



**Defense Threat Reduction Agency
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TECHNICAL REPORT

A Time-Dependent Physiological Model of Healing for a Stable, Closed Bone Fracture

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HDTRA1-14-D-0003

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UNIT CONVERSION TABLE

U.S. customary units to and from international units of measurement*

U.S. Customary Units	Multiply by Divide by[†]	International Units
Length/Area/Volume		
inch (in)	2.54 × 10 ⁻²	meter (m)
foot (ft)	3.048 × 10 ⁻¹	meter (m)
yard (yd)	9.144 × 10 ⁻¹	meter (m)
mile (mi, international)	1.609 344 × 10 ³	meter (m)
mile (nmi, nautical, U.S.)	1.852 × 10 ³	meter (m)
barn (b)	1 × 10 ⁻²⁸	square meter (m ²)
gallon (gal, U.S. liquid)	3.785 412 × 10 ⁻³	cubic meter (m ³)
cubic foot (ft ³)	2.831 685 × 10 ⁻²	cubic meter (m ³)
Mass/Density		
pound (lb)	4.535 924 × 10 ⁻¹	kilogram (kg)
atomic mass unit (AMU)	1.660 539 × 10 ⁻²⁷	kilogram (kg)
pound-mass per cubic foot (lb ft ⁻³)	1.601 846 × 10 ¹	kilogram per cubic meter (kg m ⁻³)
Pound-force (lbf avoirdupois)	4.448 222	Newton (N)
Energy/Work/Power		
electron volt (eV)	1.602 177 × 10 ⁻¹⁹	joule (J)
erg	1 × 10 ⁻⁷	joule (J)
kiloton (kT) (TNT equivalent)	4.184 × 10 ¹²	joule (J)
British thermal unit (Btu) (thermochemical)	1.054 350 × 10 ³	joule (J)
foot-pound-force (ft lbf)	1.355 818	joule (J)
calorie (cal) (thermochemical)	4.184	joule (J)
Pressure		
atmosphere (atm)	1.013 250 × 10 ⁵	pascal (Pa)
pound force per square inch (psi)	6.984 757 × 10 ³	pascal (Pa)
Temperature		
degree Fahrenheit (°F)	[T(°F) – 32]/1.8	degree Celsius (°C)
degree Fahrenheit (°F)	[T(°F) + 459.67]/1.8	kelvin (K)
Radiation		
activity of radionuclides [curie (Ci)]	3.7 × 10 ¹⁰	per second (s ^{-1‡})
air exposure [roentgen (R)]	2.579 760 × 10 ⁻⁴	coulomb per kilogram (C kg ⁻¹)
absorbed dose (rad)	1 × 10 ⁻²	joule per kilogram (J kg ^{-1§})
equivalent and effective dose (rem)	1 × 10 ⁻²	joule per kilogram (J kg ^{-1**})

*Specific details regarding the implementation of SI units may be viewed at <http://www.bipm.org/en/si/>.

[†]Multiply the U.S. customary unit by the factor to get the international unit. Divide the international unit by the factor to get the U.S. customary unit.

[‡]The special name for the SI unit of the activity of a radionuclide is the becquerel (Bq). (1 Bq = 1 s⁻¹).

[§]The special name for the SI unit of absorbed dose is the gray (Gy). (1 Gy = 1 J kg⁻¹).

^{**}The special name for the SI unit of equivalent and effective dose is the sievert (Sv). (1 Sv = 1 J kg⁻¹).

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Executive Summary

The purpose of this technical report (TR) is to present a time-dependent physiological model (TDPM) governed by seven ordinary differential equations (ODEs) for the healing of closed, stable fractures. The TDPM will serve as a foundational structure for the development of physiologically driven models of combined injury¹ resulting from a nuclear detonation (NUDET) event. The TDPM characterizes local inflammation and repair dynamics of the fracture site and surrounding area following bone fracture. Local inflammation is based on a more general wound healing framework consistent with other previously developed TDPMs (Jennings et al. 2022). This provides a unifying mechanistic framework with which to develop new models for single injuries without considerable overhaul and, more importantly, their combinations for use in the Health Effects from Nuclear and Radiological Environments (HENRE), a casualty assessment tool developed by the Defense Threat Reduction Agency (DTRA). Numerical simulations with the TDPM will demonstrate the ability of the model to produce biologically sound results and to discuss model dynamics in the case of normal fracture healing. Some initial conditions and parameters of the TDPM will then be perturbed from their baseline values to display changes in model behavior and the progression of fracture healing over time in various scenarios. This TR will not discuss in detail the parameter estimation process or the process of fracture healing for higher severity fractures beyond minor-to-moderate, as these will be discussed in future work. Future efforts beyond this will focus on improving the TDPM by way of global sensitivity analysis and integration into a TDPM for combined injury.

¹ Combined injury, more generally, refers to the injury resulting from two or more insults (blast, thermal, radiation, or other) impacting the body simultaneously or near so.

Section 1. Introduction

In a NUDET event, the subsonic blast wind pressure that follows the leading shock front can cause bone fracture by secondary or tertiary mechanisms (Yazgan and Aksu 2016; Jahangiri et al. 2018; Walilko et al. 2021). Blast wind can move debris resulting in secondary blast injury if the displaced debris impacts the body; in this case, injuries such as bone fracture occur when tissues deform beyond their failure tolerance (Foege et al. 1985). Tertiary blast injuries occur and may result in bone fracture when the same blast wind displaces human bodies, throwing them into surrounding objects or to the ground (Reeves 2018).

The qualitative severity of bone fracture critically determines the downstream inflammatory response and corresponding ability to recover correctly and in a timely manner. Injury severities are defined by injury codes such as the Abbreviated Injury Scale (AIS), the Military Combat Injury Scale (MCIS), or the International Statistical Classification of Diseases and Related Health Problems (ICD-10) (Lawnick et al. 2013). This concept of categorizing the severity of a specific injury is a crucial aspect of the blast-induced injury modeling framework for HENRE (Wagner and Rolfs 2022) because severities can be correlated to functional outcomes and performance. For instance, bone fracture severities identified via MCIS coding are mapped to the Military Functional Incapacity Scale (MFIS) to indicate the ability of an injured individual to perform their duties. Fractures with a severity index of 1 (minor) are superficial with minimal functional impairment. However, fractures with a severity index of 2 (moderate) are accompanied by impairment, but the injured party is likely able to perform mission tasks. At severities of 3 (serious) and above, the fracture causes functional impairment that needs to be treated within six hours and prevents task performance (Wagner and Rolfs 2022). Thus, these codes inform functional deficit outcomes based on the defined level of severity.

In the current time-dependent physiological modeling development work, the authors chose to focus on closed, stable fractures because the wound healing process is the simplest of the fracture types. Closed, stable fractures almost always heal without grave complications as they can be stabilized without surgery (Einhorn 1998; Hoppenfeld and Murthy 2000; Betts et al. 2013; Muire et al. 2020); that is, the injury is closed and skin around the fracture remains intact reducing the risk of infection (Betts et al. 2013). Further, stability ensures there is little displacement of the fracture itself, which limits risk of improper healing (Naimark et al. 1980; Hoppenfeld and Murthy 2000; Calori et al. 2007). Additionally, closed, stable fractures are minor to moderate severity in the AIS and MCIS coding systems, possibly resulting in a performance decrement while more severe fractures may prevent task performance entirely. Modeling less severe fracture healing therefore provides a more refined measurement of performance decrement, whereas modeling severe fracture healing would be more appropriate for longer term predictions and medical planning.

The presented TDPM modeling efforts will first examine the inflammatory and early proliferation activity initiated by these stable, closed fracture types with the goal of ensuring dynamics predicted by the model replicate normal fracture healing before considering other injuries that could greatly exacerbate the healing process. This understanding will provide a foundation of “healthy” dynamics that can be expanded to incorporate different pathological profiles.

Section 2. Background

Following bone fracture, a complex biological process is initiated to achieve a full reconstruction of the bone, including restoration of sustained function and anatomic continuity (Cruess and Dumont 1975; Einhorn 1998; Hoppenfeld and Murthy 2000; Claes et al. 2012). Healing of a bone fracture is typically described in three overlapping phases: inflammation, repair, and remodeling (Cruess and Dumont 1975; Claes et al. 2012). These phases align with those of the general healing framework, with hemostasis being encapsulated within the inflammatory stage, and “repair” in this case considered to be equivalent to the proliferative phase of wound healing.

A bone fracture occurs when the structural continuity of the bone cortex is breached resulting in a fracture gap and residual cellular debris from bone loss (Sheen and Garla 2020). Initially, bone fracture causes blood vessels in the bone and the surrounding fracture site to rupture, resulting in a hematoma (Cruess and Dumont 1975; Hoppenfeld and Murthy 2000; Kolar et al. 2010; Betts et al. 2013; Sheen and Garla 2020). The hematoma clots and acts as a temporary scaffold for the active invasion of inflammatory cells (Hoppenfeld and Murthy 2000; Kolar et al. 2010; Sheen and Garla 2020). Unlike thermal burns, where no bleeding occurs (Abazari et al. 2020), this immediate response following bone fracture closely resembles hemostasis. Although not explicitly called out as hemostasis in literature, the hematoma serves the same function: stop the bleeding resulting from the wounding event and allow for active invasion of the inflammatory cells (Hoppenfeld and Murthy 2000; Kolar et al. 2010; Sheen and Garla 2020).

Neutrophils are the first predominant leukocyte recruited at the local bone fracture site (Chung et al. 2006; Goldberg and Diegelmann 2010; Kolar et al. 2010; Muire et al. 2020), after which macrophages dominate to become the most prevalent (Glynne Andrew et al. 1994; Kolar et al. 2010). Macrophages are first polarized to a pro-inflammatory M1 phenotype (Schlundt et al. 2018). Both neutrophils and pro-inflammatory M1 macrophages work to clear dead cells and debris from the fracture site (Goldberg and Diegelmann 2010; Kolar et al. 2010; Muire et al. 2020). As healing progresses, macrophages gradually transition to an anti-inflammatory M2 phenotype (Schlundt et al. 2018). M2 macrophages are responsible for down-regulating and resolving inflammation (Mills and Ley 2014; Schlundt et al. 2018), and therefore a proper transition between the pro- and anti-inflammatory states is needed to ensure timely fracture healing (Schlundt et al. 2018; Muire et al. 2020). This phase of healing concludes by stimulating angiogenesis, signaling for revascularization, and promoting differentiation of mesenchymal stem cells (MSCs)², all of which contribute to instigation of the next stage of fracture healing (Sheen and Garla 2020).

The repair phase begins with the differentiation of MSCs into fibroblasts, chondrocytes, and osteoblasts (Bahney et al. 2019; Sheen and Garla 2020; Muire et al. 2020). Following their proliferation, fibroblasts and chondrocytes release new extracellular matrix (ECM) to fill the fracture gap with fibrocartilage, while osteoblasts deposit new bone (Hoppenfeld and Murthy 2000; Bahney et al. 2019; Sheen and Garla 2020; Muire et al. 2020). Following thermal burn injury, fibroblasts are the primary cellular component during the proliferative

² Mesenchymal stem cells are multipotent stromal cells most frequently sourced from bone marrow.

stage (Abazari et al. 2020); however, for healing of bone fractures, osteoblasts replace fibroblasts as the primary cellular component during this phase (Hoppenfeld and Murthy 2000; Sheen and Garla 2020). Additionally, during this phase, progenitors from the monocyte/macrophage lineage differentiate into osteoclast precursors (OCP) that eventually become osteoclasts in preparation for the final phase of fracture healing, bone remodeling (Udagawa et al. 1990; Wei et al. 2011; Zhao et al. 2015; Huang et al. 2017).

The remodeling phase is characterized by systematic tissue degradation and production by osteoclasts and osteoblasts, respectively (Cruess and Dumont 1975; Hoppenfeld and Murthy 2000; Marsell and Einhorn 2011; Sheen and Garla 2020; Muire et al. 2020). During this process, fibrocartilage and woven bone are removed and replaced by functional bone (Cruess and Dumont 1975; Hoppenfeld and Murthy 2000; Einhorn 2005; Marsell and Einhorn 2011; Bahney et al. 2019; Sheen and Garla 2020; Muire et al. 2020). The physiological processes that underscore remodeling can take place over a span of months to years, but usually ends with full recovery to the pre-injury state (Cruess and Dumont 1975; Marsell and Einhorn 2011; Claes et al. 2012). The outcome of fracture healing is typically defined by union or non-union, i.e., the fracture gap fully fills with bone or not, respectively. These more generally define healthy or impaired healing, respectively, where a non-union that eventually heals without intervention is referred to as a “delayed union” and is still considered impaired rather than normal healing (Naimark et al. 1980; Frölke and Patka 2007).

Section 3. TDPM of Bone Fracture

3.1 TDPM Compartments and Variables

Healing following a closed, stable fracture occurs locally with immune cells being recruited to the site of injury (see Figure 1A, a reprint from (Muire et al. 2020)). While certain fracture severities can disrupt systemic cell dynamics, here only those that do not are considered; that is, it is assumed that cell levels in the bone marrow, and blood vessels remain constant following injury. This assumption parallels that made previously for the superficial thermal burn model (Jennings et al. 2022). Further, the TDPM presented here purposefully describes dynamics at the fracture site as well as surrounding tissue to align with the data in (Baratchart et al. 2022). This way, initial manual parameterization to conduct preliminary numerical simulations will be successful.

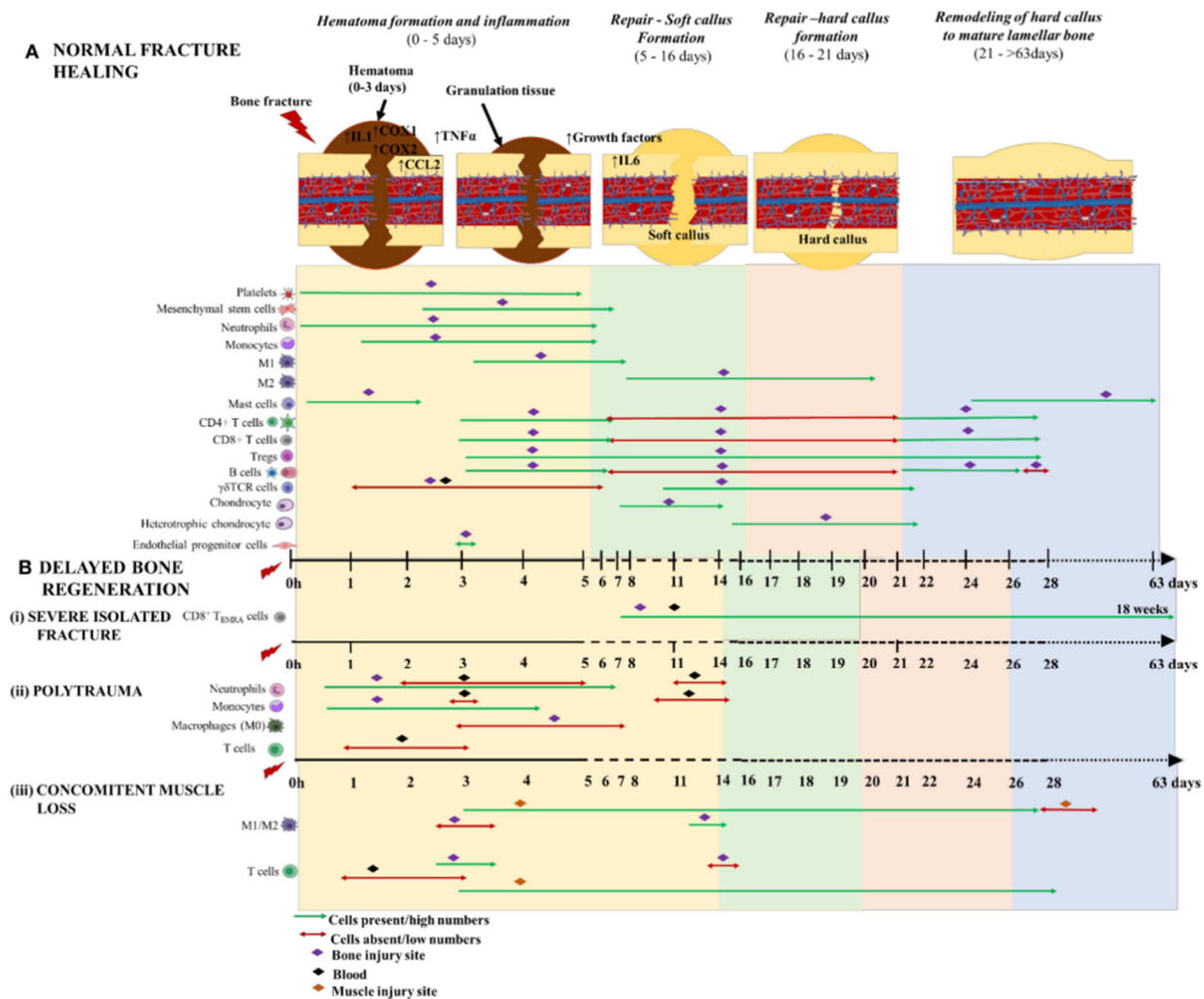


Figure 1. Reprint of Figure 4 in (Muire et al. 2020). Schematic illustration of inflammatory, immune, and skeletal cells during (A) normal and (B) delayed bone fracture healing.

The TDPM for bone fracture consists of seven (7) ODEs. Figure 2 provides a schematic of the state (or dynamic) variables³ and their relationships. State variables are represented using circles and squares. The solid black lines indicate transitions between different cellular states. Solid lines that feedback into the state variable itself indicate proliferation. Initiated cellular interactions are captured by dotted lines and remain locally concentrated. Blue and white colors are used to distinguish between up-regulatory (i.e., increasing in response to the cells themselves) and destructive/inhibitory relationships, respectively.

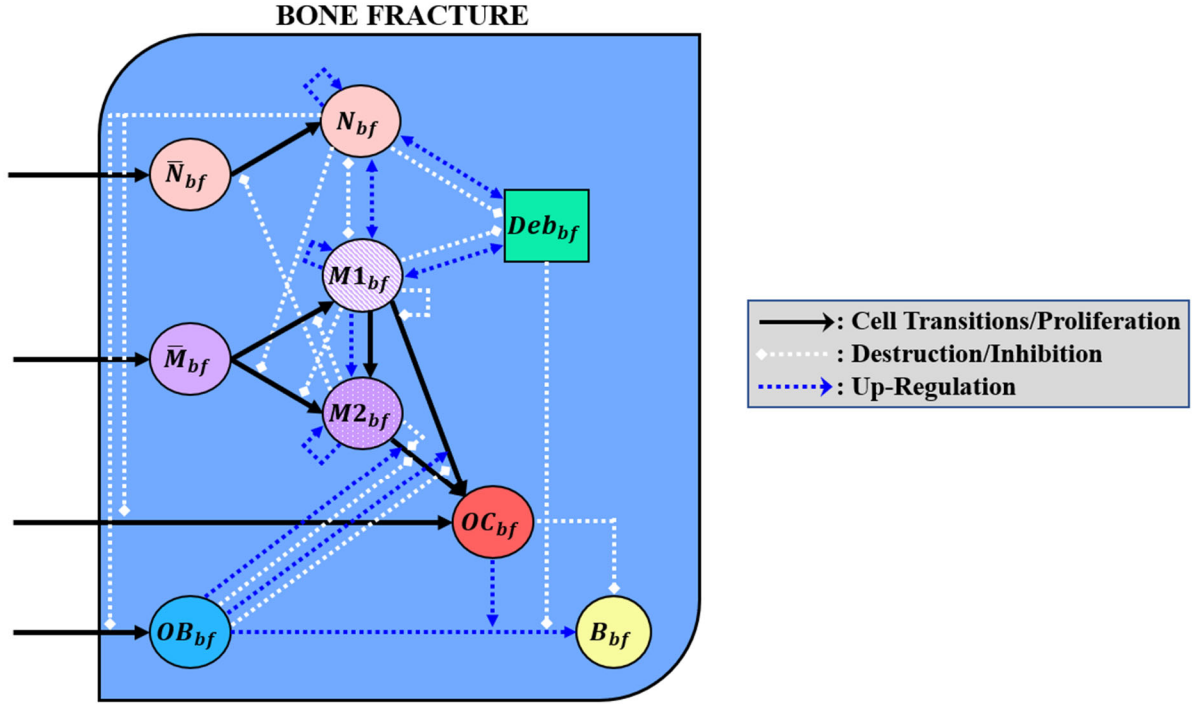


Figure 2. Schematic diagram of bone fracture, the state variables, and their relationships.

Table 1 lists the symbols and corresponding descriptions of the state variables included in the TPDM. State variables are assumed to represent the cell types and their signaling molecules. An ODE was constructed for each state variables based on known physiological behaviors and interactions supported by peer-reviewed literature. Let N , M , OB , and OC denote neutrophils, monocytes/macrophages (collectively referred to as macrophages), osteoblasts, and osteoclasts, respectively, with subscripts (bf) indicating the bone fracture site and surrounding area.

³ State (or dynamic) variables refer to those quantities whose temporal evolution will eventually be described by a system of ordinary differential equations (ODEs), reflecting that their levels change with time.

Table 1. TDPM state variable symbols and definitions.

SYMBOL	DESCRIPTION
t	Time (in hours)
<i>Local Bone Fracture</i>	
Deb_{bf}	Debris
B_{bf}	Bone volume
N_{bf}	Activated neutrophils at the fracture site
$M1_{bf}$	Classically activated macrophages (M1) at the fracture site
$M2_{bf}$	Alternatively activated macrophages (M2) at the fracture site
OB_{bf}	Differentiated osteoblasts at the fracture site
OC_{bf}	Differentiated osteoclasts at the fracture site

3.2 Model Equations

The previously developed TDPM for superficial thermal burn wounds (Jennings et al. 2022) serves as the basis for the presented TDPM for bone fracture. In alignment with the model in (Jennings et al. 2022), systemic neutrophils and monocytes are assumed to have a quasi-steady-state (see last paragraph of Section 5 Discussion for further details). However, to study the inflammatory response and repair activity at and adjacent to the fracture site several modifications must be made to delineate healing for bone fractures from that for superficial burns. These distinctions are discussed in greater detail in this section.

The TDPM for bone fracture accounts for seven state variable dynamics using the system of ODEs given by Equations (1)-(7).

$$\begin{aligned} \frac{dDeb_{bf}}{dt} = & k_{dn}\Omega_H(N_{bf}, 6, N_H) + k_{dm1}\Omega_H(M1_{bf}, 6, M1_H) - k_{dnp}N_{bf}\Omega_H(Deb_{bf}, 1, Deb_H) \\ & - k_{dm1p}\Omega_i(M1_{bf}; N_{bf}, N_1^\infty)\Omega_H(Deb_{bf}, 1, Deb_H) \\ & - k_{dm2p}\Omega_i(M2_{bf}; N_{bf}, N_1^\infty)\Omega_H(Deb_{bf}, 1, Deb_H) - d_{deb}Deb_{bf} \end{aligned} \quad (1)$$

$$\frac{dN_{bf}}{dt} = \frac{s_{nr}R_n}{R_n + d_{nr}} - k_{nm1p}N_{bf}\Omega_i(M1_{bf}; N_{bf}, N_1^\infty) - k_{nm2p}N_{bf}\Omega_i(M2_{bf}; N_{bf}, N_1^\infty) - d_nN_{bf} \quad (2)$$

$$\begin{aligned} \frac{dM1_{bf}}{dt} = & \frac{s_{mr}R_{m1}}{R_{m1} + R_{m2} + d_{mr}} - \frac{(k_{m1oc}M1_{bf})OB_{bf}}{1 + \left(\frac{M1_{bf}}{M1_2^\infty}\right)^2 + \left(\frac{M2_{bf}}{M2_2^\infty}\right)^2 + \left(\frac{OB_{bf}}{OB_2^\infty}\right)^2} \\ & - \theta_{m1m2}[k_{nm1p}N_{bf}\Omega_i(M1_{bf}; N_{bf}, N_1^\infty)] \\ & + \theta_{m2m1}M2_{bf} - d_{m1}M1_{bf} \end{aligned} \quad (3)$$

$$\begin{aligned} \frac{dM2_{bf}}{dt} = & \frac{s_{mr}R_{m2}}{R_{m1} + R_{m2} + d_{mr}} - \frac{(k_{m2oc}M2_{bf})OB_{bf}}{1 + \left(\frac{M1_{bf}}{M1_2^\infty}\right)^2 + \left(\frac{M2_{bf}}{M2_2^\infty}\right)^2 + \left(\frac{OB_{bf}}{OB_2^\infty}\right)^2} \\ & + \theta_{m1m2}[k_{nm1p}N_{bf}\Omega_i(M1_{bf}; N_{bf}, N_1^\infty)] \\ & - \theta_{m2m1}M2_{bf} - d_{m2}M2_{bf} \end{aligned} \quad (4)$$

$$\frac{dOB_{bf}}{dt} = \frac{s_{ob}(1 + k_{obm1}M1_{bf} + k_{obm2}M2_{bf})}{1 + \left(\frac{N_{bf}}{N_2^\infty}\right)^2} - d_{ob}OB_{bf}B_{bf} \quad (5)$$

$$\begin{aligned} \frac{dOC_{bf}}{dt} = & \frac{s_{oc}}{1 + \left(\frac{N_{bf}}{N_2^\infty}\right)^2} + \frac{(k_{m1oc}M1_{bf} + k_{m2oc}M2_{bf})OB_{bf}}{1 + \left(\frac{M1_{bf}}{M1_2^\infty}\right)^2 + \left(\frac{M2_{bf}}{M2_2^\infty}\right)^2 + \left(\frac{OB_{bf}}{OB_2^\infty}\right)^2} \\ & - d_{oc}OC_{bf}(1 + k_{ocm1}M1_{bf} + k_{ocm2}M2_{bf}) \end{aligned} \quad (6)$$

$$\frac{dB_{bf}}{dt} = \frac{\rho_b(1 + k_bOC_{bf})OB_{bf}}{1 + \left(\frac{Deb_{bf}}{Deb^\infty}\right)^2} - d_bOC_{bf}B_{bf} \quad (7)$$

The auxiliary functions R_n , R_{m1} , R_{m2} , $\Omega_i(\cdot)$ and $\Omega_H(\cdot)$ are defined in Equations (8) – (12) below.

$$R_n = \Omega_i(k_{nd}Deb_{bf} + k_{nn}N_{bf}; M2_{bf}, M2_1^\infty) \quad (8)$$

$$R_{m1} = \Omega_i(k_{m1d}Deb_{bf} + k_{m1n}N_{bf} + k_{m1m1}M1_{bf}; M2_{bf}, M2_1^\infty) \quad (9)$$

$$R_{m2} = \Omega_i(k_{m2m1}M1_{bf} + k_{m2m2}M2_{bf}; N_{bf}, N_1^\infty, M1_{bf}, M1_1^\infty) \quad (10)$$

$$\Omega_i = (X; Y_1, Y_1^\infty, \dots, Y_m, Y_m^\infty) = \frac{X}{1 + \left(\frac{Y_1}{Y_1^\infty}\right)^2 + \dots + \left(\frac{Y_m}{Y_m^\infty}\right)^2} \quad (11)$$

$$\Omega_H = (X, n, X_H) = \frac{X^n}{X_H^n + X^n} \quad (12)$$

The state variable symbols and definitions for the bone fracture TDPM are described in Table 1. Each state variable is assumed to represent not only the cell type but also its signalling molecules, such as cytokines and chemokines. Thus, each state variable is more indicative of functionality for the aggregated population than a specific cell. All parameters are assumed to be non-negative, and their descriptions and biological units are summarized in Table 2.

Table 2. Description and assigned baseline values for parameters of TDPM given by Equations (1)-(7).

PARAMETER		DESCRIPTION	UNITS	BASELINE VALUE
Inhibition	Deb^∞	Constant controlling the effectiveness of Deb_{bf} at inhibiting bone repair	D -units	0.7
	N_1^∞	Constant controlling the effectiveness of N_{bf} at inhibiting the activity of other immune cells	N -units	0.1417
	N_2^∞	Constant controlling the effectiveness of N_{bf} at inhibiting the source of OB_{bf} and OC_{bf}	N -units	0.5
	$M1_1^\infty$	Constant controlling the effectiveness of $M1_{bf}$ at inhibiting activation of $M2_{bf}$	M -units	0.1457
	$M1_2^\infty$	Constant controlling the effectiveness of $M1_{bf}$ at inhibiting activation of OC_{bf}	M -units	0.0843
	$M2_1^\infty$	Constant controlling the effectiveness of $M2_{bf}$ at inhibiting activation of N_{bf} and $M1_{bf}$	M -units	0.4734
	$M2_2^\infty$	Constant controlling the effectiveness of $M2_{bf}$ at inhibiting activation of OC_{bf}	M -units	0.2367
	OB^∞	Constant controlling the effectiveness of OB_{bf} at inhibiting activation of OC_{bf}	OB -units	0.5314
Debris	N_H	Hill constant for tissue damage associated with N_{bf}	N -units	0.06
	$M1_H$	Hill constant for tissue damage associated with $M1_{bf}$	M -units	0.06
	Deb_H	Hill constant controlling the effectiveness of Deb_{bf} at inhibiting phagocytic removal of debris by N_{bf} , $M1_{bf}$ and $M2_{bf}$	D -units	0.0610
	k_{dn}	Maximum collateral tissue damage rate caused by N_{bf} at the local wound site	D -units/h	1.75e-04
	k_{dm1}	Maximum collateral tissue damage rate caused by $M1_{bf}$ at the local wound site	D -units/h	8.75e-05
	k_{dnp}	Phagocytosis rate of Deb_{bf} by N_{bf}	D -units/(N -units·h)	0.0087
	k_{dm1p}	Phagocytosis rate of Deb_{bf} by $M1_{bf}$	D -units/(M -units·h)	0.0117
	k_{dm2p}	Phagocytosis rate of Deb_{bf} by $M2_{bf}$	D -units/(M -units·h)	0.0067
	d_{deb}	Decay rate of Deb_{bf}	1/h	1.2e-04

PARAMETER		DESCRIPTION	UNITS	BASELINE VALUE
Neutrophils	s_{nr}	Recruitment rate of circulating neutrophils to the local fracture site	N -units/h	0.0137
	k_{nd}	Activation rate of $\bar{N}_{bf}$ to N_{bf} by Deb_{bf}	$1/(D\text{-units}\cdot h)$	0.2432
	k_{nn}	Activation rate of resting neutrophils $\bar{N}_{bf}$ to N_{bf} by N_{bf} and its byproducts	$1/(N\text{-units}\cdot h)$	0.0169
	k_{nm1p}	Phagocytosis rate of N_{bf} by $M1_{bf}$	$1/(M\text{-units}\cdot h)$	0.1207
	k_{nm2p}	Phagocytosis rate of N_{bf} by $M2_{bf}$	$1/(M\text{-units}\cdot h)$	0.3628
	d_{nr}	Decay rate of $\bar{N}_{bf}$	1/h	0.0190
	d_n	Decay rate of N_{bf}	1/h	0.0206
Macrophages	s_{mr}	Recruitment rate of fixed tissue and circulating monocytes to the local fracture site	M -units/h	3.5150
	k_{m1d}	Activation rate of $\bar{M}_{bf}$ to $M1_{bf}$ by Deb_{bf}	$1/(D\text{-units}\cdot h)$	6.9924e-06
	k_{m1n}	Activation rate of $\bar{M}_{bf}$ to $M1_{bf}$ by N_{bf} and its byproducts	$1/(N\text{-units}\cdot h)$	0.0035
	k_{m1m1}	Activation rate of $\bar{M}_{bf}$ to $M1_{bf}$ by $M1_{bf}$ and its byproducts	$1/(M\text{-units}\cdot h)$	3.7879e-05
	k_{m2m1}	Activation rate of $\bar{M}_{bf}$ to $M2_{bf}$ by $M1_{bf}$ and its byproducts	$1/(M\text{-units}\cdot h)$	0.0110
	k_{m2m2}	Activation rate of $\bar{M}_{bf}$ to $M2_{bf}$ by $M2_{bf}$ and its byproducts	$1/(M\text{-units}\cdot h)$	6.7667e-04
	θ_{m1m2}	Transition rate of $M1_{bf}$ to $M2_{bf}$	$M\text{-units}/N\text{-units}$	0.6901
	θ_{m2m1}	Transition rate of $M2_{bf}$ to $M1_{bf}$	$M\text{-units}$	1.0e-08
	d_{mr}	Decay rate of $\bar{M}_{bf}$	1/h	0.0201
	d_{m1}	Decay rate of $M1_{bf}$	1/h	0.0497

PARAMETER		DESCRIPTION	UNITS	BASELINE VALUE
	d_{m2}	Decay rate of $M2_{bf}$	1/h	0.1236
Osteoblasts	s_{ob}	Non-injury recruitment rate of OB_{bf} to the local fracture site	OB -units/h	1303.24
	k_{obm1}	Constant controlling the upregulation of OB_{bf} proliferation by $M1_{bf}$	$1/(M\text{-units}\cdot h)$	0.4330
	k_{obm2}	Constant controlling the upregulation of OB_{bf} proliferation by $M2_{bf}$	$1/(M\text{-units}\cdot h)$	0.4330
	d_{ob}	Decay rate of OB_{bf}	1/h	0.0133
Osteoclasts	s_{oc}	Non-injury recruitment rate of OC_{bf} to the local fracture site	OC -units/h	1943.33
	k_{m1oc}	Transition rate of $M1_{bf}$ to OC_{bf}	M -units	2.2292e-06
	k_{m2oc}	Transition rate of $M2_{bf}$ to OC_{bf}	M -units	2.2292e-06
	k_{ocm1}	Constant controlling the upregulation of OC_{bf} decay by $M1_{bf}$	$1/(M\text{-units}\cdot h)$	0.0160
	k_{ocm2}	Constant controlling the upregulation of OC_{bf} decay by $M2_{bf}$	$1/(M\text{-units}\cdot h)$	0.0520
	d_{oc}	Decay rate of OC_{bf}	1/h	0.0221
Bone	ρ_b	Baseline proliferation and activation rate of bone	$\text{mm}^3/(OB\text{-units}\cdot h)$	2.1134e-10
	k_b	Constant controlling the upregulation of B_{bf} proliferation by OC_{bf}	$1/(OC\text{-units}\cdot h)$	0.0120
	d_b	Decay rate of B_{bf}	1/h	2.4958e-08
Notation: D , N , M , OB , and OC denote the units of debris (Deb_{bf}), neutrophils ($\bar{N}_{bf}$ and N_{bf}), macrophages ($\bar{M}_{bf}$, $M1_{bf}$ and $M2_{bf}$), osteoblasts (OB_{bf}), and osteoclasts (OC_{bf}), respectively. Note, $\bar{N}_{bf}$ and $\bar{M}_{bf}$ are defined as neutrophils and monocytes (resting and fixed tissue) recruited to the fracture site, respectively.				

In the following subsections, each ODE constituting the model is described in greater detail. Moreover, the corresponding equation(s) is also reprinted first for reading ease.

3.2.1. Debris Equation

The equation for debris at the bone fracture site (Deb_{bf}) is given by the following:

$$\begin{aligned} \frac{dDeb_{bf}}{dt} = & k_{dn}\Omega_H(N_{bf}, 6, N_H) + k_{dm1}\Omega_H(M1_{bf}, 6, M1_H) - k_{dnp}N_{bf}\Omega_H(Deb_{bf}, 1, Deb_H) \\ & - k_{dm1p}\Omega_i(M1_{bf}; N_{bf}, N_1^\infty)\Omega_H(Deb_{bf}, 1, Deb_H) \\ & - k_{dm2p}\Omega_i(M2_{bf}; N_{bf}, N_1^\infty)\Omega_H(Deb_{bf}, 1, Deb_H) - d_{deb}Deb_{bf} \end{aligned} \quad (1)$$

Debris dynamics follow the formulation of that in the superficial thermal burn TDPM (Jennings et al. 2022), except that effects from T lymphocytes are not included which is discussed in greater detail in Section 4.3. Unlike thermal burn, a closed fracture is defined by loss of bone and fracture gap in place of tissue damage as described in Section 3. Therefore, the present TDPM assumes debris to be representative of the fracture gap since the debris caused by initial bone loss would be proportional to the fracture gap.

3.2.2. Bone Equation

Changes in bone volume at the fracture site (B_{bf}) over time are characterized in the TDPM by the following equation:

$$\frac{dB_{bf}}{dt} = \frac{\rho_b(1 + k_b OC_{bf})OB_{bf}}{1 + \left(\frac{Deb_{bf}}{Deb^\infty}\right)^2} - d_b OC_{bf} B_{bf} \quad (7)$$

The TDPM includes bone volume at the fracture site as a dynamic state variable instead of damage to accurately model bone fracture healing. Hence, the damage equation previously defined for thermal burn is no longer included, and instead the dynamics of bone replacement takes precedent. The two terms of the bone volume dynamics represent firstly bone formation by osteoblasts and secondly bone resorption by osteoclasts (Cruess and Dumont 1975; Hoppenfeld and Murthy 2000; Marsell and Einhorn 2011; Sheen and Garla 2020; Muire et al. 2020). The model assumes both processes to be proportional to the osteoblasts/osteoclast populations, respectively. The formation term is also upregulated by osteoclasts since they are known to promote bone formation by osteoblasts through releasing osteoblastic mediators while degrading bone (Jones et al. 2011). The formation term is lastly inhibited by debris to ensure bone does not properly form if debris persists.

3.2.3. Neutrophil and Macrophage Equations

Equations for the neutrophils (N_{bf}) and macrophages ($M1_{bf}$ and $M2_{bf}$) at the bone fracture site are given by the following:

$$\frac{dN_{bf}}{dt} = \frac{s_{nr}R_n}{R_n + d_{nr}} - k_{nm1p}N_{bf}\Omega_i(M1_{bf}; N_{bf}, N_1^\infty) - k_{nm2p}N_{bf}\Omega_i(M2_{bf}; N_{bf}, N_1^\infty) - d_n N_{bf} \quad (2)$$

$$\begin{aligned} \frac{dM1_{bf}}{dt} = & \frac{s_{mr}R_{m1}}{R_{m1} + R_{m2} + d_{mr}} - \frac{(k_{m1oc}M1_{bf})OB_{bf}}{1 + \left(\frac{M1_{bf}}{M1_2^\infty}\right)^2 + \left(\frac{M2_{bf}}{M2_2^\infty}\right)^2 + \left(\frac{OB_{bf}}{OB_2^\infty}\right)^2} \\ & - \theta_{m1m2}[k_{nm1p}N_{bf}\Omega_i(M1_{bf}; N_{bf}, N_1^\infty)] \\ & + \theta_{m2m1}M2_{bf} - d_{m1}M1_{bf} \end{aligned} \quad (3)$$

$$\begin{aligned} \frac{dM2_{bf}}{dt} = & \frac{s_{mr}R_{m2}}{R_{m1} + R_{m2} + d_{mr}} - \frac{(k_{m2oc}M2_{bf})OB_{bf}}{1 + \left(\frac{M1_{bf}}{M1_2^\infty}\right)^2 + \left(\frac{M2_{bf}}{M2_2^\infty}\right)^2 + \left(\frac{OB_{bf}}{OB_2^\infty}\right)^2} \\ & + \theta_{m1m2}[k_{nm1p}N_{bf}\Omega_i(M1_{bf}; N_{bf}, N_1^\infty)] \\ & - \theta_{m2m1}M2_{bf} - d_{m2}M2_{bf} \end{aligned} \quad (4)$$

The equations for these inflammatory cells are nearly identical their counterparts in the superficial thermal burn TDPM (Jennings et al. 2022). Here, however, T lymphocytes are not considered and as a result, their effects on N_{bf} and $M1_{bf}$ are omitted. The main difference for these cell dynamics is the removal of T lymphocytes from the system and therefore the associated lymphatic effects previously seen on these dynamics. T lymphocytes are not included in previously developed bone fracture models (Kojouharov et al. 2017; Trejo et al. 2019; Baratchart et al. 2022) and experimental studies in mice have shown that removal of the adaptive immunity results in faster (Toben et al. 2011), but weaker bone development (Khassawna et al. 2017). Thus, adaptive immunity was observed to potentially affect quality and speed of bone development with unknown effects on functionality and no obvious effects on overall bone volume. Additionally, Bastian et al., found no significant differences in blood lymphocyte in multi-trauma patients with normal versus impaired bone fracture healing, indicating that the systemic lymphocyte count is not affected by bone fracture even with additional trauma (Bastian et al. 2016b). The only remaining difference for these inflammatory cells equations is the inclusion of the second term on both macrophage differential equations to account for their differentiation to osteoclasts (Udagawa et al. 1990; Zhao et al. 2015; Huang et al. 2017). This is explained in more detail in a later paragraph on the osteoclast state variable dynamics.

It is important to note that the inclusion of neutrophils distinguishes the model presented here from those published in the literature that also aim to describe inflammation (Kojouharov et al. 2017; Trejo et al. 2019). Neutrophils are well recognized as having a critical role in fracture healing (Einhorn and Gerstenfeld 2015; Kovtun et al. 2018; Peiseler and Kubes 2019). They have been found in fracture hematoma (Bastian et al. 2016a; Hoff et al. 2016; Kovtun et al. 2018) arriving within minutes following injury (Kovtun et al. 2018), suggesting they initiate the inflammatory cascade. Moreover, fracture healing has been found to be impaired in their absence (Kovtun et al. 2016).

3.2.4. Osteoblast Equation

A key difference in thermal burn and bone fracture healing is the primary cells involved in tissue regeneration. For thermal burn, epithelial tissue regeneration requires primarily fibroblasts while bone tissue regeneration for fracture healing requires osteoblasts. Although fibroblasts are still involved in bone fracture healing, their primary function is aiding in soft callus (i.e., fibrocartilage) formation that temporarily fills the fracture gap. Over

time, the soft callus mineralized to become bone by way of osteoblasts (Bahney et al. 2019; Sheen and Garla 2020; Muire et al. 2020). In a stable, closed fracture, the soft callus formation occurs normally and in a timely manner therefore removing the necessity to model and measure fibroblasts and/or fibrocartilage formation. For this reason, here, fibroblasts are replaced by osteoblasts (OB_{bf}), tailoring the superficial burn TDPM to bone fracture healing. Osteoblast dynamics over time are characterized in the bone fracture TDPM by:

$$\frac{dOB_{bf}}{dt} = \frac{s_{ob}(1 + k_{obm1}M1_{bf} + k_{obm2}M2_{bf})}{1 + \left(\frac{N_{bf}}{N_2^\infty}\right)^2} - d_{ob}OB_{bf}B_{bf} \quad (5)$$

The first term of the osteoblast equation represents the source of homeostatic levels from circulating osteoblasts in the surrounding tissue (Baratchart et al. 2022) as well as additional differentiation of MSC to osteoblast by upregulation of macrophages (Zhang et al. 2017). The first term is also inhibited by presence of neutrophils to ensure delayed onset of osteoblast-driven repair phase after early inflammation. The second term in the osteoblast ODE is a typical exponential decay to represent decline in the osteoblast population due to apoptosis or further differentiation of osteoblasts into osteocytes (Sawa et al. 2019). This term is proportional to B_{bf} (bone volume) to ensure a rapid decrease in OB_{bf} as more bone is created and osteoblasts are no longer needed.

3.2.5. Osteoclast Equation

Additionally unique to bone fracture healing is the remodeling phase defined by the coupled relationship of osteoblasts and osteoclasts to produce and degrade bone, respectively. Therefore, osteoclasts are included as a dynamic state variable (OC_{bf}) in the TDPM, and the equation characterizing their role in bone fracture healing is given by:

$$\begin{aligned} \frac{dOC_{bf}}{dt} = & \frac{s_{oc}}{1 + \left(\frac{N_{bf}}{N_2^\infty}\right)^2} + \frac{(k_{m1oc}M1_{bf} + k_{m2oc}M2_{bf})OB_{bf}}{1 + \left(\frac{M1_{bf}}{M1_2^\infty}\right)^2 + \left(\frac{M2_{bf}}{M2_2^\infty}\right)^2 + \left(\frac{OB_{bf}}{OB_2^\infty}\right)^2} \\ & - d_{oc}OC_{bf}(1 + k_{ocm1}M1_{bf} + k_{ocm2}M2_{bf}) \end{aligned} \quad (6)$$

Similar to osteoblasts, there are homeostatic levels of circulating osteoclasts in the surrounding tissue (Baratchart et al. 2022) represented by the first source term in the equation, which- again- like osteoblasts is inhibited by neutrophils to delay osteoclast presence until early inflammation has concluded and the repair phase has begun. In addition to circulating osteoclasts, osteoclast precursors (OCPs) are derived from macrophages becoming osteoblasts by way of osteoclastogenesis (Udagawa et al. 1990; Zhao et al. 2015; Huang et al. 2017). Osteoclastogenesis is dependent on the osteoblast-produced cytokine, receptor activator of nuclear factor kappa- β ligand (RANKL), which binds to OCP RANK to activate osteoclasts. This process is captured by the second term in Equation (6) which is proportional to OB_{bf} and the total available macrophages. It is known that T lymphocytes also produce RANKL and IL-7 that promote osteoclastogenesis; however, Sato et al. (Sato et al. 2006) found osteoblast to be the primary provider of RANKL to promote osteoclastogenesis. This further reinforced the decision to exclude T lymphocytes from the TDPM.

The second term of Equation (6) additionally includes inhibition by macrophages and osteoblasts. Pro-inflammatory macrophages target NFATc1 to limit osteoclastogenesis (Zhao et al. 2015; Yamaguchi et al. 2016) and anti-inflammatory macrophages inhibit early osteoclastogenesis by IL-10 production (Lampiasi et al. 2016) while osteoblasts are known to produce osteoprotegerin RANKL decoy receptors limiting macrophage differentiation to osteoclasts (Kostenuik 2005; Eriksen 2010). Lastly included is the osteoclast decay term which is upregulated by macrophages through M1-produced IL-12 (Yamaguchi et al. 2016) and M2-produced TGF- β 1 that both increase OC_{bf} apoptosis (Houde et al. 2009).

Section 4. Results

Numerical simulations were performed to not only illustrate dynamics of the TDPM governed by Equations (1)-(7), but also to demonstrate the model's ability to reproduce observed, known biological phenomena. The first objective of the TDPM is to reproduce normal bone fracture healing dynamics, referred to as our baseline case. Parameters were primarily obtained from prior modeling efforts and the existing literature. Their assigned (baseline) values are provided in Table 2.

. Additional included experiments involve perturbations of either the initial conditions or the parameters of the model to reflect known environmental or biological phenomena, and each were assessed for their effect on baseline normal bone fracture healing dynamics.

All simulations were performed in MATLAB (2022).

4.1 Initial Conditions

Current understanding of the literature indicates no existence of human data on the cellular dynamics that define normal bone fracture for a closed, stable fracture (see fourth paragraph in Section 5 Discussion for more detail). For this reason, normal healing dynamics for the presented TDPM are based on high-fidelity murine data collected in (Baratchart et al. 2022) that measured pro- and anti-inflammatory macrophages, osteoclasts, osteoblasts, and bone volume over time.

In alignment with (Baratchart et al. 2022), the TDPM includes the fracture site and surrounding area. Thus, the model assumes the same non-zero initial conditions for osteoblasts and osteoclasts as in (Baratchart et al. 2022). Additionally, both debris and bone volume assume non-zero initial conditions to account for fracture gap and residual cellular debris from bone loss. The initial values for bone volume and debris are the same as defined in (Baratchart et al. 2022), however, these values are modified to include more bone loss in Section 4.4.1 to account for fracture severity. All other cells are assumed to have initial condition of zero. Initial conditions for all state variables are summarily listed in Table 3.

Table 3. Baseline initial conditions for the TDPM based on those measured in murine data (Baratchart et al. 2022).

SYMBOL	DESCRIPTION	INITIAL VALUE
t	Time (in hours)	-
<i>Local Bone Fracture</i>		
Deb_{bf}	Debris (mm ³)	2.8
B_{bf}	Bone volume (mm ³)	0.353
N_{bf}	Activated neutrophils at the fracture site	0
$M1_{bf}$	Classically activated macrophages (M1) at the fracture site	0
$M2_{bf}$	Alternatively activated macrophages (M2) at the fracture site	0
OB_{bf}	Differentiated osteoblasts at the fracture site	3.1e04
OC_{bf}	Differentiated osteoclasts at the fracture site	8.8e04

SYMBOL	DESCRIPTION	INITIAL VALUE
Note: The healed steady state, denoted as $(Deb^*, N^*, M1^*, M2^*, OB^*, OC^*, B^*)^t$ corresponds to all state variables being zero except OB^*, OC^*, B^* , i.e.,		
$Deb^* = N^* = M1^* = M2^* = 0$, $OB^* = \frac{s_{ob}}{d_{ob}B^*}$, $OC^* = \frac{s_{oc}}{d_{oc}}$, and $B^* = \frac{\rho_b(1 + k_b OC^*) OB^*}{d_b OC^*}$		
Where OB^* and OC^* equal the initial (baseline) values listed, and $B^* = B_{bf}(0) + Deb_{bf}(0)$ since initial debris represents the loss of bone from fracture needed to be restored in steady state.		

4.2 Healing Time

The key metric of bone fracture healing is the time to “healed.” To identify this endpoint from the transients generated by the TDPM, a fracture is defined as “healed” when the state variable for bone volume, B_{bf} , is within some $\varepsilon > 0$ of its steady-state value (see third paragraph of Section 5 Discussion for more detail on this assumption). Clearly stated, the healing time is the timepoint $t_h > 0$ such that $|B^* - B_{bf}(t)| \leq \varepsilon$ for all $t \geq t_h$. Here, ε is a variable parameter depending on how restrictive the bounds need to be defined and $B_{bf}^* = B_{bf}(0) + Deb_{bf}(0)$ since it is assumed that debris represents loss of bone due to fracture. All steady-state values are noted in Table 3.

4.3 Baseline Parameters and Normal Healing Dynamics

To conduct numerical simulations, baseline values for each parameter were first determined. Pointwise estimates⁴ for the superficial thermal burn TDPM reported in (Jennings et al. 2022) serve as initial values for inflammatory parameters. Similarly, due to analogous mathematical representations in the ODEs, values reported in published studies were used for other unknown parameters related to bone fracture repair (Wei et al. 2011; Yona et al. 2013; Patel et al. 2017; Baratchart et al. 2022). These initial estimates were then manually calibrated to qualitatively matched to observed normal bone fracture healing dynamics (Baratchart et al. 2022). The resulting baseline values can be found in Table 2.

Figure 3 displays the dynamics of all state variables predicted by the TDPM under the baseline parameter values. Notably, N_{bf} , $M1_{bf}$, $M2_{bf}$, Deb_{bf} all approach zero while B_{bf} steadily increases to ultimately approach steady state indicating resolution of inflammation and full healing of the fracture, respectively. Inflammatory response behaves as expected where N_{bf} peaks initially, followed by $M1_{bf}$ and lastly by $M2_{bf}$ as described in the third paragraph of Section 2 Background. Particularly unique to bone fracture healing dynamics is the two-phase increase of pro-inflammatory $M1_{bf}$ observed in previous murine data (Baratchart et al. 2022) that is replicated by the TDPM. Additionally, the TDPM captures the qualitative dynamics of OB_{bf} that gradually increase then decrease to approach initial value and opposing dynamics of OC_{bf} that gradually decrease then increase to approach initial

⁴ Here, pointwise estimates specifically refers to the representative set computed via Brute-Force with Latin-Hypercube Sampling reported in (Jennings et al. 2022).

value (Baratchart et al. 2022). Thus, the TDPM with the defined baseline parameter values can model normal healing of a bone fracture.

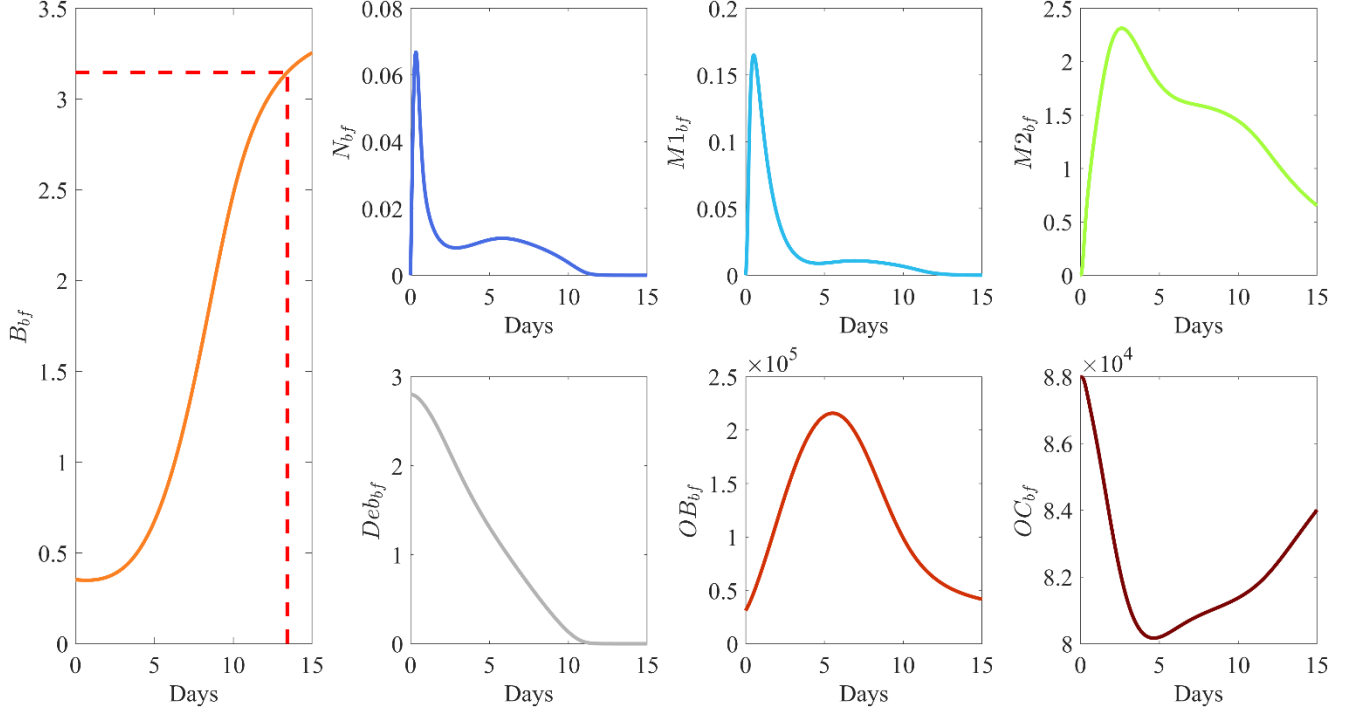


Figure 3. Normal (baseline) healing dynamics for a closed, stable fracture. Model initial conditions and parameter values were assigned the baseline values from Table 3 and Table 2. The red dashed line in the bone (B_{bf}) plot denotes when the fracture is healed.

4.4 Impaired Healing Dynamics

The ability to reproduce improper healing dynamics is of particular interest to ensuring robustness of the TDPM for bone fracture. Specifically, the model should be able to capture delayed union or non-union provided varying initial conditions or parameters representing different realistic scenarios. For this reason, these values were modified to evaluate differences in corresponding dynamics when fracture bone loss is increased, when neutrophils are removed from the system, and when all inflammatory cells are removed from the system.

4.4.1. Sensitivity to Initial Bone Loss

One cause of delayed union is increased severity of fracture (Calori et al. 2007). In order to account for altered dynamics based on increased fracture severity, the initial values of bone (B_{bf}) and debris (Deb_{bf}) were altered. Specifically, increase in severity is modeled by decreasing the initial value of bone while proportionally increasing the initial value of debris assumed to arise from additional bone loss with increased severity. However, as previously stated, the presented TDPM is only considered for minor-to-moderate closed stable fractures in which dynamics outside the immediate site of injury remain unperturbed; it is anticipated by the authors that more severe fractures may disrupt systemic levels that the model would need to additionally consider (see last paragraph of Section 5 Discussion for more detail).

Therefore, the increase of bone loss (i.e., increase of debris and proportional decrease of bone initial values) is representative of a change from minor to moderate level of fracture severity in this case. Here, minor severity is represented by baseline initial values listed in Table 3 and moderate severity is represented by doubling the amount of debris ($Deb(0) = 4.8\text{mm}^3$) and defining bone to be $B(0) = 0.6051\text{mm}^3$ ensuring identical proportion of debris to bone as used with baseline values..

Figure 4 shows the comparison of minor (solid lines, baseline) and moderate (dashed lines) severity dynamics. Differences arise for all state variables when changing the fracture severity, particularly a clear delay in bone increase to eventual steady-state with moderate fracture compared to minor. Both fracture types do heal properly, however, the timing is altered between different bone loss quantities. Thus, the TDPM captures delayed union in the expected scenario when a fracture is considered more severe by way of additional bone loss.

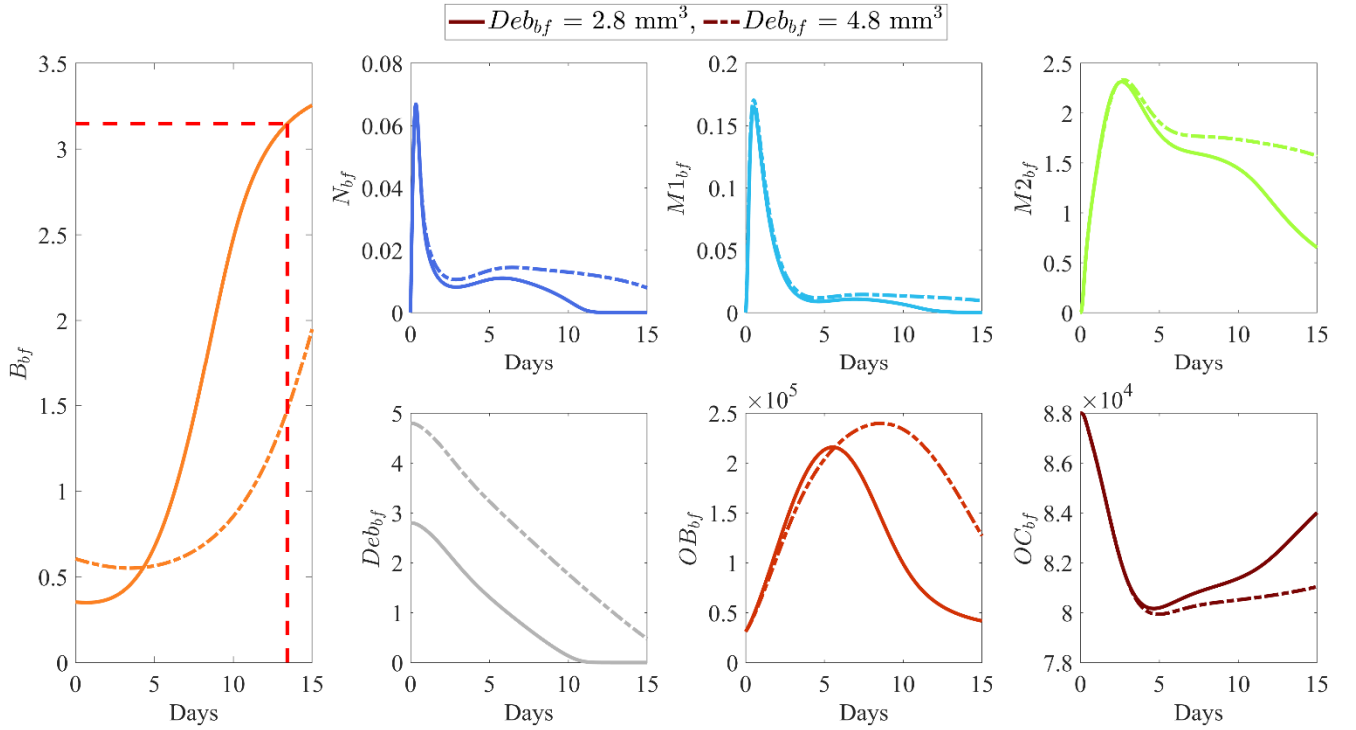


Figure 4. Transients for numerical simulations (dashed lines) compared to baseline normal healing dynamics (solid lines). In the simulation, $B_{bf}(0) = 0.6051\text{mm}^3$ and $Deb_{bf}(0) = 4.8\text{mm}^3$ while all other TDPM initial conditions and parameter values match baseline values from Table 3 and Table 2. The red dashed line in the bone (B_{bf}) plot denotes when the fracture is healed.

4.4.2. Healing in the Absence of Neutrophils

Prior studies have shown neutrophils play a vital role in bone fracture healing (Chung et al. 2006; Xing et al. 2010; Bastian et al. 2016a; Kovtun et al. 2016), but no mathematical ODE model to date includes neutrophils as a part of the defined system. The presented TDPM includes neutrophils as a state variable for the first time, so the necessity of their inclusion needed to be evaluated. To do so, the system was modeled with and without neutrophils to investigate any differences. This concept was previously considered in the TDPM for thermal

burn (Jennings et al. 2022) where the wound healed with or without neutrophils, however, there was a delay in healing when neutrophils were excluded. Similarly, it is expected that lack of neutrophils will cause a delay in union for bone fracture.

Numerical simulations of the TDPM were performed with the production rate of circulating, resting neutrophils set to zero ($s_{nr} = 0$) to ensure no neutrophils entered the local fracture site; all other parameters are set to their baseline values listed in Table 2. As seen in Figure 5, all state variable dynamics change without neutrophils (dashed lines) from the system with neutrophils (solid lines, baseline). As expected, B_{bf} increase and Deb_{bf} decrease is shifted later in time when neutrophils are excluded indicating a delay in union. Overall, the inflammatory response ($M1_{bf}$ and $M2_{bf}$) is weakened without neutrophils which may give rise to the ultimate delay of repair cell OB_{bf} and subsequently bone B_{bf} . These results further reinforce the need to include neutrophils when modeling bone fracture.

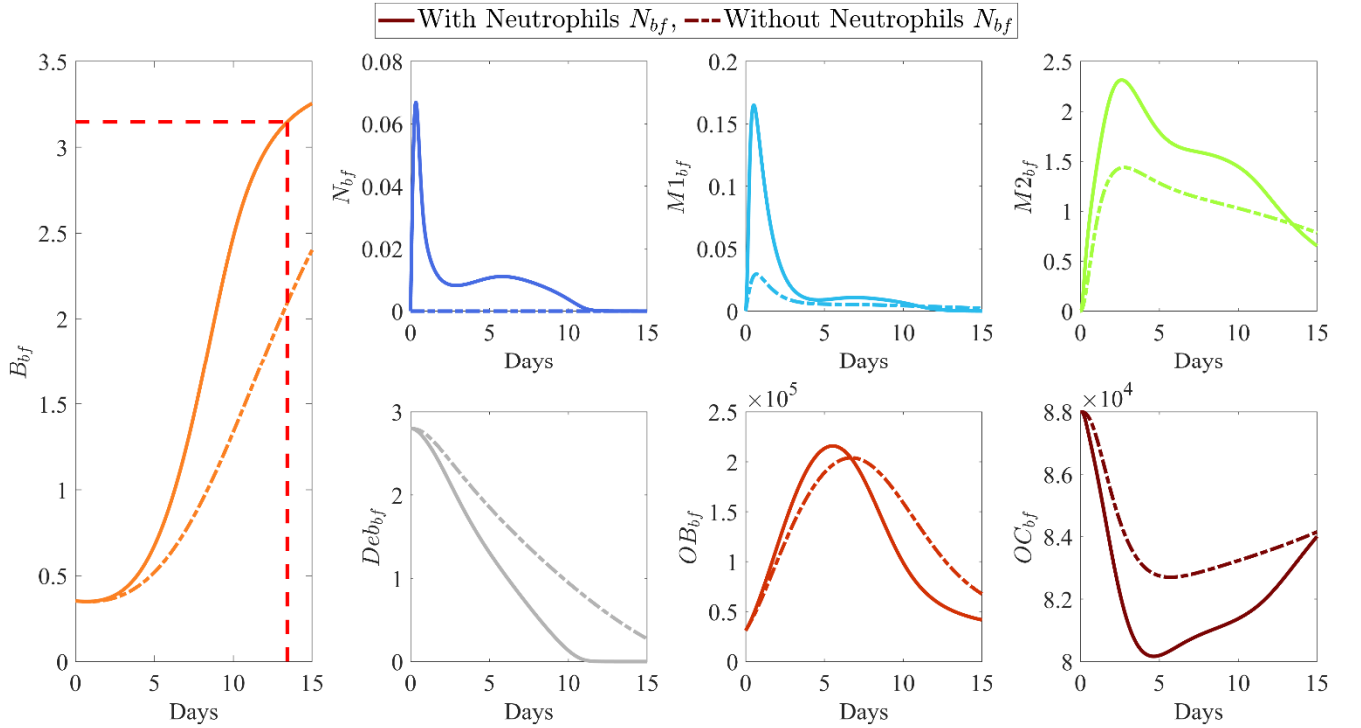


Figure 5. Transients for numerical simulations (dashed lines) compared to baseline normal healing dynamics (solid lines). In the simulation, $s_{nr} = 0$ while all other TDPM initial conditions and parameter values match baseline values from Table 3 and Table 2. The red dashed line in the bone (B_{bf}) plot denotes when the fracture is healed.

4.4.3. Healing in the Absence of Inflammation

Another consideration is the critical role of all inflammatory cells (N_{bf} , $M1_{bf}$, and $M2_{bf}$) in proper healing of bone fracture. Similar to neutrophils, the system is considered with and without the inflammatory cells to measure the resulting effects on healing dynamics. This is also done analogously by setting the production rate of circulating, resting neutrophils and macrophages to zero ($s_{nr} = 0$ and $s_{mr} = 0$); all other parameters are set to their baseline values listed in Table 2. However, the expectation without any inflammatory cells is that the bone should not heal, and fracture should result in a non-union.

Shown in Figure 6, the result of removing the inflammatory cells (dashed lines) is as expected where debris does not resolve, and bone does not reach steady state. These transients were evaluated up to 1 year (365 days) after fracture (results not shown for conciseness) by which time bone was not at steady-state and debris persisted. Although through that time, debris and bone did slowly decrease and increase, respectively, however, this is due to the intrinsic decay and proliferation that exists for the respective variable. In reality, a fracture that has not healed in a year's time will require surgical intervention in alignment with the clinical definition of a non-union (Frölke and Patka 2007). Thus, the system without inflammatory cells does not heal properly and results in a non-union as anticipated.

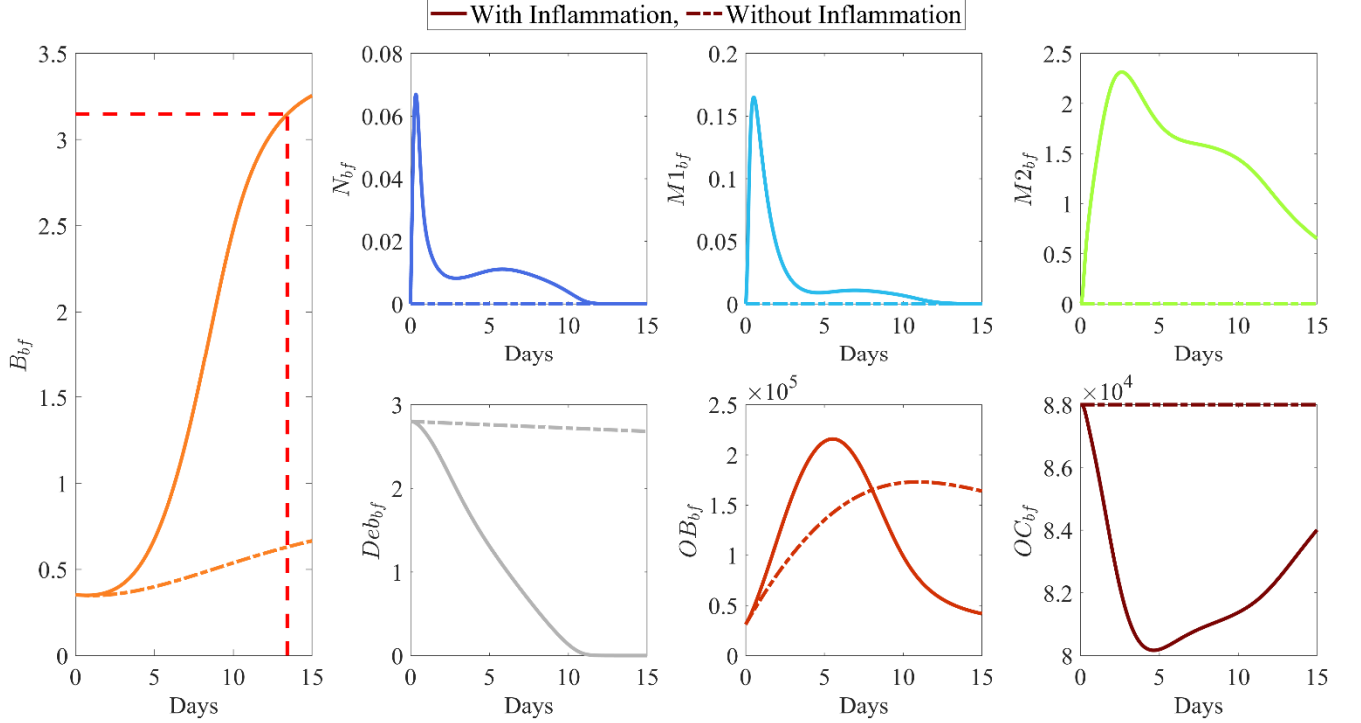


Figure 6. Transients for numerical simulations (dashed lines) compared to baseline normal healing dynamics (solid lines). In the simulation, $s_{nr} = 0$ and $s_{mr} = 0$ while all other TDPM initial conditions and parameter values match baseline values from Table 3 and Table 2. The red dashed line in the bone (B_{bf}) plot denotes when the fracture is healed.

Section 5. Discussion

In this technical report, the mathematical formulation and numerical results of a TDPM for bone fracture healing was presented. This model aims to capture the inflammatory and repair processes at the fracture site and surrounding area initiated by a closed, stable injury. It should be noted that this model assumes immunocompetency (i.e., the ability to produce a normal immune response following injury). Distinguishing features of this model compared to those for an existing superficial thermal burn TDPM were highlighted. However, the number of consistencies between the two models tangibly demonstrated for the first time the generalizability of the modeling being anchored by wound healing to capture the pathophysiology following different NUDET injuries (here, thermal burn and bone fracture). Such a healing-centric approach permits the opportunity to construct a unifying mechanistic framework for HENRE that moves away from the need to start from scratch when developing new models for single injuries, and their combinations.

Previous efforts have attempted to model bone fracture healing dynamics as defined by a system of ODEs (Kojouharov et al. 2017; Trejo et al. 2019; Baratchart et al. 2022). However, most did not separately account for distinct dynamics of osteoblasts and osteoclasts, nor did they include any supporting data to ensure the modeled dynamics were biologically realistic (Kojouharov et al. 2017; Trejo et al. 2019). To the authors' best knowledge, no model to date has included neutrophils as a state variable and their downstream effects on inflammation and bone repair, despite their crucial role in bone fracture healing (Chung et al. 2006; Xing et al. 2010; Bastian et al. 2016a; Kovtun et al. 2016). Basing the TDPM on the general framework for wound healing not only drives the inclusion of neutrophils for the first time but provides additional realism to inflammatory dynamics. It is important to note that existing models were constructed for a different purpose, specifically, to investigate the role of macrophages in healing more critically, and the omission of neutrophils supports that purpose. Additionally, the model accounts for other cellular interactions observed in the literature, but not included in other models, while retaining the capability of replicating dynamics seen in *in vivo* murine data (Baratchart et al. 2022).

Although previous models of bone fracture have excluded T lymphocytes (Kojouharov et al. 2017; Trejo et al. 2019; Baratchart et al. 2022), and exclusion of a T lymphocyte population from the TDPM presented herein is justifiable (see Section 3.2.3), it is worth noting that there does appear to be a correlation between local tissue lymphocyte number and fracture repair (Hurtgen et al. 2016). As such, the inclusion of lymphocyte dynamics in the bone fracture TDPM may be worth further investigation as the model is expanded to include other injuries and/or more severe bone fractures in future work.

Two of the previous models of bone fracture (Kojouharov et al. 2017; Trejo et al. 2019) define three healing outcomes of their model: "successful" union, non-union, or delayed union. However, they do not provide any empirical data to support their timescales, dynamics, or measures. For the singular previous model that does fit to murine data, there is no defined outcome of healing (Baratchart et al. 2022). Rather, one could infer that due to the nature of fractures studied, that the dynamics are those of expected "healthy" healing dynamics. To this end, the TDPM assumes the dynamics observed in (Baratchart et al. 2022) are typical of "healthy" healing dynamics.

Further, our model is determined as a “union” or “healed” at the timepoint when bone has reached and remains within ε -bounds of its steady-state value. As noted in the last paragraph of Section 3, full remodeling of bone can take months to years, but a fracture is often functional well before this timespan (Frölke and Patka 2007). Thus, the TDPM assumes that if bone is within reasonably close bounds (where ε can easily be adjusted to redefine “reasonably”) of its steady state, then it is a functional union to be deemed healed. This is a large assumption, but necessary since defining “union” or “healed” bone fracture can be arbitrary even in a clinical setting (Frölke and Patka 2007). Typically, clinicians rely on imperfect measures (e.g., radiographs, range of motion, pain response, full weight bearing ability, etc.) that are the only available methods to date (Frölke and Patka 2007); therefore, our model also required including a simplified and modifiable assumption to define “healed” bone fracture until consistent definitions are developed.

In addition to no clear definition for “union,” there is also a gap in the literature for defining human cellular dynamics and levels at the fracture site for stable, closed fractures. Figure 1 outlines the timing of human bone fracture healing phases and respective cells including neutrophils and macrophages (both pro- and anti-inflammatory) for normal (Figure 1A) and delayed (Figure 1B) timelines (Muire et al. 2020). Nevertheless, this does not include specific dynamics of neutrophils or macrophages, and lacks information for osteoclasts, osteoblast, and overall bone reformation.

Normal healing levels of systemic neutrophil and monocytes over time have been measured in humans (Bastian et al. 2016b); however, these were measured in patients who experienced multi-trauma and therefore may differ from the levels observed with a closed, stable fracture. Accurate application of the presented model requires additional studies measuring human-specific cellular dynamics. In the intermediary, the presented simplifying assumptions allow for an initial basis from which to model bone fracture healing with eventual extension to human dynamics.

It should be further noted that the dynamics of bone fracture healing are dependent upon injury attributes, such as fracture location and specific bone tissue characteristics (Einhorn 1998), as well as demographic characteristics such as age (Sheen and Garla 2020). The model presented herein was developed in cognizance of a military-aged population, but is meant to be a generalized model, not focused on a specific fracture location and amalgamating differences in healing between cortical and trabecular bone layers; this aspect of the characterization of injury will be investigated further in future work, especially as the model is extended to include other types of injury and/or levels of injury severity.

Lastly, the systemic effects from a closed, stable fracture are not well defined to date. For simplicity, a quasi-steady-state for systemic monocytes and neutrophils is assumed in alignment with the previously developed TDPM for superficial burn (Jennings et al. 2022). This assumption has been observed in rats for monocytes in open, stable fractures (Mangum et al. 2019) suggesting the same could hold true for closed, stable fractures.

However, the same study also found significant differences in circulating lymphocytes and granulocytes within 24 hours of fracture compared with healthy rats (Mangum et al. 2019). This difference was found to no longer be significant after 72hrs and may be due to requiring additional immune cells to repair the open wound site rather than the fracture alone.

However, there is no clear data in the current literature to indicate the strength of this assumption beyond monocytes or extension to human healing dynamics. As a result, in the first formulation, the model assumes the systemic dynamics remain unperturbed following a stable, closed fracture. Further investigation into this aspect is currently ongoing.

Section 6. Future Work

The next steps to refine the TDPM for bone fracture will be to conduct a global sensitivity analysis using the Extended Fourier Amplitude Sensitivity Test (eFAST) approach prior to parameter estimation. All model parameters will be varied following the previously used approach (Creel et al. 2022), then aggregated eFAST results will determine the critical set of parameters to be included in parameter estimation. All other parameters not determined critical by the eFAST results will remain fixed. As a starting point, the distribution of each parameter will be defined as uniform, with bounds either pulled from previous modeling studies (e.g., (Kojouharov et al. 2017; Trejo et al. 2019; Baratchart et al. 2022)) or calibrated from numerical solutions of the TDPM across combinations from its potential parameter space.

The parameters that drive model dynamics (i.e., those identified as influential by sensitivity analysis) will likely be initially estimated using a qualitative method, such as Brute-Force with Latin-Hypercube Sampling, in which model predictions are matched to criteria extracted from the literature and/or trends observed in experimental studies such as (Baratchart et al. 2022). The advantage of this approach when pooling data across studies was previously demonstrated in (Glynne Andrew et al. 1994; Claes et al. 1998; Bastian et al. 2016b, 2016a, 2018a, 2018b; Hesselink et al. 2018; Mangum et al. 2019; Jennings et al. 2022). Via Bayesian methods for parameter estimation, the data from in (Baratchart et al. 2022) can then be used to obtain “informed” distributions for each parameter with results from the qualitative approach acting as refined priors. The aim being to result in a TDPM capable of mimicking fracture healing dynamics as seen experimentally in rats and will ultimately require additional human experiments to comprehensively model human bone fracture healing.

The ultimate objective of the TDPM for bone fracture is integration with other TDPMs for thermal burn and radiation to model combined injury for use in HENRE. Although these individual TDPMs are based on a unifying framework, no attempt has been made to fully integrate multiple injury models for measuring combined injury. This may result in many unexpected challenges beyond those known to happen such as requiring parameter fine-tuning. Specifically, it is known that combined injuries can cause synergistic effects (Brooks et al. 1952; Alpen et al. 1954), which presents an additional complication in modeling the combined injury as well as an increase in model variables and parameters. Future models are thus expected to be sizable as it is attempted to replicate the complex dynamics of combined injury. Despite concerns regarding the enormity of these models, these TDPMs of combined injury will eventually provide increasingly accurate predictions for combat readiness as well as the opportunity for specialized predictions tailored to distinct combinations and intensities of injuries.

Section 7. References

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Appendix A. Abbreviations and Acronyms

AIS	Abbreviated Injury Scale
DTRA	Defense Threat Reduction Agency
HENRE	Health Effects from Nuclear and Radiological Environment
ICD-10	International Statistical Classification of Diseases and Health Related Problems
MCIS	Military Combat Injury Scale
MFIS	Military Functional Incapacity Scale
NUDET	Nuclear Detonation
OCP	Osteoclast Precursor
ODE	Ordinary Differential Equation
<i>RANKL</i>	Receptor Activator of Nuclear Factor Kappa- β Ligand
TDPM	Time-Dependent Physiological Model
TR	Technical Report