Inclusion of the Effects of Systemic TNF-alpha in a Local Differential Equation Model of Acute Wound Healing

Virginia Commonwealth University BBSI Final Report
Student: Cynthia H.T. Edwards
Supervisor: Tarynn M. Witten, Ph.D.

Abstract

The effects of a high initial concentration of TNF-α were added to a differential equation model for acute wound healing in order to simulate wound healing after traumatic injury. Addition of TNF-α yielded heightened inflammatory and fibroblastic responses, which in turn decreased the time required to clear pathogen from the wound. These results suggest that wound healing following traumatic injury may reach higher levels of inflammation, but that heightened inflammation is not necessarily harmful due to the beneficial effects on pathogen reduction.

Introduction

Acute wound healing is a balanced process which requires conditions to fall within a range of acceptable values in order to progress normally. It is therefore of importance to investigate how common pre- and co-existing conditions affect wound healing. Severe trauma, sepsis, and cancer are three types of conditions which increase levels of pro-inflammatory mediators in the blood (Brown et al., 2008; Menges et al., 2008). While an increased local concentration of pro-inflammatory factors can lead to a chronic wound healing state, it is unknown how an increased systemic concentration of pro-inflammatory factors might influence wound healing (Menke, Ward, Witten, Bonchev, & Diegelmann, 2007). The purpose of the current study is to try and determine how local wound healing is affected by an initially high systemic level of pro-inflammatory cytokine (specifically, TNF-α) using a mathematical model.

TNF-α is a pro-inflammatory cytokine with many local effects related to wound healing. It has been shown to stimulate proliferation of fibroblasts and to induce degranulation and increase the phagocytic capacity of neutrophils. In addition, TNF-α has been shown to induce expression of other wound healing-related factors such as PDGF, IL-1, and collagenase (Werner & Grose, 2003). Acute wound healing does not elevate the circulating concentration of TNF-α in any predictable manner, making elevated initial systemic TNF-alpha due to trauma a good

independent candidate for inclusion in the modified Pitt wound healing model (Kiecolt-Glaser et al., 2005).

The original Pitt wound healing model (PWHM) describes acute wound healing in terms of Fibroblasts (F), Inflammation (N), Damage (D) and Pathogen (P). It was modified in a previous study to include the effects of systemic cortisol by adding equations for adrenal cortisol production and plasma cortisol concentration. The resulting six-equation model is referred to in this paper as the modified Pitt wound healing model (mPWHM).

Methods

A mathematical model developed by Pitt et al and modified by Witten et al was used as the base wound healing model for addition of systemic TNF-α. During the first summer of this research the program XPPAUT was used to analyze the system of differential equations, and during this second summer of the research MATLAB was used. MATLAB code generated in this study is included in Appendix B. along with descriptions of the functions used in the model.

Model Description

The modified Pitt wound healing model consists of the following six nonlinear differential equations and parameters, with parameter values listed in Appendix A.:

$$\frac{dP}{dt} = p_{growth}(O_2) * P(t)\left(1 - \frac{P}{p_{Infinity}}\right) - \frac{kpm * sm * P}{\mu m + kmp * P} - kpn * f(N,F) * P(t) * \sigma 1(C_p)$$

$$\frac{dN}{dt} = \left(\frac{snr * R(P,N,D,F)}{\mu nr + R(P,N,D,F)} - \mu n * N(t) - \mu fn * F(t) * N(t)\right) * \sigma 2(C_p, t)$$

$$\frac{dF}{dt} = sf + \frac{kfn * f(N(t) + kfnd * D(t), F(t))}{1 + f(N(t) + kfnd * D(t), F(t))} - \mu f * F(t)$$

$$\frac{dD}{dt} = kdn * fs\left[f\left(N(t), F(t)\right)\right] * \sigma 4\left(C_p\right) - \mu d * D(t) - \mu df * D(t) * F(t) * \sigma 5\left(C_p\right) + \beta_d * \left(g\left(O_2\right) - \alpha\right)$$

$$\frac{dC_p}{dt} = \beta_1 * C_a(t) - \beta_c * C_p(t)$$

$$\frac{dC_a}{dt} = -\beta_1 * C_a(t) + \kappa A_{\max} * \mu(t)$$

Functions of the system are defined below:

$$\sigma 1\left(C_p\right):\left\{C_p > \beta, \sigma 1 = 0.5\right\},\left\{C_p \le \beta, \sigma 1 = 1.0\right\}$$

$$\sigma 4\left(x\right):\left\{x > \beta, \sigma 4 = 0.5\lambda\right\},\left\{x \le \beta, \sigma 4 = \lambda\right\}$$

$$\sigma 5\left(x\right):\left\{x > 25, \sigma 5 = \left(\frac{4240}{4750}\right)\right\},\left\{x \le 25, \sigma 5 = 1.0\right\}$$

$$\sigma 6\left(x\right):\left\{x \le 0, \sigma 6 = 0\right\},\left\{x > 0, \sigma 6 = 1.0\right\}$$

$$\sigma 7\left(t\right):\left\{t < 28, \sigma 7 = 1\right\},\left\{t \ge 28, \sigma 7 = 0\right\}$$

$$fs\left(x\right) = \frac{x^6}{xdn^6 + x^6}$$

$$g\left(x\right) = e^{\left(\frac{O_2}{O_{2crit}}\ln\alpha\right)}$$

$$f\left(x,y\right) = \frac{x}{1+\left(\frac{y}{y_{Infinity}}\right)^2}$$

$$p_{growth}\left(O_2\right):\left\{O_2 > O_{2crit}, p_{growth} = kpg\right\},\left\{O_2 \le O_{2crit}, kpg + \beta_p * \left(1 - \frac{O_2}{O_{2crit}}\right)\right\}$$

$$\mu\left(t\right) = c_{[1]} + \sum_{r=1}^{2}\left(c_{[r+1]}\cos\left[\frac{2\pi rt}{24}\right] + d[r]\sin\left[\frac{2\pi rt}{24}\right]\right) + baselineshift$$

$$Deg\left(t\right) = T_o e^{\left(\frac{-\log(2)}{t_{half} * t}\right)}$$

The following equations are changed or added in order to include systemic TNF-α :

$$\frac{dN}{dt} = \left(\frac{snr * R(P,N,D,F)}{\mu nr + R(P,N,D,F)} - \mu n * N(t) - \mu fn * F(t) * N(t)\right) * \sigma 2(C_p,t) + knmig * T(t)$$

$$\frac{dF}{dt} = sf + \frac{kfn * f(N(t) + kfnd * D(t), F(t))}{1 + f(N(t) + kfnd * D(t), F(t))} - \mu f * F(t) + ktpro * T(t) * \sigma 7\left(t\right)$$

$$\frac{dT}{dt} = \left(-Deg(t) - kcort * Cp(t)\right) * \sigma 6\left(T(t)\right)$$

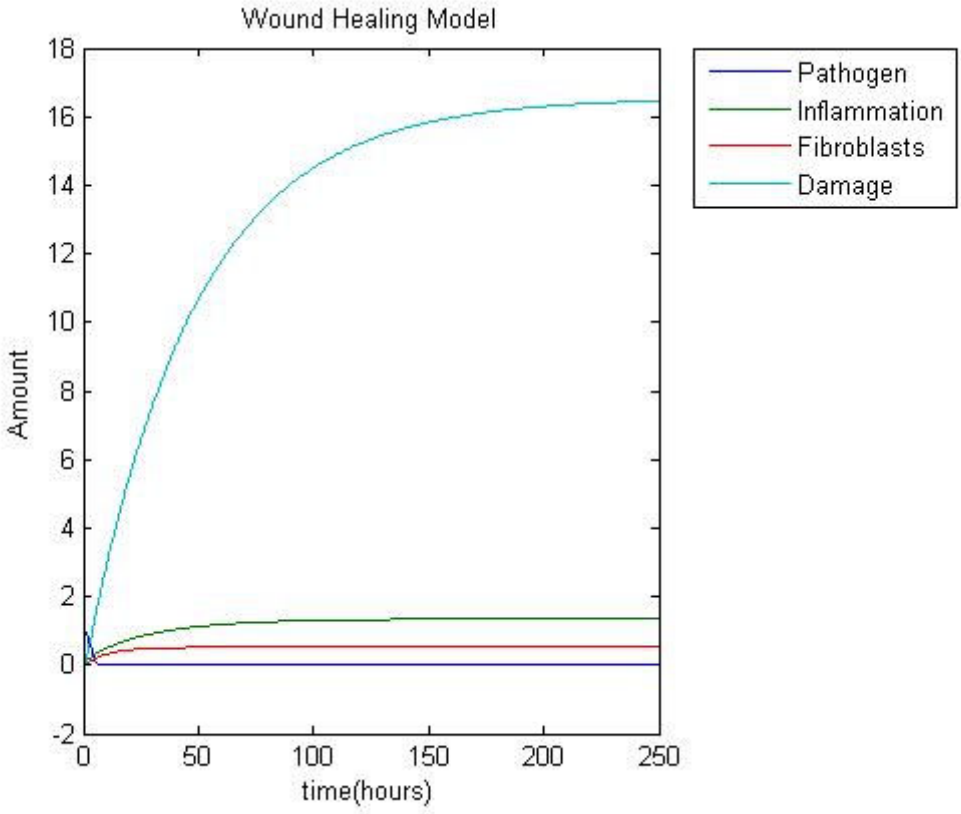
A term was added to the inflammation (N) equation because TNF-α is involved in leukocyte-endothelial interactions which allow immune cells to migrate to the wound site(Chandrasekharan et al., 2007). This increased migration is modeled here by an effective

increase in inflammation modulated by the constant *knmig*. The value used for knmig was chosen arbitrarily from a range of acceptable values. A term was added to the fibroblast (F) equation in order to take into account TNF-α's growth factor-like effect on fibroblasts. TNF-α causes an increase in fibroblast proliferation, with peak DNA expression in cultured primary fibroblasts at approximately 28 hours after receipt of the TNF-α signal(Battegay, Raines, Colbert, & Ross, 1995).

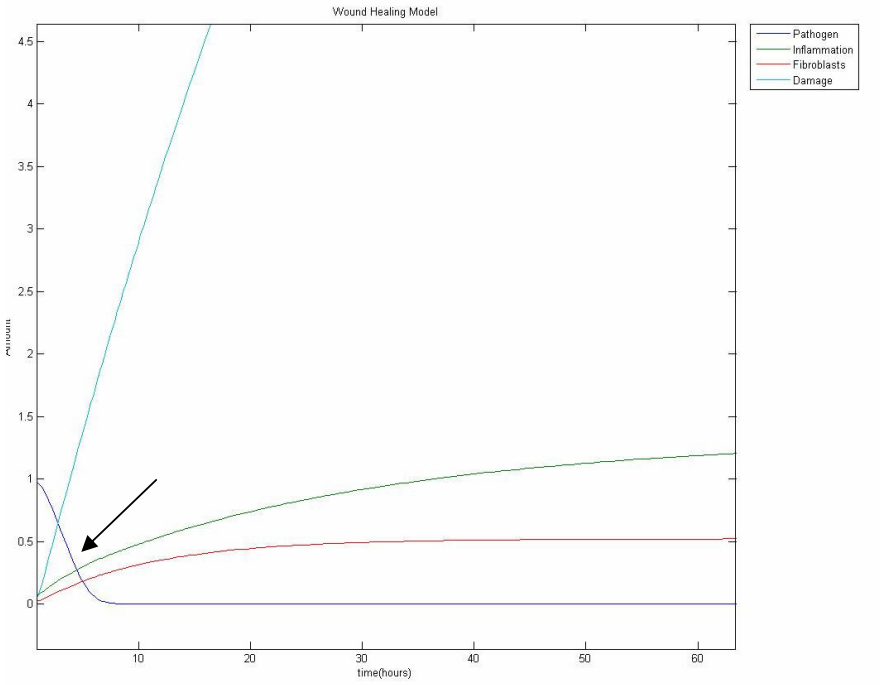
The *Deg(t)* function in the TNF-α equation describes the degradation of TNF-α in the blood over time. A value for the half-life of TNF-α in plasma was used(Tsutsumi et al., 1996). *kcort* is a constant which describes the extent to which plasma cortisol decreases plasma TNF-α.

Results

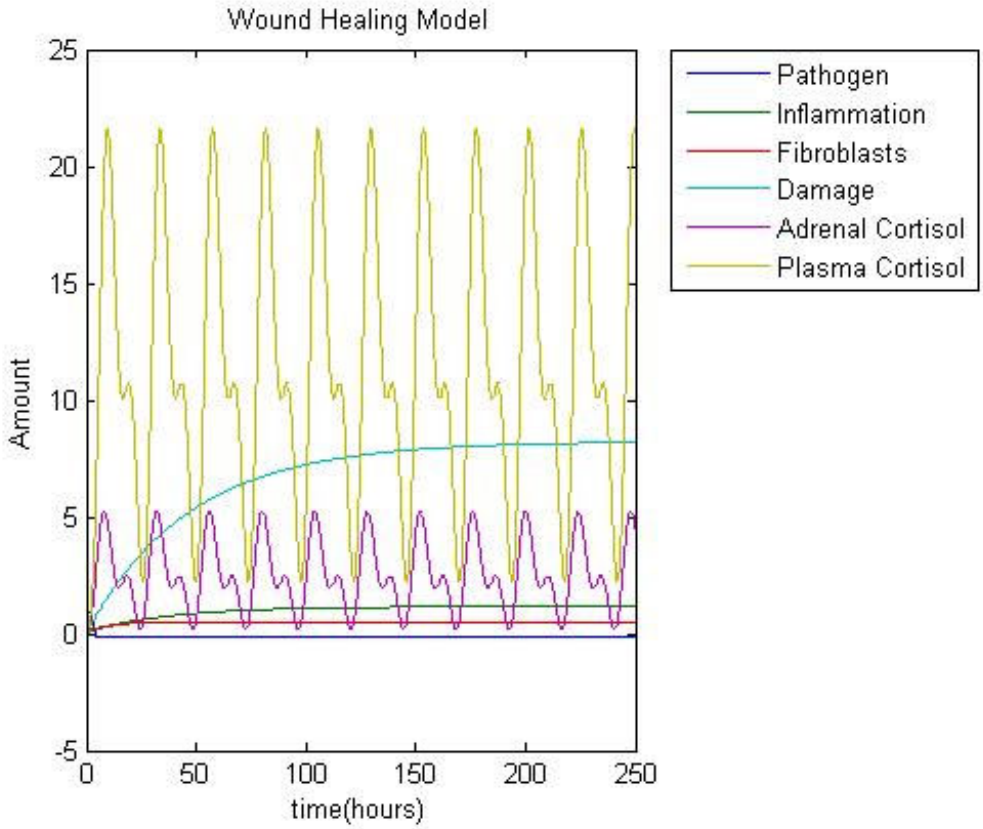
The original Pitt wound healing model, integrated over 250 hours, yielded the following result:



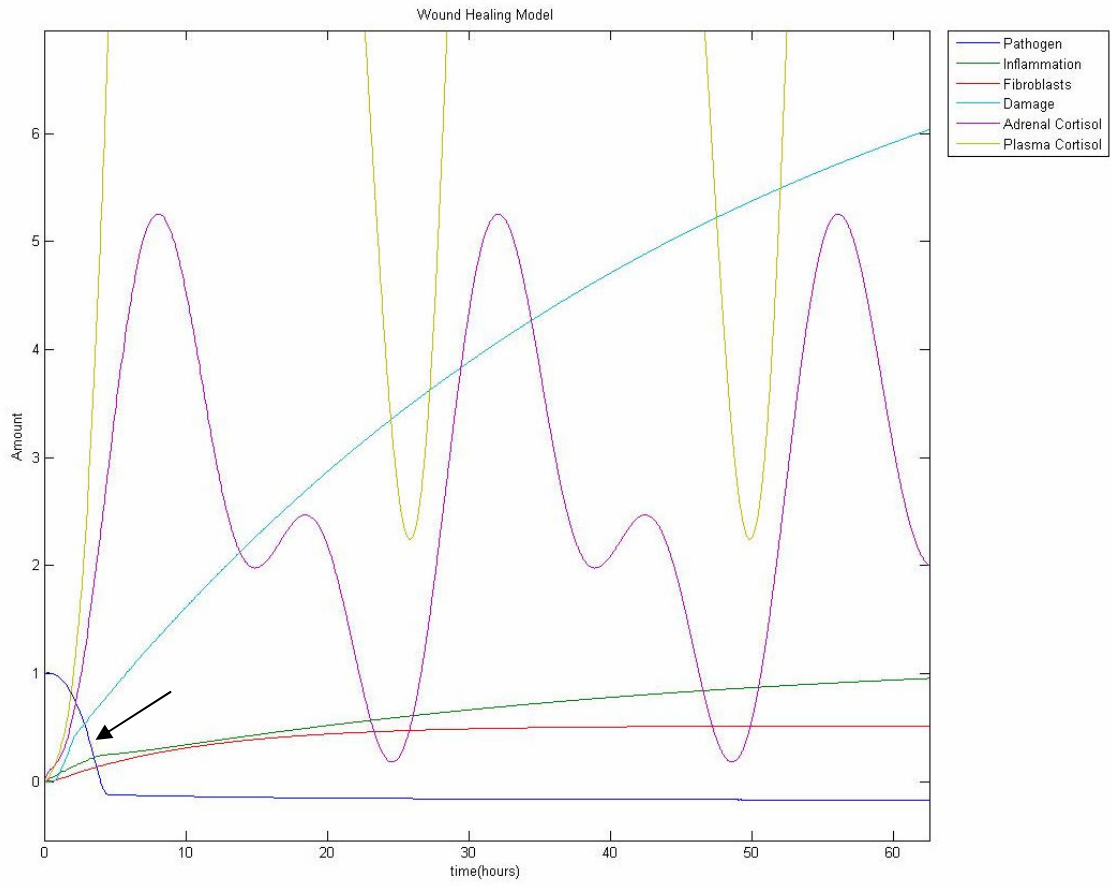
A zoomed-in view shows the behavior of the pathogen equation:



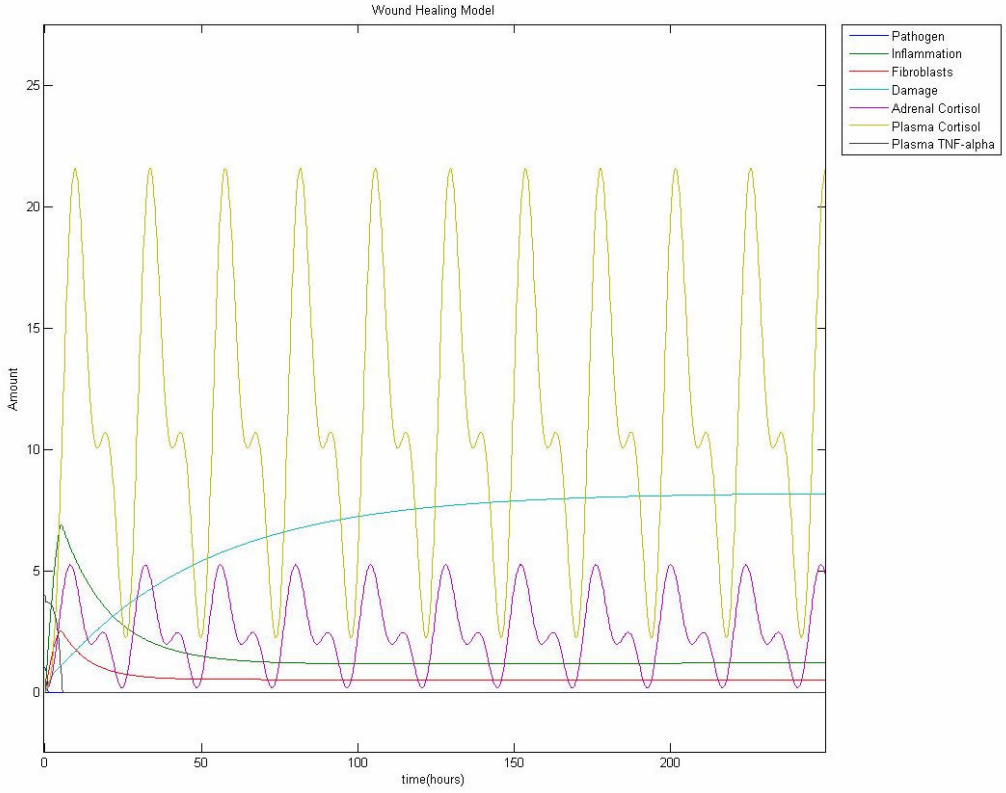
After cortisol was added to the model, with the same parameter values it yielded the following:



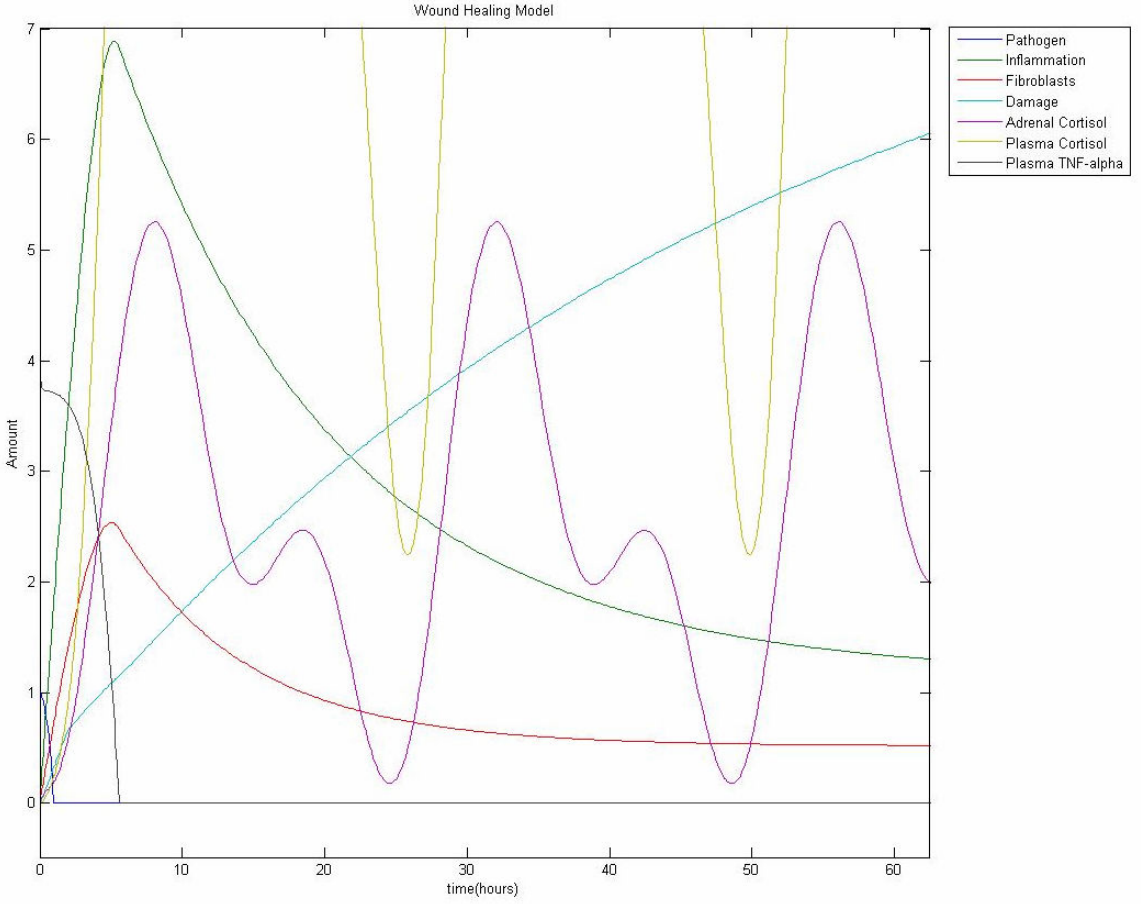
A zoomed-in view again shows the behavior of the pathogen equation:



Adding TNF-alpha to the cortisol model yielded the following:



A zoomed-in view shows the behavior early on in the model:



Discussion

Addition of the effects of a high initial TNF-α concentration to the mPWHM yielded increased maximum values for inflammation (N) and fibroblasts (F). By modifying the N and F equations, systemic TNF-α also decreased the amount of time it took for pathogen (P) to be cleared from the system. These results suggest that traumatic wounds may reach a higher level of inflammation due to the action of pro-inflammatory mediators, but that this is not harmful since pathogen is cleared more quickly.

In order to check the accuracy of this prediction, the modified Pitt wound healing model should be more closely examined and written so that the damage, inflammation, and fibroblast equations return to equilibrium at zero, indicating that the wound is healed. In addition, the TNF-α equation should be added to the original wound healing model without cortisol and the results should be analyzed to determine exactly how the TNF-α equation is behaving on its own.

Acknowledgements

This work was funded by an undergraduate research grant from the National Science Foundation to the Virginia Commonwealth University Bioinformatics and Bioengineering Summer Institute.

Appendix A. Model Parameters

Parameter	Value
pInfinity	20.00
O_2	10.00
O_{2crit}	5
kpg	0.3
kpm	0.60
sm	0.005
μm	0.002
kmp	0.010
kpn	1.80
β	1.00
snr	0.080
μnr	0.012
μn	0.050
μfn	0.002
knp	0.10
knn	0.010
knd	0.020
λ	1.00
kdn	0.35
μd	0.020
μdf	0.002
β_d	0.020
xdn	0.060
α	0.100
sf	0.013
kfn	0.040
kfnd	48.00
μf	0.100
c	[6.10 -4.75 -3.76]
d	[3.93 -2.53]
γ_A	0.50
β_I	2.70
β_C	0.60
κA_{max}	0.75
baselineshift	3.00

Appendix B. MATLAB Code and Function Descriptions

Main file:

```
%mainWHMc_plusT.m
%full wound healing model including cortisol and tnf-alpha

%Clear all variables
clear all

%assign global values so all programs can see the parameters
global pInfinity o2 o2crit kpg kpm sm mum kmp kpn betaP beta snr munr mun
mufn knp knn knd lambda kdn mud mudf betaD xdn alpha sf kfn kfnd muf c d
gammaA betaI betaC kamax baselineshift

%define parameters
pInfinity = 20.0;
o2 = 10.0;
kpm = 0.6;
sm = 0.005;
mum = 0.002;
kmp = 0.01;
kpn = 1.8;
beta = 1; %can also be 25 mg/dl
snr = 0.08;
munr = 0.012;
mun = 0.05;
mufn = 0.002;
knp = 0.1;
knn = 0.01;
knd = 0.02;
gamma = 1.0;
kdn = 0.35;
mud = 0.02;
mudf = 0.002;
betaD = 0.02;
sf = 0.0125;
kfn = 0.04;
kfnd = 48.0;
muf = 0.1;
c = [6.10 -4.75 -3.76];
d = [3.93 -2.53];
gammaA = 0.5; %dimensionless
betaI = 2.7; %units are 1/min
betaC = 0.6; %units are 1/min
kAmax = 0.75; %units of ug/dl

%define whm initial conditions
whm10=1.0;
whm20=0;
whm30=0;
whm40=0;
whm50=0;
whm60=0;
whm70=4.0;
```

```
% (1) = pathogen
% (2) = inflammation
% (3) = fibroblasts
% (4) = damage
% (5) = adrenal cortisol
% (6) = plasma cortisol

%tell it to solve the ode system in ODEwhmc.m from t=0 to t=150
[t,whm]=ode45('ODEwhmc_plusT',[0 200],[whm10; whm20; whm30; whm40; whm50;
whm60;
whm70],[],pInfinity,o2,o2crit,kpg,kpm,sm,mum,kmp,kpn,betaP,beta,snr,munr,mun,
mufn,knp,knn,knd,lambda,kdn,mud,mudf,betaD,xdn,alpha,sf,kfn,kfnd,muf,c,d,gamm
aA,betaI,betaC,kamax,baselineshift);

%plot the results
plot(t,whm);
%
legend('Pathogen','Inflammation','Fibroblasts','Damage','Adrenal
Cortisol','Plasma Cortisol','Plasma TNF-
alpha','location','northeastoutside');
xlabel('time(seconds)');
ylabel('Amount');
title('Wound Healing Model');
```

ODE File:

```
%ODEwmc_plusT.m
%define a function that solves ODE's using the following variables
%the first variable is independent, the second variable is dependent
function whm_dot = ODEwhmc_plusT(t,y)

global pInfinity o2 kpm sm mum kmp kpn beta snr munr mun mufn knp knn knd kdn
mud mudf betaD sf kfn kfnd muf c d gammaA betaI betaC kcort ktpro knmig
%redefining these got rid of an error even though they are global so they
%should not need to be redefined

%define parameters
pInfinity = 20.0;
o2 = 10.0;
kpm = 0.6;
sm = 0.005;
mum = 0.002;
kmp = 0.01;
kpn = 1.8;
beta = 1; %can also be 25 mg/dl
snr = 0.08;
munr = 0.012;
mun = 0.05;
mufn = 0.002;
knp = 0.1;
knn = 0.01;
knd = 0.02;
kdn = 0.35;
mud = 0.02;
mudf = 0.002;
betaD = 0.02;
```

```
sf = 0.0125;
kfn = 0.04;
kfnd = 48.0;
muf = 0.1;
c = [6.10 -4.75 -3.76];
d = [3.93 -2.53];
gammaA = 0.5; %dimensionless
betaI = 2.7; %units are 1/min
betaC = 0.6; %units are 1/min
kAmax = 0.75; %units of ug/dl
kcort = 0.2; %for now this is arbitrary
ktpro = 0.2; %another arbitrary constant describing the tnf-a effect on
fibroblast proliferation
knmig = 0.5; %arbitrary constant for the neutrophil migration effect

%tell it how many equations
whm_dot = zeros(6,1);

%feed it the differential equation for pathogen
whm_dot(1)= (Pgrowth(o2)*y(1)*(1-(y(1)/pInfinity))-
(kpm*sm*y(1))/(mum+kmp*y(1))-kpn*F(y(2),y(3)*y(1)*S1(y(6))))*S6(y(1));

%feed it the differential equation for inflammation
whm_dot(2)= ((snr*R(y(1),y(2),y(4),y(3)))/(munr+R(y(1),y(2),y(4),y(3)))-
mun*y(2)-mufn*y(3)*y(2))*S2(y(6),t)+knmig*y(7);

%feed it the differential equation for fibroblasts
whm_dot(3)= sf+(kfn*F(y(2)+kfnd*y(4),y(3)))/(1+F(y(2)+kfnd*y(4),y(3)))-
muf*y(3)+ktpro*y(7)*S7(t);

%feed it the differential equation for damage
whm_dot(4)= kdn*FS(F(y(2),y(3)))*S4(y(6))-mud*y(4)-
mudf*y(4)*y(3)*S5(y(6))+betaD*G(o2)*(y(4)/(y(4)+1));

%feed it the differential equation for adrenal cortisol
whm_dot(5)= -betaI*y(5)+kAmax*MU(t);

%feed it the differential equation for plasma cortisol
whm_dot(6)= betaI*y(5)-betaC*y(6);

%feed it the differential equation for systemic tnf-alpha
whm_dot(7)= (-Deg(t)-kcort*y(6))*S6(y(7));

% y(1) = pathogen
% y(2) = inflammation
% y(3) = fibroblasts
% y(4) = damage
% y(5) = adrenal cortisol
% y(6) = plasma cortisol
% y(7) = plasma tnf-alpha
```

Function Files (each is a separate .m file):

```
%F.m
function f = F(b,c)
fInfinity = 0.28;
f = b/(1+(c/fInfinity)^2);

%FS.m
function f = FS(k)
xdn = 0.06;
f = k^6/(xdn^6+k^6);

%R.m
function f = R(e,f,g,h)
knp = 0.1;
knn = 0.01;
knd = 0.02;
f = F((knp*e+knn*f+knd*g),h);

%Pgrowth.m
function f = Pgrowth(d)
o2crit = 5.0;
kpg = 0.3;
betaP = 0.3;
if (d > o2crit)
    f = kpg;
else
    f = kpg + betaP*(1-(d/o2crit));
end

%G.m
function f = G(l)
o2crit = 5.0;
alpha = 0.1;
f = exp((o2/o2crit)*log(alpha));

%MU.m
function f = MU(o)
baselineshift = 3; %defined as mu in the paper by Brown et al, 2001
b = [1 1];
c = [6.10 -4.75 -3.76];
d = [3.93 -2.53];
for i = 1:2
    b(i)= c(i+1)*cos(2*pi*i*o/24)+ d(i)*sin(2*pi*i*o/24);
end
f = c(1)+ sum(b)+ baselineshift;

%Deg.m
function f = Deg(t)
whm70 = 4.0; %this should always be the same value as set in the main code
for the initial condition of the TNF-alpha equation
thalf = (2.8/60); %half life of TNF-alpha converted to hours
f = whm70*exp(-log(2)/thalf*t);
```

```
%S1.m
function f = S1(a)
beta = 1; %can also be 25 mg/dl
if (a > beta)
    f = 0.5;
else
    f = 1;
end

%S2.m
function f = S2(i,j)
if (i <= 25)
    f = 1;
else
    if (-1 <= j < 48)
        f = 0.5;
    elseif (48 <= j < 72)
        f = 0.25;
    elseif (72 <= j < 96)
        f = (1/3);
    elseif (96 <= j < 120)
        f = 0.5;
    elseif (120 <= j < 144)
        f = (2/3);
    elseif (144 <= j < 168)
        f = 0.75;
    elseif (j >= 168)
        f = 1;
    else
        f = 2;
    end
end

%S4.m
function f = S4(m)
beta = 1; %can also be 25 mg/dl
lambda = 1;
if (m > beta)
    f = 0.5*lambda;
else
    f = lambda;
end

%S5.m
function f = S5(n)
if (n > 25)
    f = (4240/4750);
else
    f = 1;
end
```

```
%S6.m
function f = S6(a)
if (a <= 0)
    f = 0;
else
    f = 1;
end
%This function is multiplied by the tnf-alpha equation to keep it from
%going negative since there are no production terms

%S7.m
function f = S7(t)
if (t < 28)
    f = 1;
else
    f = 0;
end
%This function is used to delay the fibroblast proliferation response until
28 hours
```

References

Battegay, E. J., Raines, E. W., Colbert, T., & Ross, R. (1995). TNF-alpha stimulation of fibroblast proliferation. Dependence on platelet-derived growth factor (PDGF) secretion and alteration of PDGF receptor expression. *J Immunol, 154*(11), 6040-6047.

Brown, E. R., Charles, K. A., Hoare, S. A., Rye, R. L., Jodrell, D. I., Aird, R. E., et al. (2008). A clinical study assessing the tolerability and biological effects of infliximab, a TNF-alpha inhibitor, in patients with advanced cancer. *Ann Oncol, 19*(7), 1340-1346.

Chandrasekharan, U. M., Siemionow, M., Unsal, M., Yang, L., Poptic, E., Bohn, J., et al. (2007). Tumor necrosis factor alpha (TNF-alpha) receptor-II is required for TNF-alpha-induced leukocyte-endothelial interaction in vivo. *Blood, 109*(5), 1938-1944.

Kiecolt-Glaser, J. K., Loving, T. J., Stowell, J. R., Malarkey, W. B., Lemeshow, S., Dickinson, S. L., et al. (2005). Hostile marital interactions, proinflammatory cytokine production, and wound healing. *Arch Gen Psychiatry, 62*(12), 1377-1384.

Menges, T., Konig, I. R., Hossain, H., Little, S., Tchatalbachev, S., Thierer, F., et al. (2008). Sepsis syndrome and death in trauma patients are associated with variation in the gene encoding tumor necrosis factor. *Crit Care Med, 36*(5), 1456-1462, e1451-1456.

Menke, N. B., Ward, K. R., Witten, T. M., Bonchev, D. G., & Diegelmann, R. F. (2007). Impaired wound healing. *Clin Dermatol, 25*(1), 19-25.

Tsutsumi, Y., Kihira, T., Tsunoda, S., Kamada, H., Nakagawa, S., Kaneda, Y., et al. (1996). Molecular design of hybrid tumor necrosis factor-alpha III: polyethylene glycol-modified tumor necrosis factor-alpha has markedly enhanced antitumor potency due to longer plasma half-life and higher tumor accumulation. *J Pharmacol Exp Ther, 278*(3), 1006-1011.

Werner, S., & Grose, R. (2003). Regulation of wound healing by growth factors and cytokines. *Physiol Rev, 83*(3), 835-870.