval(ODE,t,w);

end

function k = dipth(t)
% Not used in this script. Thus, the function is set to zero.
k = 0;

end

function day = findDay(t)
% Finds current day depending on given t.
% Needed for periodic damage functions to know which element of the
% severity matrix to use.
day = 0;

    while t > day + 1
        day = day + 1;

    end

end

function dwdt = FNSys(t,w,para,IC)
% A paired down version of DynSys. This allows calculation of Normal and

```

```

% Fibrous velocities which are needed for phase portraits.

%    Assign friendly names to depended variables.
F = w(1);
N = w(2);
R = w(3);
M2c = w(4);
M2a = w(5);
Md = w(6);

%    Assign friendly names to parameters.
d1 = para(6);
d2 = para(7);
d6 = para(11);

Mc0 = IC(6);

%    Calls to a user defined function that can be set for different damage
%    outputs.
f = inputDamage(t);

%    The Normal and Fibrous ODE system.
dF_dt = d1*M2c*R - d6*F*Md/(M2c - Mc0 + 1) + d2*M2a*R;
dN_dt = d6*F*Md/(M2c - Mc0 + 1) - f*N;

%    Assemble the vector with derivatives
dwdt=[dF_dt; dN_dt];
end

function dwdt = DynSys(t,w,para,IC)

%    Assign friendly names to depended variables.
F = w(1);
R = w(2);
N = w(3);
D = w(4);
M1 = w(5);
M2c = w(6);
M2a = w(7);
M3 = w(8);
Mb = w(9);
Md = w(10);

%    Assign friendly names to parameters.

```

```

a1 = para(1); a2 = para(2); a3 = para(3); a4 = para(4);
a5 = para(5); d1 = para(6); d2 = para(7); d3 = para(8);
d4 = para(9); d5 = para(10); d6 = para(11); k1 = para(12);
k2 = para(13); k3 = para(14); k4 = para(15); k5 = para(16);
k6 = para(17); k7 = para(18); k8 = para(19); k9 = para(20);

Mc0 = IC(6);
Ma0 = IC(7);
Mb0 = IC(9);

% Calls to a user defined function that can be set for different damage
% outputs.
f = inputDamage(t);

% Calls to a user defined function that can be set for diptheria's inhibiting M_1
% macrophage recruitment.
g = diphth(t);

% The FRiND model ODE system
dF_dt = d1*M2c*R - d6*F*Md/(M2c - Mc0 + 1) + d2*M2a*R;
dR_dt = d3*M1*D - d1*M2c*R + d4*M2a*D + d5*M2c*D - d2*M2a*R;
dN_dt = d6*F*Md/(M2c - Mc0 + 1) - f*N;
dD_dt = f*N - d3*M1*D - d4*M2a*D - d5*M2c*D;

dM1_dt = k1*D^2 - k2*M1*D - k3*M1*M2c + k4*M1*M3 - g*M1;
dM2c_dt = k2*M1*D - k5*(M2c - Mc0)*D - k8*(M2c - Mc0)*M2a;
dM2a_dt = k5*(M2c - Mc0)*D + k6*M2a*M3 + k8*(M2c - Mc0)*M2a - k7*M1*M2a - k9*(M2a -
dM3_dt = k3*M1*M2c + k7*M1*M2a - k4*M1*M3 - k6*M2a*M3;

dMb_dt = a1*M1*Mb - a2*(Mb - Mb0)*M2a;
dMd_dt = a2*(Mb - Mb0)*M2a - (a3*Md*F)/(M2c - Mc0 + 1) - a4*Md - a5*M1*Md;

% Assemble the vector with derivatives
dwdt=[dF_dt; dR_dt; dN_dt; dD_dt; dM1_dt; dM2c_dt; dM2a_dt; dM3_dt; dMb_dt; dMd_dt];
end

```

APPENDIX F

FIGURES_4_7.M

```
% Code used to create Figures 4.7
% f(t) = (h/sigma*sqrt(2*pi)*(t+1))*exp((-1/(2*sigma^2))*(ln(t+1)-m)^2)
% g(t) = 50 0.3<t<2 and 0 everywhere else.
% Creates one figure for acute damage function and M_1 inhibition:
% Figure 1: Time course for all substrates.
% By Matthew T. Houston

% Set the number of days for the time course.
    t_min=0; t_max = 14;

% Step size of ABM solver
    h=.0001;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Initial Conditions %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
    IC = [0 0 100 0 0 200 200 0 100 0];

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Setting Model Parameters %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
    para = zeros(1,21);

%      a1 =
para(1) = 0.0003420387898;

%      a2 =
para(2) = 0.00027805269;

%      a3 =
para(3) = 5.926671787;

%      a4 =
para(4) = 0.1685466672;

%      a5 =
```

```

para(5) = 0.0004735032862;

%      d1 =
para(6) = 0.0001;

%      d2 =
para(7) = 0.000199043595;

%      d3 =
para(8) = 1.041101141e-05;

%      d4 =
para(9) = 0.0005912309411;

%      d5 =
para(10) = 0.000103662336;

%      d6 =
para(11) = 0.310343;

%      k1 =
para(12) = 0.9167670584;

%      k2 =
para(13) = 0.007512675612;

%      k3 =
para(14) = 0.01113163887;

%      k4 =
para(15) = 0.01332585023;

%      k5 =
para(16) = 0.002183270219;

%      k6 =
para(17) = 0.001135187023;

%      k7 =
para(18) = 0.0005064492007;

%      k8 =
para(19) = 0.0002272581636;

%      k9 =

```

```

para(20) = 0.6179192106;

% h =
% Different from step size.
para(21) = 9.466706714;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Plot Results %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Calls the function ABM by Matthew T. Houston.
% ABM requires:
% ABM(ODE handle, [start time,end time],time step,initial conditions)
w = ABM(@(t,w) DynSys(t,w,para,IC),[t_min,t_max],h,IC);

% ABM outputs a matrix of estimated solution points.
% w = (F(t); R(t); N(t); D(t); M1(t); M2c(t); M2a(t); M3(t); Mb(t); Md(t))

% Covertng w matrix into friendly variables
F=w(1,:);R=w(2,:); N=w(3,:); D=w(4,:);
M1=w(5,:); M2c=w(6,:); M2a=w(7,:); M3=w(8,:);
Mb=w(9,:); Md = w(10,:);

% Creating the time variable based on [start time,end time] used in ABM
t = t_min:h:t_max;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Time Course of the FRiND Model %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Figure 4.7 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
figure(1)

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Muscle Tissue %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
subplot(2,2,[1,2]);

h = plot(t,F,'k');
set(h,'linewidth',2);
axis([t_min t_max 0 100])

hold on

h = plot(t,R,'b');
set(h,'linewidth',2);

h = plot(t,N,'g');

```

```

set(h,'linewidth',2);

h = plot(t,D,'r');
set(h,'linewidth',2);

xlabel('Time in Days');
ylabel('Percent of Tissue');
xticks(t_min:1:t_max);
yticks(0:20:100);
set(gca,'FontSize',20);

legend('Fibrotic Tissue','Regenerating Tissue','Normal Tissue','Damage Tissue')

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Macrophages %%%%%%%%%
subplot(2,2,3);

h = plot(t,M1,'r');
set(h,'linewidth',3);

hold on

h = plot(t,M3,'k');
set(h,'linewidth',3);

plot(t,M2c,'b','linewidth',3);
hold on

plot(t,M2a,'g','linewidth',3);

axis([t_min t_max 0 7000])
legend('M_1','M_3','M_{2c}','M_{2a}');
xlabel('Time in Days');
ylabel('Cells per mm^3');
axis([t_min t_max 0 7000])
xticks(t_min:1:t_max);
yticks(0:1000:7000);
set(gca,'FontSize',20);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Myocytes %%%%%%%%%
subplot(2,2,4);

```

```

h = plot(t,Mb,'b');
set(h,'linewidth',3);

hold on

h = plot(t,Md,'r');
set(h,'linewidth',3);

xlabel('Time in Days');
ylabel('Cells per mm^3');
axis([t_min t_max 0 5000])
xticks(t_min:1:t_max);
yticks(0:1000:5000);
set(gca,'FontSize',20);

legend('Myoblast','Differentiated Myoblasts');
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

function f = inputDamage(t,h)

    %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Lognormal Damage %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
    % This is used to simulate CTX damage to muscle.
    % sigma is the standard deviation of damage over time.
    % m is the log(time) at which average damage occurs.
    % h modifies peak damage.

    sigma = .01;
    m = .2;

    f = (h./((t+1).*sigma.*sqrt(2*pi))).*exp(-1*(log(t+1)-m).^2/(2*sigma^2));

end

function k = diphth(t)
% Used to simulate introduction of diphtheria toxin (DT) as described in
% Arnold et al (2007)
% DT is introduced at t=0.3 days and is sufficiently gone by t=2 days.
% A simple constant function is used to approximate this toxin's effects.

if(t<.3)
    k = 0;
elseif(t<2)

```

```

        k = 50;
    else
        k = 0;
    end

end

end

function dwdt = DynSys(t,w,para,IC)

%    Assign friendly names to depended variables.
F = w(1);
R = w(2);
N = w(3);
D = w(4);
M1 = w(5);
M2c = w(6);
M2a = w(7);
M3 = w(8);
Mb = w(9);
Md = w(10);

%    Assign friendly names to parameters.
a1 = para(1); a2 = para(2); a3 = para(3); a4 = para(4);
a5 = para(5); d1 = para(6); d2 = para(7); d3 = para(8);
d4 = para(9); d5 = para(10); d6 = para(11); k1 = para(12);
k2 = para(13); k3 = para(14); k4 = para(15); k5 = para(16);
k6 = para(17); k7 = para(18); k8 = para(19); k9 = para(20);
h = para(21);

Mc0 = IC(6);
Ma0 = IC(7);
Mb0 = IC(9);

%    Calls to a user defined function that can be set for different damage
%    outputs.
f = inputDamage(t,h);

%    Calls to a user defined function that can be set for diptheria's inhibiting M_1
%    macrophage recruitment.
g = dipth(t);

%    The FRiND model ODE system

```

```

dF_dt = d1*M2c*R - d6*F*Md/(M2c - Mc0 + 1) + d2*M2a*R;
dR_dt = d3*M1*D - d1*M2c*R + d4*M2a*D + d5*M2c*D - d2*M2a*R;
dN_dt = d6*F*Md/(M2c - Mc0 + 1) - f*N;
dD_dt = f*N - d3*M1*D - d4*M2a*D - d5*M2c*D;

dM1_dt = k1*D^2 - k2*M1*D - k3*M1*M2c + k4*M1*M3 - g*M1;
dM2c_dt = k2*M1*D - k5*(M2c - Mc0)*D - k8*(M2c - Mc0)*M2a; % M_2c(0) = 200
dM2a_dt = k5*(M2c - Mc0)*D + k6*M2a*M3 + k8*(M2c - Mc0)*M2a - k7*M1*M2a - k9*(M2a -
dM3_dt = k3*M1*M2c + k7*M1*M2a - k4*M1*M3 - k6*M2a*M3;

dMb_dt = a1*M1*Mb - a2*(Mb - Mb0)*M2a; % M_b(0) = 100
dMd_dt = a2*(Mb - Mb0)*M2a - (a3*Md*F)/(M2c - Mc0 + 1) - a4*Md - a5*M1*Md;

% Assemble the vector with derivatives
dwdt=[dF_dt; dR_dt; dN_dt; dD_dt; dM1_dt; dM2c_dt; dM2a_dt; dM3_dt; dMb_dt; dMd_dt];
end

```

APPENDIX G

SENSITIVITYMATRIX.M

```
% Code used to create the sensitivity matrix for Chapter 3
% The script will create a 10n by m matrix of partial derivatives where:
%   n is the number of time points we have data on.
%   m is the number of parameters.
%   10 is the number of substrates.
% We will use central difference formulas to approximate partial derivatives.
% By Matthew T. Houston

% Set the number of days for the time course.
    t_min=0; t_max = 14;

% Step size of ABM solver
    h=.0001;

% Time points we have data on.
    Time = [2,4,7,14];

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Initial Conditions %%%%%%%%%%
    IC = [0 0 100 0 0 200 200 0 100 0];

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Setting Model Parameters %%%%%%%%%%
    para = zeros(1,21);

%     a1 =
para(1) = 0.0003420387898;

%     a2 =
para(2) = 0.00027805269;

%     a3 =
para(3) = 5.926671787;
```

```

%      a4 =
para(4) = 0.1685466672;

%      a5 =
para(5) = 0.0004735032862;

%      d1 =
para(6) = 0.0001;

%      d2 =
para(7) = 0.000199043595;

%      d3 =
para(8) = 1.041101141e-05;

%      d4 =
para(9) = 0.0005912309411;

%      d5 =
para(10) = 0.000103662336;

%      d6 =
para(11) = 0.310343;

%      k1 =
para(12) = 0.9167670584;

%      k2 =
para(13) = 0.007512675612;

%      k3 =
para(14) = 0.01113163887;

%      k4 =
para(15) = 0.01332585023;

%      k5 =
para(16) = 0.002183270219;

%      k6 =
para(17) = 0.001135187023;

%      k7 =
para(18) = 0.0005064492007;

```

```

%      k8 =
para(19) = 0.0002272581636;

%      k9 =
para(20) = 0.6179192106;


% h =
% Different from step size.
para(21) = 9.466706714;


%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Plot Results %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%   Calls the function ABM by Matthew T. Houston.
%   ABM requires:
%   ABM(ODE handle, [start time,end time],time step,initial conditions)
w = ABM(@(t,w) DynSys(t,w,para,IC),[t_min,t_max],h,IC);


%   Creating the time variable based on [start time,end time] used in ABM
t = t_min:h:t_max;


% Stores the location of time points that we have data on.
tt(1) = find(t==Time(1));
tt(2) = find(t==Time(2));
tt(3) = find(t==Time(3));
tt(4) = find(t==Time(4));


%   n is the number of time points we have data on.
%   m is the number of parameters.
n = size(tt,2);
m = size(para,2);


% The size of perturbation in the parameter space used to approximate a
% partial derivative.
h1 = h/100;


% Creating the sensitivity matrix.
SM = zeros(10*n,m);


% Iterates through the partial of the substrates in respect to each parameter
% (i)

```

```

for i=1:m
%   Store original parameter sets each iteration.
    paraUp1 = para;
    paraUp2 = para;

%   Perturbates a specific parameter each iteration.
    paraUp1(i) = paraUp1(i) + h1;
    paraUp2(i) = paraUp2(i) - h1;

%   Calculates the time course with the perturbed parameter set.
    g1 = ABM(@(t,w) DynSys(t,w,paraUp1,IC),[t_min,t_max],h,IC);
    g2 = ABM(@(t,w) DynSys(t,w,paraUp2,IC),[t_min,t_max],h,IC);

%   Calculates the partial derivatives for each time point and substrate.
    for j=1:n
        SM(j:4:40,i) = (g1(:,tt(j)) - g2(:,tt(j)))/(2*h1);
    end
end

% Calculates the Fisher Matrix according to equation 3.14
Fisher = SM'*SM;

% Gives the determinate of the Fisher matrix. A small or zero determinate
% would imply the model is unidentifiable.
det(Fisher)

function f = inputDamage(t,h)

    %%%%%%%%%%%%%%% Lognormal Damage %%%%%%%%%%%%%%%
% This is used to simulate CTX damage to muscle.
% sigma is the standard deviation of damage over time.
% m is the log(time) at which average damage occurs.
% h modifies peak damage.

    sigma = .01;
    m = .2;

    f = (h./((t+1).*sigma.*sqrt(2*pi))).*exp(-1*(log(t+1)-m).^2/(2*sigma^2));

end

```

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

```
function k = diphth(t)
% Not used in this script. Thus, the function is set to zero.
k = 0;
```

```
end
```

```
function dwdt = DynSys(t,w,para,IC)
% Assign friendly names to depended variables.
F = w(1);
R = w(2);
N = w(3);
D = w(4);
M1 = w(5);
M2c = w(6);
M2a = w(7);
M3 = w(8);
Mb = w(9);
Md = w(10);

% Assign friendly names to parameters.
a1 = para(1); a2 = para(2); a3 = para(3); a4 = para(4);
a5 = para(5); d1 = para(6); d2 = para(7); d3 = para(8);
d4 = para(9); d5 = para(10); d6 = para(11); k1 = para(12);
k2 = para(13); k3 = para(14); k4 = para(15); k5 = para(16);
k6 = para(17); k7 = para(18); k8 = para(19); k9 = para(20);
h = para(21);

Mc0 = IC(6);
Ma0 = IC(7);
Mb0 = IC(9);

% Calls to a user defined function that can be set for different damage
% outputs.
f = inputDamage(t,h);

% Calls to a user defined function that can be set for diptheria's inhibiting M_1
% macrophage recruitment.
g = diphth(t);
```

```

%           The FRiND model ODE system
dF_dt = d1*M2c*R - d6*F*Md/(M2c - Mc0 + 1) + d2*M2a*R;
dR_dt = d3*M1*D - d1*M2c*R + d4*M2a*D + d5*M2c*D - d2*M2a*R;
dN_dt = d6*F*Md/(M2c - Mc0 + 1) - f*N;
dD_dt = f*N - d3*M1*D - d4*M2a*D - d5*M2c*D;

dM1_dt = k1*D^2 - k2*M1*D - k3*M1*M2c + k4*M1*M3 - g*M1;
dM2c_dt = k2*M1*D - k5*(M2c - Mc0)*D - k8*(M2c - Mc0)*M2a; % M_2c(0) = 200
dM2a_dt = k5*(M2c - Mc0)*D + k6*M2a*M3 + k8*(M2c - Mc0)*M2a - k7*M1*M2a - k9*(M2a -
dM3_dt = k3*M1*M2c + k7*M1*M2a - k4*M1*M3 - k6*M2a*M3;

dMb_dt = a1*M1*Mb - a2*(Mb - Mb0)*M2a; % M_b(0) = 100
dMd_dt = a2*(Mb - Mb0)*M2a - (a3*Md*F)/(M2c - Mc0 + 1) - a4*Md - a5*M1*Md;

% Assemble the vector with derivatives
dwdt=[dF_dt; dR_dt; dN_dt; dD_dt; dM1_dt; dM2c_dt; dM2a_dt; dM3_dt; dMb_dt; dMd_dt];
end

```