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Abstract: A new mathematical model for wound healing is introduced and applied to three sets of experimental data. The model is easy to implement but can accommodate a wide range of factors affecting the wound healing process. The data sets represent the areas of trace elements, diabetic wounds, growth factors, and nutrition within the field of wound healing. The model produces an explicit function accurately representing the time course of healing wounds from a given data set. Such a function is used to study variations in the healing velocity among different types of wounds and at different stages in the healing process. A new multivariable model of wound healing capable of analyzing the effects of several variables on accelerating the wound healing process is also introduced. Such a model can help to formulate appropriate strategies to treat wounds. It also would enable us to evaluate the efficacy of different treatment modalities during the inflammatory, proliferative, and tissue remodelling phases.

Keywords: Wound healing, hyperbolastic models, diabetes, growth factors, trace elements

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Section 1. Introduction

The healing of tissue wounds is an important area of biological research which has been extensively studied using mathematical modelling. We present a new mathematical model of wound healing which is highly accurate in representing the time course of the healing and which can be used as a tool in the study of the underlying biological events. We believe this model should be very useful to researchers in accurately representing the time course of the wound healing and the rate of healing, both as a function of additional experimental variables and as the rate varies over the course of the healing.

Although the healing of wounds is a biological process fundamental to normal functioning and repair of the human body, this biological response is highly complex, involving both the inflammatory response of the immune system, signaling among various cytokines, action of various signaling cascades, and migration of many cell types, including neutrophils, macrophages, fibroblasts, and keratinocytes. Gillitzer and Goebler [18] describe wound healing as "one of the most complex biological events after birth" as a result of the interplay between many different cell types and tissue structures and the complex array of signaling. During the initiation of the wound healing sequence of events, many of these cell types needed in the healing are required to migrate to the region of the wound. Migration continues its importance as the healing proceeds, as cells must migrate into the bed of the wound for purposes of angiogenesis, fibroplasia, and reepithelialization. The process of wound healing has three overlapping stages plus the preliminary clotting stage, each with multiple substeps, and there are complex interactions and dependence relations among these various processes. The action of these separate phases, and each of their individual steps, must be properly coordinated. A number of pathologies in wound healing occur when certain steps are not completed in their turn or when certain factors are out of balance. Although a great deal is known about the proper course of healing of a wound, aspects such as locomotion and matrix production are not yet fully understood [24].

Furthermore, there is much less known about the pathological cases where wound healing does not proceed properly. In particular, it would be highly desirable to understand the underlying processes to a level where intervention in a non-healing wound could restore the proper balance to allow the normal healing process to proceed.

Wound healing is an important area of biomedical research at the intersection of several other important areas including cellular migration, cellular signaling, and action of cytokines. It also has important relations to medical areas such as aging and cellular senescence [30, 32] as well as angiogenesis and the metastatic environment for cancer [9, 14, 27, 42]. Advances in wound healing have a positive societal impact, both in terms of time and resources in the medical system [20] and in terms of human suffering, especially in cases of ulcerous wounds [13, 37]. The healing of diabetic ulcers and other types of ulcerous wounds are areas of particular concern in wound healing research.

We briefly outline the sequence of events underlying the process of healing of a tissue wound. There are three separate stages in the process of wound healing, with overlap between successive stages and with many biological signals from one stage which have a considerable impact on the progression of the next stage. The first stage in wound healing is the inflammatory stage, followed by proliferation stage, with the process finally completed in the tissue remodelling stage. These are all preceded by a necessary pre-stage of hemostasis in which the platelets stop the bleeding as the body regains homeostasis. This is necessary before repair can begin, and it also initiates several important cell signaling events and other processes leading into the inflammatory and later phases. In order for wound healing to proceed along the normal pathways, there are many players which must be coordinated effectively, and there are many points at which the normal process can be disturbed or even break down. Two important pathologies in wound healing are ulcers, in which the wound is stuck in a state of chronic inflammation and the wound bed is filled with destructive enzymes, and fibrotic

wounds in which excess deposition of matrix and lack of remodelling lead to hypertrophic or keloid scar formation [13].

During the inflammatory stage, the main task is the removal of debris, damaged tissue, and bacteria by neutrophils and macrophages in the process of phagocytosis. Pro-inflammatory factors and growth factors are released, beginning the alteration in the local environment needed for wound healing. The pro-inflammatory factors help to attract other cells needed for healing and furthermore stimulate proliferation of cells. The growth factors are needed for a number of purposes, including in the proliferative stage to stimulate cellular division, migration, and angiogenesis.

The main events of the proliferative stage include angiogenesis, in which new blood vessels are formed, followed by formation of granulation tissue and epithelialization. This phase ends with the contraction of the wound in which the wound edges are pulled together. The process of angiogenesis is pushed forward by low oxygen content in the wound and by cytokines released by macrophages in the late inflammatory stage. In angiogenesis endothelial cells migrate into the wound area and form new blood vessels and capillaries, bringing oxygen and nutrients to the wound site. The process of fibroplasia, in which granulation tissue is deposited in the wound bed, is also based on migration and is also stimulated by hypoxia and action of cytokines. As the fibroblasts migrate into the wound and deposit granulation tissue for a temporary ECM (extracellular matrix), they also release growth factors to attract epithelial cells, leading to reepithelialization. The keratinocytes which proliferate at the boundary of the wound cannot begin reepithelialization until granulation material on which they can migrate is available. Proliferation of the epithelial cells at the wound margin continues as more cells are produced to cover the entire wound surface. Finally, in contraction myofibroblasts form adhesions to the ECM at the wound edges. They pull together the edges of the wound by a mechanism similar to smooth muscle, reducing the wound size.

During the final stage of wound healing, the underlying tissue laid down in the earlier phases is reorganized and replaced with stronger, more stable material, while some of the excess vascular tissue formed in wound healing is removed via apoptosis. The strength of the wounded tissue continues to increase during this stage. The collagen III laid down during the proliferative phase is replaced by collagen I. The collagen I are able to bond and form cross-linkages forming an organized network of collagen and a stronger tissue. Note that certain other mathematical models, such as the study of Cobbold and Sherratt [11], have demonstrated that the course of this phase of tissue remodelling is directly related to the events in the earlier phases of inflammation and proliferation. One of the main themes in wound healing is the interrelatedness between the phases and the impact of one phase upon another, while another is the delicateness of the proper course of healing.

The majority of the extensive research in mathematical modelling of wound healing has used the approach of systems of partial differential equations which model specific functional relationships between specific predetermined variables such as collagen production, amount of oxygen, level of a growth factor, etc. A good example of a microscopic model for a wound healing disease based on interactions at the cellular level can be found in Bianca [5]. Over the past 27 years, Sherratt and his collaborators have been very influential in modelling of wound healing using microscopic models based on systems of PDEs, with numerous papers. A short summary of this direction of research can be found in Murray [28], chapters 8 through 10. As Murray mentions, “we are without question a long way from being able to reliably simulate actual wounds” by using this approach. However the importance of such models instead lies in the ability to formulate a “hypothetical mechanism and examine its consequences in the form of a mathematical model”. As such, these models interact with science by allowing mathematical investigation of hypotheses which can be further explored by scientific investigation.

Our approach is somewhat different from the above microscopic approach in which variables and relations are constructed mathematically based upon interactions at the molecular or cellular levels. We instead focus on attaining an accurate mathematical description of the wound healing process from experimental data, and in particular we are concerned with analysis of the temporal dynamics of the wound healing rate. The goal is to accurately represent experimental data from the healing of wounds in the form of explicitly defined functions. By producing explicit functions accurately approximating the wound healing rates observed in experimental data, this type of model allows for direct interaction of scientific investigation in formulation of the underlying mathematical relationships. These models become a means through which empirical data can be taken up into the more theoretical level of mathematics.

Our model falls into the category of supermacroscopic models, which may be described as mathematical models in which the interactions are based on observable data at the macroscopic, or tissue level, in this case the size of the healing wound remaining to be healed. For more details about macroscopic models, please see the article by Fusi [16]. See also Bellomo *et al*. [4] for examples of tissue models based on an underlying biological description at the cellular level.

The hyperbolastic models introduced in **Section 2** will be applied to model the healing of wounds in three separate data sets. **Section 3** models length wounds treated with magnesium hydroxide. **Section 4** compares diabetic wounds without treatment, diabetic wounds treated with placental growth factor, and non-diabetic wounds. **Section 6** compares the healing of wounds under different levels of the nutrient zinc in the diet. In analyzing the time course of the healing for these sets of data, the time course of healing represented in the data is related to the regular course of healing represented in the stages described above. In particular, the wounds treated with magnesium have a marked impact on the course of the proliferative phase, supplements of zinc have an impact on the early inflammatory

phase, and diabetic wounds experience a deficient inflammatory phase which also affects the healing in the remaining stages.

In its focus of finding an effective mathematical representation and understanding of actual experimental or clinical data, the focus of our model is comparable to the focus in several recent papers. The work of Cukjati *et al*. [12], which models rate of healing of many chronic wounds in a clinical setting, is the closest to our study in the current paper. These authors fit a number of three- and four-parameter models to a large collection of data from healing of chronic wounds. They selected the delayed exponential model on the basis of not only accuracy of fit, but also biophysical significance and predictive capability. The paper of Wallenstein and Brem [41] also addresses wound healing from a point of view similar to ours. The authors utilize a nonlinear model for statistical analysis of actual data on closure of wounds with the goal of calculating expected rate of healing as a function of time. Such a model can be useful for "identifying factors, evaluating treatments, and improving our understanding of the variables that affect the wound-healing process." Cardinal *et al*. [8] consider modelling the healing or non-healing of venous leg ulcers using an exponential model. In the cases when the exponential models fits the data well, parameter estimates allow a predication of time until wound closure, and the authors believe that "similar models may someday reach routine use in comparing the efficacy of various treatments in routine practice and in product registration trials." These models relying upon experimental data have also been very successful in describing and understanding the healing process.

In Cukjati et al. [12] the authors undertake a comparative analysis of mathematical models for healing of wounds and select the delayed exponential, primarily upon the basis of accuracy of fit and biological meaning of the model and parameters. We believe that the hyperbolastic models presented in this paper give better results than the delayed exponential, both in accuracy and in meaningful expression of the biological reality. Each of the parameters in these models can be assigned a meaning

in the context of wound healing, as expressed in **Section 2**. However, we obtain in addition an explicit function whose derivative represents the rate of healing as a function of time. This dynamic characterization of healing rate is closer to the biological reality where the healing rate varies over time, at different stages of the healing process, and with other factors. The hyperbolastic models have much flexibility in the rate of healing over the time course of the wound, whereas the delayed exponential will always predict a fixed rate of exponential decay, where that rate is determined by the parameter estimate. In the case studies of **Sections 3**, **4**, and **6**, we are able to observe how the dynamic rate of healing is closer to the reality found in experimental data. The healing rate can furthermore be related directly to the underlying biological processes.

This accurate representation of the dynamics of wound healing in an explicit function allows researchers to study the effect of a given treatment, or other variable, on the velocity and acceleration of healing at any stage of the healing process. Using the multivariable model of Section 5, it is possible to also describe how the rate changes with additional explanatory variables. One of the strengths of these models is the ability to represent variation in the rate of healing, both over the course of the above stages in the healing process, and with any additional variables that may affect the healing. Thus the models can be used with experimental data as a tool to study the effects of different treatments or other variables on the rate of healing at different stages in the healing process. These features of this new model for wound healing did not exist in previous models.

Uncovering the dynamics of the rate of healing is an important area of study that has been proposed by previous researchers. For instance, Robson *et al*. [33] describe the importance of consideration of the entire trajectory of wound healing in analyzing the effectiveness of wound healing agents. The new model for wound healing presented in **Section 2** makes this possible. For the data sets in **Sections 3**, **4**, and **6**, we illustrate how the trajectory of the wound healing relates to each treatment.

Section 2. Hyperbolastic Growth Models

Tabatabai *et al*. [40] first introduced the hyperbolastic growth models, and these have been successfully applied to model a range of biological data including cancer growth [15,40] and stem cell proliferation [7,39]. Here we give the form of these models, H1, H2, and H3, and some of their properties. The models will be used to describe the wound healing data in **Sections 3**, **4**, and **6**. In this setting of wound healing, the function P(t) will represent either the amount of the wound that has healed or the amount of the wound that remains to be healed.

2.1 The Hyperbolastic Model H1

The first of the three differential equations is called the hyperbolastic growth rate of type I (H1) which is a nonlinear differential equation of the form

$$\frac{dP(t)}{dt} = \frac{P(t)}{M}(M - P(t))\left(\delta + \frac{\theta}{\sqrt{1+t^2}}\right), \tag{2.1}$$

with the initial condition $P(t_0) = P_0$, where $P(t)$ is the size at time t, the constant M is the parameter representing carrying capacity, and δ and θ are real constants. These parameters δ and θ jointly determine the growth rate. The parameter δ gives the intrinsic growth rate, while the size of |θ| represents the distance from symmetric sigmoidal growth. These both can take any real values. Solving the equation (2.1) for the population size P gives

$$P(t) = \frac{M}{1 + \alpha\, EXP\left[-\delta t - \theta \operatorname{arcsinh}(t)\right]}, \tag{2.2}$$

where $\alpha = \dfrac{M - P_0}{P_0} EXP\left[\delta t_0 + \theta \operatorname{arcsinh}\left(t_0\right)\right]$

and arcsinh(t*)* is the inverse hyperbolic sine function of t. We call the function *P*(*t*) of equation (2.2) the hyperbolastic growth model of type I, or simply H1.

2.2 The Hyperbolastic Model H2

The following nonlinear differential equation, from Tabatabai *et al*. [40], is called the hyperbolastic growth rate of type II (H2)

$$\frac{dP(t)}{dt} = \alpha\delta\gamma\, P^2(t)\, t^{\gamma-1} \tanh\left[\frac{M - P(t)}{\alpha P(t)}\right] / M, \qquad (2.3)$$

with the initial conditions $P(t_0) = P_0$, where tanh[.] stands for the hyperbolic tangent function, and M, δ, and γ are parameters, which are real constants. M represents the carrying capacity or limiting value, while δ and γ jointly determine the growth rate. The value of δ represents the intrinsic growth rate, while γ is an allometric constant. Each of these parameters can take any real value. Solving the equation (2.3) for population size *P* gives

$$P(t) = \frac{M}{1 + \alpha \operatorname{arcsinh}\left[EXP\left(-\delta t^{\gamma}\right)\right]}, \qquad (2.4)$$

where $\alpha = \dfrac{M - P_0}{P_0 \operatorname{arcsinh}\left[EXP\left(-\delta t_0^{\gamma}\right)\right]}$.

We call the function P(t) of equation (2.4) the hyperbolastic growth model of type II, or simply H2.

2.3 The Hyperbolastic Model H3

Finally, we consider the third growth curve through the following nonlinear hyperbolastic differential equation of the form

$$\frac{dP(t)}{dt} = (M - P(t))\left(\delta\gamma t^{\gamma-1} + \frac{\theta}{\sqrt{1+\theta^2 t^2}} \right), \qquad (2.5)$$

with the initial condition $P(t_0) = P_0$ where M, δ, γ and θ are parameters, which are real constants. M represents the carrying capacity or limiting value, while δ, γ, and θ jointly determine the rate of growth. The size of |θ| represents the distance from symmetric sigmoidal growth, γ is an allometric constant, and δ is the intrinsic rate. Each of these can take any real value. We refer to the model (2.5) as the hyperbolastic ordinary differential equation of type III or H3. The solution to the equation (2.5) is

$$P(t) = M - \alpha\, EXP\left[-\delta\, t^{\gamma} - \operatorname{arcsinh}(\theta t) \right], \qquad (2.6)$$

where $\alpha = (M - P_0) EXP\left[\delta\, t_0^{\gamma} + \operatorname{arcsinh}(\theta t_0) \right]$.

We call the function $P(t)$ of equation (2.6) the hyperbolastic growth model of type III or simply H3. For additional details about these models and their derivation, please see Tabatabai *et al*. [40].

These models are similar in concept to other growth models such as logistic, Weibull, Gompertz, and Richards; however the hyperbolastic models, especially H3, have proven to be more flexible, leading to a higher degree of accuracy for a wider range of data. As models to represent wound healing, the hyperbolastic models offer a model which is easy for scientists to implement and which remains highly accurate when studying the wide variety of factors that influence the healing of wounds. Each of the hyperbolastic models can be easily implemented using the nonlinear regression package of SPSS to estimate the parameters. After entering the formula for (2.2), (2.4), or (2.6) into the box for Model Expression, it is then necessary to enter the initial value estimates for the parameters. In SPSS the arcsinh(x) function must be entered using its definition in terms of logarithms:

$\operatorname{arcsinh}(x) = \ln\left(x + \sqrt{1 + x^2}\right)$. An example of the source code used to estimate the parameters in SAS can be found in the additional file of [40].

Section 3. Magnesium and Trace Elements in Wound Healing

In this section we analyze the data from Alimohammad *et al*. [2] describing the effects of magnesium hydroxide on wound healing. We first outline some of the known effects of magnesium in the wound healing process, such as production of collagen, proliferation of endothelial cells, and facilitation of cellular migration. Senni *et al*. [36] observe the role of magnesium in the production of collagen, the principal agent produced to fill in the wound bed and the source of strength in the repaired tissue. In the study of Geesin *et al*. [17] magnesium was shown to stimulate the production of collagen. It is also observed in Senni *et al*. [36] that magnesium plays a role in the maintenance of proteoglycans, an important component of the extracellular matrix (ECM). Senni *et al*. [36] also discuss the role of the divalent cations Ca^{2+} and Mg^{2+} in cellular migration. These authors discuss the role of magnesium in binding of keratinocytes and fibroblasts to type I collagen and to laminins in the extracellular membrane. They further raise the possibility that magnesium may have some role in modulation of the activity of matrix metalloproteinases (MMPs), another means of control over cellular migration.

The role of magnesium in cellular migration is of fundamental importance for wound healing, and the paper of Lange *et al*. [21] demonstrates the role of Mg in wound healing by showing Mg^{2+} and Ca^{2+} affect the adhesion of fibroblasts and keratinocytes to the extracellular matrix (ECM). Banai *et al*. [3] identify several essential roles of magnesium in cellular function as related to cellular migration. The role of magnesium in regulation of cyclic AMP is conjectured by these authors as one means by which magnesium affects cellular migration, and another possible method is assembly of actin monomers and myosin ATPase activity, two components of the motor behind cellular migration.

The research of Grzesiak and Pierschbacher [19] suggest the Mg^{2+} and Ca^{2+} increase the ability of the cells to migrate, either on their own through a combined action with certain growth factors. The study of Lange *et al*. [22] similarly suggest that the type of concentration gradient in Mg^{2+} and Ca^{2+} found in tissue injury is needed for the cellular migration that is a part of wound healing. Furthermore Lange *et al*. [21] demonstrate *in vitro* that cellular adhesion to and deadhesion from the extracellular matrix (ECM) increases with the concentration of Mg^{2+} while higher levels of Ca^{2+} concentration suppressed the effect of Mg^{2+}. As observed in Schultz *et al*. [34], action of MMP's and TIMP's is essential to the migration of fibroblasts and other cells through the ECM (extracellular matrix). Fibroblasts bind to components of the matrix such as collagen, fibronectin, vitronectin, and fibrin, using integrin receptors. In order to migrate, these bonds must be broken, which is accomplished by proteases, particularly MMPs. Schulze-Tanzil *et al*. [35] have identified a co-localization of integrins and MMPs in the extracellular matrix (ECM).

Here we analyze the data from healing of a wound that has been treated with magnesium hydroxide on the surface of the wound. The hyperbolastic growth model H3 is applied to model the time course of the healing of this wound. In this case, using data from Alimohammad *et al*. [2] for a length wound treated with magnesium hydroxide cream, we model the time progression of the wound using the models H1, H2, and H3. These results are truly extraordinary, with a nearly perfect fit to the data. Table 1 presents the data for the time course of the wound healing as predicted by each of these models. Notice that although H3 has the most accurate fit, the remaining hyperbolastic models are exceptionally accurate.

Table1. Observed and estimated percentage values for the wound healing treated with magnesium hydroxide

Day	Observed Percentage	Estimated Percentage H3	Estimated Percentage H2	Estimated Percentage H1
3	11.63	11.63	11.63	11.63
6	49.75	49.75	49.75	49.72
9	94	94.00	94.01	94.01
12	100	99.99	99.88	99.84
15	100	100.01	100.11	100.14
		R^2 =1.000	R^2 =1.000	R^2 =1.000
		Residual Mean Square=.000	Residual Mean Square=.013	Residual Mean Square=.022

Next we analyze the time course of wound healing as predicted by the H3 model both for the data from the length wound treated with magnesium hydroxide cream and for the control case of an untreated length wound. This H3 model yields an equation describing precisely the time course of the wound, and the derivatives of this equation will also describe the rate of this healing, as well as its acceleration or deceleration at every stage of the wound healing process. We can then make a comparative analysis and describe the impact of the hydroxide cream in the healing at the various stages. We first present the parameter values when the wound healing data is fit by H3 in Table 2.

Table 2. Parameter estimates for the wound healing using hyperbolastic model H3

	Control Wound		MgOH treated Wound	
Parameter	Estimate	Std. Err.	Estimate	Std. Err.
M	100.124	6.483	100.0118	0.009
δ	6.717 E-11	0.000	0.0004	0.000
γ	9.38891	8.963	3.9808	0.003
θ	0.0562481	0.015	0.0306	0.000

In Figures 1 through 3 we present the graphs of the modelling of this wound healing data by H3. In each of these graphs, the solid line represents the wound treated by MgOH, while the dashed line represents the untreated wound. The first graphs show the time course of the healing.

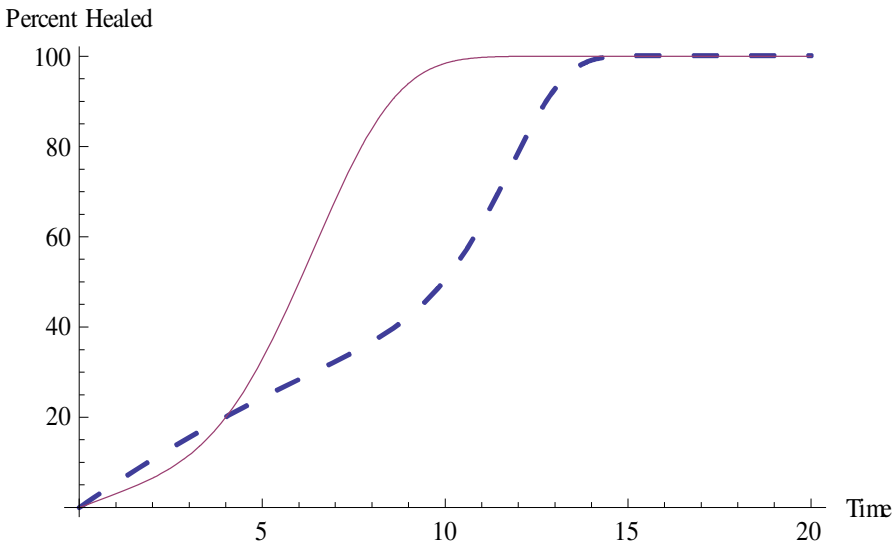


Figure 1 Healing of Wound

This is followed by two graphs describing the velocity and the acceleration of the healing.

Figure 2 Velocity of Healing

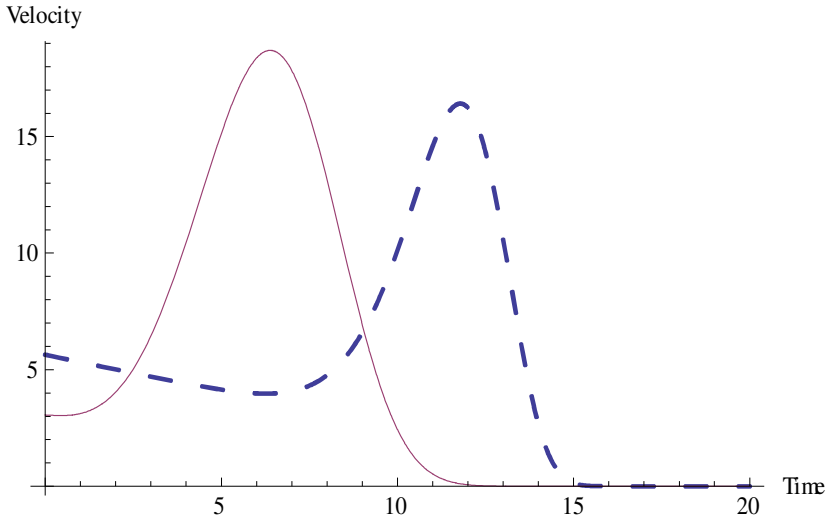
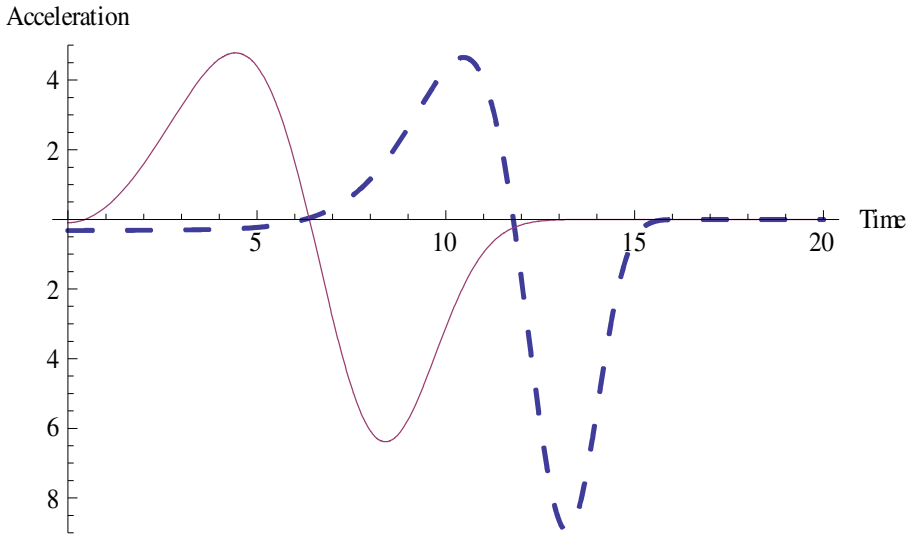


Figure 3 Acceleration of Healing



We recall from the introduction that magnesium plays several roles in the wound healing process. Much of its action is related to cellular migration. First the Mg^{2+} ions play an important role in binding of keratinocytes and fibroblasts to the ECM. This binding and release forms an important part of cellular migration. It was furthermore suggested the Mg^{2+} and Ca^{2+} concentration gradient found in tissue injuries also contributes to the cellular migration. Other studies have shown magnesium has an impact on the production of collagen used in forming the new tissue. All of these events take place during the proliferative stage of the wound healing. Here we make a comparative study between the wounds treated with magnesium and the control wounds to analyze the impact of the magnesium.

The first difference that we notice between the healing of the treated and untreated wounds is the total healing time. The treated wounds heal in just under 10 days, while the untreated wounds take approximately 15 days to heal completely. Comparing the healing velocities of the treated and untreated wounds, we see that the wound treated with magnesium reaches a slightly higher total velocity than does the untreated wound, and furthermore, this maximum velocity occurs much earlier in the wound healing process, providing for a much faster total healing time. For the treated wound, the

period with a high rate of healing is between days 5 and 9, with the maximum velocity occurring at 6.39 days. This is during the proliferative stage of the wound, beginning with the early proliferative stage. During this phase of the wound many cell types are migrating into the wound bed. We can assume the magnesium is acting by accelerating the production of collagen used in the new tissue and by facilitating the migration of fibroblasts, endothelial cells, and keratinocytes along the extracellular matrix from the wound margin into the body of the wound. In contrast, in the untreated wound the high rate of healing does not occur until days 10 through 14, much later in the process and after the treated wound has already healed completely. In this case the proliferative stage lasts longer, and we can assume that without the extra magnesium the migration of cells involved in the wound healing is delayed. Therefore, there is not as much production of collagen until a later time, and the completion of the proliferative phase is delayed in general as the necessary cells and components take longer to migrate.

Section 4. Diabetic Wounds and Growth Factors

Healing of diabetic wounds is one of the primary frontiers in research on wound healing, and it continues to be an area of active interest. From a scientific perspective there is the desire to explain the mechanisms for the impairment in healing of diabetic wounds, and much of the underlying interest is based upon the human interest of ending the suffering for the many who suffer from ulcerous diabetic wounds. Furthermore the expense within the medical system is considerable. From one point of view, diabetic ulcers are thought of as being stuck in the inflammatory stage, and this explanation successfully describes much of the experimental data.

Diabetic wounds are well known to have difficulty healing, with delayed healing sometimes leading to an ulcerous state. Although the causes are not entirely understood, diabetic wounds are characterized by diminished production of collagen, poor angiogenesis, and a prolonged and dysfunctional inflammatory response, as observed in Cianfarani *et al*. [10]. Some of the defect in healing is related to

reduced production of growth factors; thus an important area of research has been use of growth factors in an attempt to correct the healing dysfunction. However, the prolonged inflammatory phase is also a critical point, and the lengthened inflammatory phase can cause much damage to the wound environment. Schultz *et al*. [34] describe how a lengthened inflammatory phase can cause an imbalance in levels of proteases, such as MMPs, elastase, plasmin, and thrombin. The high levels of these chemicals causes damage to the ECM, which is critical for proper healing, and also damage the growth factors that are produced in the wound, leading to a chronic state. Recent research with advanced glycation endproducts (AGEs) has also implicated these in complicating the inflammatory response, interfering with proper cellular function and with the ECM, as described in Pierce [31]. One common method to treat chronic diabetic wounds is surgical debridement to clear the bad cells from the environment and to create a new wound bed where proper healing can take place. The paper of Gillitzer and Goebeler [18] suggests a detailed analysis of the roles of chemokines, particularly in regard to migration of inflammatory cells, in order to determine the proper factors to be administered at the proper times.

In this paper we focus on a more recently discovered cytokine, placenta growth factor (PlGF) and its role in wound healing, particularly in the area of stimulation of angiogenesis. Odorisio *et al*. [29] investigated the role of PlGF in skin repair, both in the embryonic stage and in post-natal life, concluding its strong angiogenic properties accelerate wound healing. This study was followed by the study of Cianfarani *et al*. [10] in which these angiogenic function of PlGF is used to improve the healing in diabetic wounds. Shyu *et al*. [38] studied treatement of wounds with hyperbaric oxygen, finding both an increase migration of mesenchymal stem cells and an increase in expression of PlGF. The data from Cianfarani *et al*. [10] compares wound healing in untreated diabetic mice and healthy mice with wound healing in diabetic mice treated with PlGF. Application of H3 to this wound healing data demonstrates that this model remains highly accurate for diabetic wounds, which follow a different pattern of healing.

It furthermore accommodates the healing pattern when the diabetic wound is treated with a growth factor, yielding a healing pattern somewhere between those for healthy and diabetic individuals.

Here we analyze the time course of these three types of wounds, and we make a comparison between the three cases with attention to the underlying biological processes. Considering the wound healing of the healthy individuals as the control case, the course of the healing is comparable to that for the control case in the magnesium section, the untreated individuals. The wound velocities of these two follow approximately the same pattern with the difference that the peak rate of healing occurs a few days earlier in this case, probably because of a smaller wound. The next case to consider is the diabetic wounds. It is generally known that diabetic wounds heal considerably slower than wounds in healthy individuals. Although there are many complex reasons behind the delayed healing in diabetic wounds, two of the primary reasons are a prolonged inflammatory period and reduced expression of certain necessary cytokines, as observed in Braiman-Wiksman *et al*. [6]. From the graphs, the delayed healing in the diabetic individuals is clear, and we furthermore observe that the diabetic wounds begin healing very slowly, with a low initial rate which only gradually increases. The graph of the wound healing velocity for the diabetic wound shows us that the rate stays low throughout the course of healing and is particularly low in the early stages of healing.

The wound healing for the diabetic wound treated with PlGF is somewhere in between the other two. Although the rate of healing starts out slow like the diabetic wound, the rate picks up quickly due to the action of the PlGF. We assume the PlGF is beginning to promote angiogenesis from the wound boundary, but the early impact also suggests it plays a role in the inflammatory phase. The data of Cianfarani *et al*. [10] confirms this impact of PlGF on inflammatory cell recruitment. The healing reaches its maximum rate quickly, with a maximum velocity about midway between that for the diabetic and healthy individuals. Comparing the graphs of the time course of healing for the healthy individuals and

the diabetic individuals treated with PlGF, we observe that these two stay for the first eight days in the healing process. After that the amount of closure of the diabetic individual treated with PlGF begins to lag behind that for the healthy individual. The PlGF provides enough momentum in the early stages of the healing but in the end the PlGF treated diabetic wounds do not keep pace with normal wounds. We assume that some of the inhibiting factors from the diabetic inflammatory state remains, and the increased angiogenesis of the PlGF is not enough to overcome this. These wounds may also lack some other important growth factors, and production of collagen or proliferation of kerationcytes may be lower than in normal wounds. Nevertheless, the rate of healing is significantly improved as compared to the untreated diabetic wounds and approaches that of the healthy individuals.

In Figures 4 through 6, the solid line represents the wounds in the healthy mice. The dotted line represents untreated wounds in diabetic mice, while the dashed line represents wounds in diabetic mice treated by PlGF. Figure 4 shows a graph of the time course of the healing, and Figure 5 and Figure 6 wound healing velocity and acceleration, respectively.

Figure 4 Healing of Wound

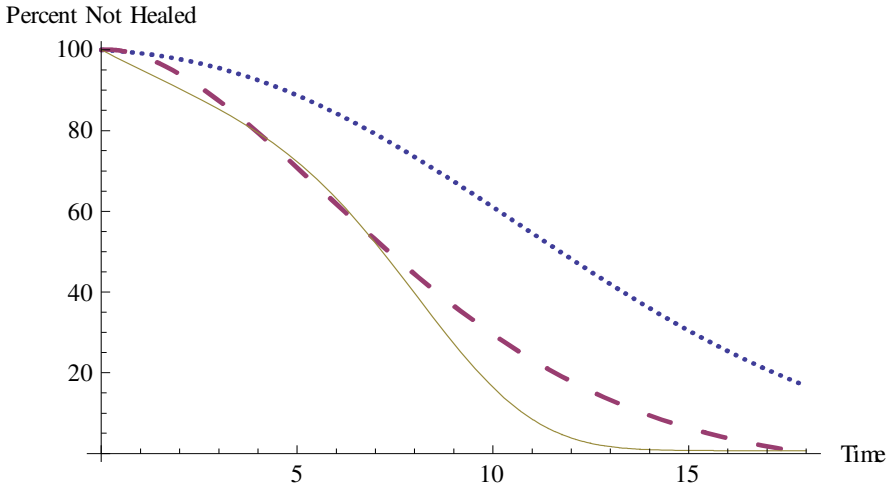


Figure 5 Velocity of Healing

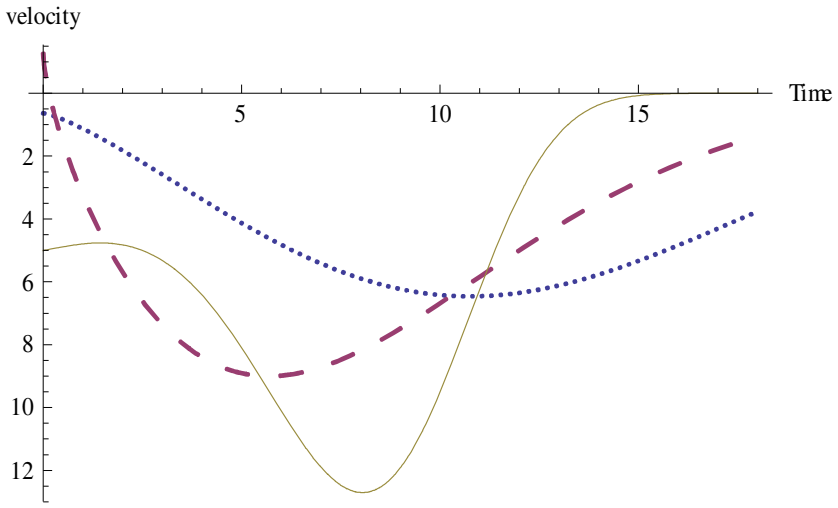


Figure 6 Acceleration of Healing

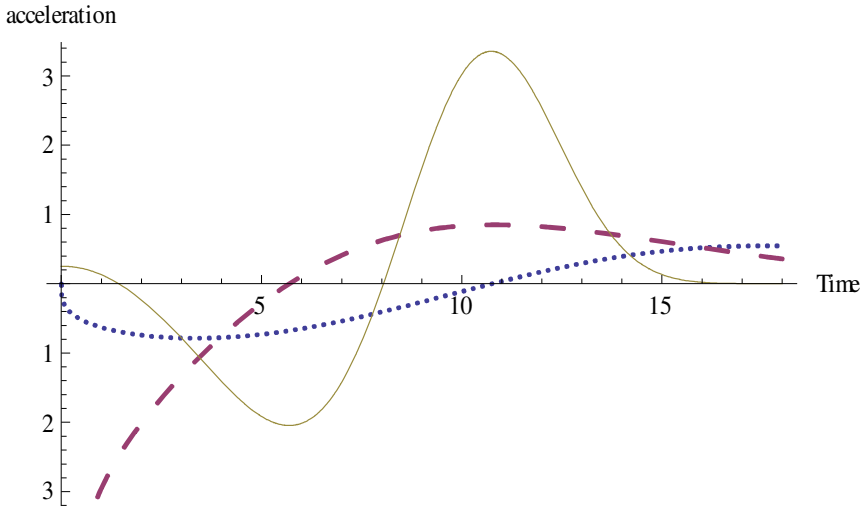


Table 3 shows the parameter values when H3 is fit to the wound healing data in Cianfarani *et al*. [10]. The table contains the parameter values from the untreated diabetic mice, the parameter values from the diabetic mice treated with PlGF, and the parameter values from healthy mice.

Table 3. Parameter estimates using hyperbolastic H3 for Diabetic Saline, Diabetic PlGF, and Non-diabetic Control

	Diabetic, Saline		Diabetic, PlGF		Non-diabetic Control	
Parameter	Estimate	Std. Dev.	Estimate	Std. Dev.	Estimate	Std. Dev.
M	-0.7436	2.555	-4.3750	2.621	0.7231	0.868
δ	0.0022	0.002	0.0256	0.019	0.0001	0.000
γ	2.2920	0.322	1.6797	0.244	4.1212	0.412
θ	0.0063	0.010	-0.01222	0.029	0.0504	0.006

Section 5. A Generalized Hyperbolastic Model for Wound Healing

Evidence suggests that in many areas of wound healing research, healing dynamics depend not only on time as a variable but also on a set of other explanatory variables such as age, amount of oxygen, or level of growth factors. These variables may affect the healing process in a variety of ways such as acceleration or deceleration of the wound healing dynamics. A means to predict the wound size while allowing for the effects of explanatory variables in accelerating the wound healing is needed. In this section we generalize the hyperbolastic model H3 to accommodate the case when multiple predictors are used in determination the healing rate. The multivariable generalization of H3 may be characterized by the rate of change of wound size with respect to each explanatory variable. These are given by the following nonlinear generalized hyperbolastic partial differential equations of type H3, of the form

$$\frac{\partial P(X;M,\theta_0,\theta_1,\lambda)}{\partial X_j} = \alpha\, EXP\Big[-g_1(X;\theta_0,\lambda) - \operatorname{arcsinh}(g_2(X;\theta_1,\lambda))\Big] * W,$$

where $W = \dfrac{\partial g_1(X;\theta_0,\lambda)}{\partial x_j} + \dfrac{1}{\sqrt{g^2{}_2(X;\theta_1,\lambda)+1}} . \dfrac{\partial g_2(X;\theta_1,\lambda)}{\partial x_j}$, (5.1)

with the initial condition $P_0 = P(X_0; M, \theta_0, \theta_1, \lambda)$ and the parameter vector λ. The vector X is a vector of explanatory variables and t is the time variable. The solution to the equation (5.1) is

$$P(X; M, \theta_0, \theta_1, \lambda) = M - \alpha\, EXP\Big[-g_1(X; \theta_0, \lambda) - \operatorname{arcsinh}(g_2(X; \theta_1, \lambda))\Big], \tag{5.2}$$

where

$$\alpha = (M - P_0) EXP\Big[g_1(X_0; \theta_0, \lambda) + \operatorname{arcsinh}(g_2(X_0; \theta_1, \lambda))\Big].$$

We call the function $P(X; M, \theta_0, \theta_1, \lambda)$ of equation (5.2) the generalized hyperbolastic model of type H3. The choice of the link functions $g_1(X; \theta_0, \lambda)$ and $g_2(X; \theta_1, \lambda)$ depends on the nature of the wound and how the variables of the model accelerate the process of healing. One possible choice of link functions has the form $g_1(X; \theta_0, \lambda) = \theta_0 EXP[\lambda_1(\lambda_2 t + \sum_{i=3}^{k+2} \lambda_i X_{i-2})]$

and

$$g_2(X; \theta_1, \lambda) = \operatorname{arcsinh}(\theta_1 EXP(t) + \sum_{i=3}^{k+2} \lambda_i X_{i-2}).$$

We use this choice of link function to model the zinc data of **Section 6**. Other sets of data may be more effectively modeled by alternative choices for link functions. The models H1 and H2 may be similarly generalized through use of appropriate link functions.

Each of the parameters λ_i in the link function will be associated to a specific explanatory variable representing the effect of a quantity on the healing of the wound. The λ_i then represents the effect of this explanatory variable on the rate of healing. Note that $\lambda_i = 0$ corresponds to no significance for the associated variable. Using this formulation, it is possible to apply statistical tests to determine the

significance of each variable in the wound healing. Note that the precise meaning of each λ_i may depend on the link function used, and for certain link function, some values of λ_i may represent the interaction between two or more variables.

Section 6. Nutrition and Zinc in Wound Healing

As a natural response of the body to injury, wound healing is affected by general health of the body and proper levels of nutrition are important factors. Zinc is well known as an important element for the overall health of the body, and particularly for its role in immune function and its role as an antioxidant. Zinc deficiency is associated with poor wound healing and with decreased strength of the healed tissue [1]. Although the role of zinc in healing of wounds is well established, there is only a limited knowledge about the mechanism behind its action. Zinc serves a role comparable to magnesium in matrix metalloproteinases that augment autodebridement and keratinocyte migration during wound repair as observed in Lansdown *et al*. [23]. Lim *et al*. [26] explored the means by which zinc can alter the inflammatory stage in the healing of wounds, in particular investigating the roles of the transcription factor NF(kappa)B and the cytokines IL-1(beta) and TNF-(alpha). These results demonstrate that mRNA levels of IL-1(beta) and TNF-(alpha)were decreased in cases of zinc deficiency, together with a decline of infiltration of neutrophils during the inflammatory stage.

Due to the interconnectedness between the overlapping phases of wound healing and the dependence between the various stages, what happens at the outset of the inflammatory phase as a wound begins to heal can have a dramatic impact that endures throughout the course of the healing process. Thus the inflammatory stage of wound healing, which is mostly completed within the first few days is highly significant to proper healing and to the course of the remaining stages of the wound. In Lim *et al*. [26] the authors hypothesize that zinc deficiency inhibits the normal NF(kappa)B binding activity, leading to the deleterious effects of impaired cytokine production and reduced neutrophil

infiltration into the wound site. The data and analysis of [25, 26] support this hypothesis, both in terms of the rate of healing of the wounds and also in terms of the levels of mRNA expression for the pro-inflammatory cytokines IL-1(beta) and TNF-(alpha). Here we will apply the generalized hyperbolastic model H3 to model the time course of the healing of the wounds from the data in the dissertation of Lim [25]. Thus we are able to study the effects of zinc on the rate of the wound healing at any time throughout the progression of the healing of the wounds, and with particularly attention to the inflammatory phase.

This data set describes the effect of level of zinc in the diet on the rate of wound healing. Here we consider the healing of the wound as a function not only of the time variable, but also as a function of the level of zinc in the diet. This direction of research is important because it demonstrates the generalized hyperbolastic models can be used to model data in more than one variable, as suggested in **Section 6**. In this case we treat the three levels of zinc in the diet, deficient – 0 (mu)g/g, normal – 50 (mu)g/g, and supplemented – 500 (mu)g/g as three separate categories of treatment. Table 4 shows parameter values for the generalized H3 model in fitting the data of Lim [30]. The predictions of this function are highly accurate with R^2 of 0.991 and residual mean square error of 0.001.

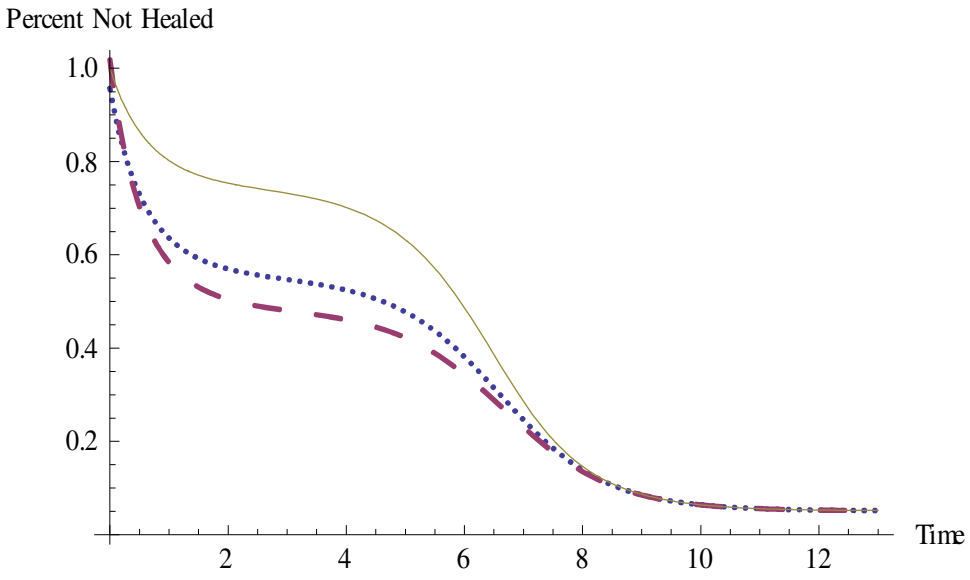
Table 4. Parameter estimates for Zinc data using generalized hyperbolastic H3

Parameter	M	θ_0	λ_1	λ_2	θ_1	λ_3	λ_4
Estimate	0.051	-0.315	1.917	-0.696	0.001	0.490	0.329
Std. Error	0.011	0.026	0.131	0.112	0.000	0.047	0.040

Figure 7 presents the time course of the wound healing for the three treatments, as well as the velocities and accelerations of the rates of healing in these cases. The solid line represents zinc

deficiency, the dotted line represents normal levels of zinc, and the dashed line represents zinc supplementation.

Figure 7 Healing of Wound



Figures 8 and 9 show the wound healing velocity and wound healing acceleration, respectively.

Figure 8 Velocity of Healing

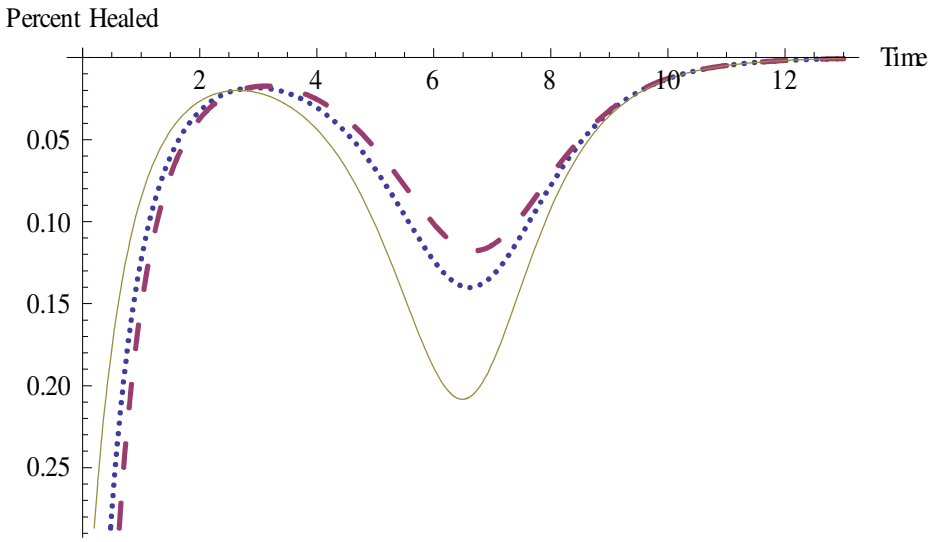
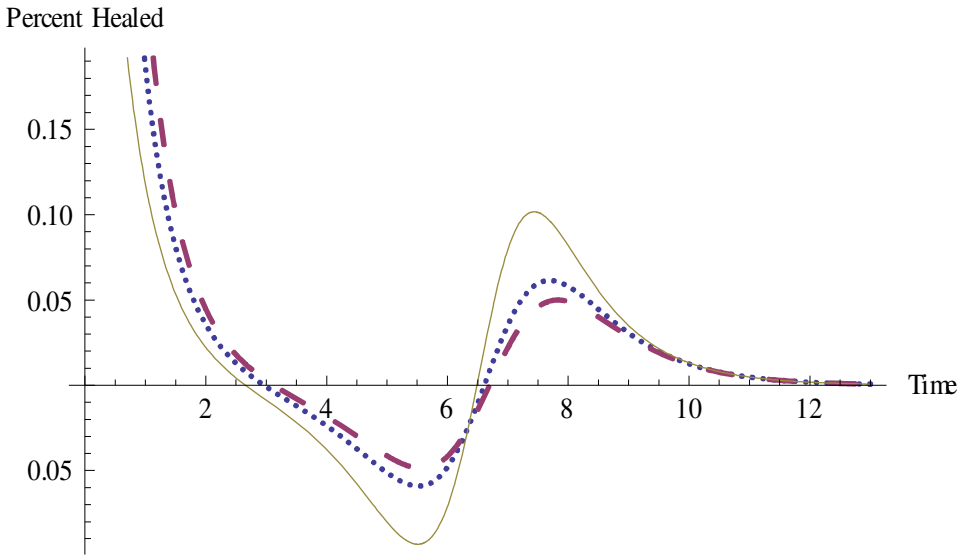


Figure 9 Acceleration of Healing



Consider Figure 7 which describes the time course of the healing, showing the percentage of the wound that remains unhealed. First observe the percentage of healing for the zinc deficient mice during the initial phase is much less than the amount of healing for others. However around day 6, as the inflammation period is ending, the rate of healing of the zinc deficient mice begins to make a significant increase, and shortly after that the healing of these mice approaches the healing for others. In all of these graphs we notice a rapid initial healing in the first day or two followed by a general slowing in the healing rate. But this trend is much stronger for the mice with normal levels of zinc or zinc supplements, in which forty to fifty percent of the healing is completed in the first two days.

This data appeared in Lim [25], together with other data involving the expression of mRNA for the pro-inflammatory cytokines IL-1(beta) and TNF-(alpha). Lim [25] and Lim *et al*. [26] concluded that healing is impaired during the early inflammatory phase for mice with zinc deficiency. Clearly the healing of the zinc deficient mice is impaired during the first 6 days, although after that point the rate increases. The wounds are practically even by day 8 in the process. The wounds started out with a high rate of healing for all three treatments, as can be observed in both the velocity graph and the graph of

the time course of the healing. However, we can also see that the rate of healing in the zinc deficient mice does not start as rapidly, and furthermore this rate slows much sooner. It appears that the zinc deficiency allows the wound to begin healing at a nearly normal rate, but that the delay in the rate begins shortly thereafter. This sequence of events is consistent with the proposed inhibition in healing due to decreased activation of NF(kappa)B and resulting reduced expression of IL-1(beta) and TNF-(alpha). The short time lag until the zinc deficient wound shows inhibited healing appears to correspond to the delay until these pro-inflammatory cytokines begin to affect the rate of healing.

For the zinc deficient mice the healing clearly proceeds slowly through remainder of inflammation phase then increases afterward. In this case zinc deficient healing has maximum rate after the inflammatory phase, and this maximum is higher for the mice with normal or supplemented levels of zinc. At this point the zinc deficient mice are completing healing that had been completed earlier in the other mice. Thus this higher rate must be interpreted as catching up to the normal progression of healing. From the velocity and acceleration of wound healing, we can determine that for the zinc deficient mice the minimum rate of healing occurs at 2.66 days, during the inflammatory phase, and the maximum occurs at 6.49 days, just after the inflammatory phase has completed. This analysis of the time course of the wounds supports the conclusions of Lim *et al*. [26] and further illustrates their point of zinc increasing the healing in the early inflammatory phase. The graphs also display the extent to which healing proceeded during the early inflammatory phase for the mice with sufficient zinc, with forty to fifty percent of the wound closed within the first two days.

Notice that the wound healing curves for the zinc deficient mice are irregularly shaped, in part due to the healing impairment during the early inflammatory phase. However, generalized H3 still models this data very effectively and accurately, providing a curve which remains close to all the data points. This illustrates our claim that the hyperbolastic growth model and the generalizations possess the necessary

flexibility to conform to the rate of healing exhibited by all varieties of wounds, and with all varieties of treatment. Clearly the shapes of other models, such as exponential or logistic, are not as free to accommodate this type of data. Furthermore we can make this statement even stronger since we were able to accommodate this data for three separate treatments using only one function dependent on both time and level of dietary zinc.

Section 7. **Conclusions**

In the course of this paper, we have demonstrated how the model H3 can be applied to accurately model the healing dynamics of wounds. Part of the power of the model is flexibility, enabling scientists to accurately model a wide variety of data sets with varying growth curves and can also accommodate a wide variety of functions influencing the healing. This flexibility combines with ease in implementation to make it a powerful and effective tool for researchers. Three separate data sets, dealing with different types of wounds and different treatments, were modeled. Although the healing patterns were different, as were the shapes of the resulting graphs, all of the cases were modeled accurately by this one model. It is important that these models can accurately predict different types of behavior associated with different types of treatments. In each of these cases the growth model H3 yields a function representing the time course of the healing, and from this function are also obtained functions for the healing velocity and acceleration. As we illustrated in each of the data sets, the time course of the healing can also be related to underlying biological events in the healing. Furthermore a comparison of the rates of healing at different stages for an untreated and a treated wound can help researchers to evaluate the treatment at various stages in the healing process.

The action of magnesium in promoting the healing of wounds is primarily due to increasing the facility of cellular migration and to increasing the production of collagen. In the wound treated with magnesium the maximum velocity was higher than the untreated wound, and this maximum healing

velocity also occurs earlier than in the untreated wound. The higher rate of healing velocity is directly related to the increased facility of cells migrating into the wound to produce the healing. Furthermore, as the components needed for healing can migrate to the wound site faster, this maximum rate of healing can occur earlier in the total process. This explains the mechanism behind the significant decrease in healing time for the wound treated with magnesium.

In the case of the diabetic wounds, the velocity graph offers a good illustration of the difference in healing between the control wound in the normal mice and the treated and non-treated diabetic wounds. The healing pattern in the control wound is like that in the other data sets where a positive initial rate of healing soon increases to a maximum rate of healing at some point in the proliferative phase, between six and ten days into the healing. In contrast, the diabetic wounds start out very lethargically, with a low rate of healing, and only gradually increase. The rate of healing in the diabetic wounds after ten days of healing has reached the initial rate for a normal wound, but the maximum rate never gets close to that for the normal wounds. This very low rate of initial healing characterizes the problem with diabetic wounds, an imbalanced inflammatory phase which lasts too long and creates problems for the later phases of healing. The treated diabetic wound overcomes these deficiencies somewhat, and it approaches but does not reach the normal course of healing. Although the velocity starts out low, there is an initial high acceleration in healing, representing the influence of the placenta growth factor on inflammation and later angiogenesis. In fact the increased angiogenesis allows the diabetic wound treated with PlGF to reach its maximum rate of healing sooner than the control wound. The healing in the treated wound nearly equals that in the control wound for the first eight days, however it is outpaced thereafter. Although the stimulation of angiogenesis by PlGF assists the healing of the wound, it does not overcome all the deficiencies of diabetic healing. The shape of its velocity remains more like the diabetic wound and distinct from the control wound. Other factors of the healing deficiency of diabetic wounds are not corrected by the application of the growth factor.

When considering the role of zinc in the diet upon healing of wounds, the information from the hyperbolastic model also supports the underlying biology. In this case the authors who collected the data concluded that zinc affects the early inflammatory stage of healing, while presenting other evidence that the mechanism of action is likely through the regulatory protein NF(kappa)B and its role in production of the pro-inflammatory cytokines IL-1(beta) and TNF-(alpha). The data showed a very high rate of healing in the early inflammatory phase for the wounds of the mice whose diet had normal or supplemented levels of zinc. The brief time lag until the healing slows conceivably corresponds to the time until the IL-1(beta) and TNF-(alpha), produced through regulation of NF(kappa)B play a role in the healing.

It is often important to consider several variables in a mathematical model, and **Section 5** presents a multivariable form of the hyperbolastic growth model H3. **Section 6** presents data which is modeled with an extra categorical variable representing either zinc deficiency, normal levels of zinc, or zinc supplementation, and the resulting predictions from H3 remain highly accurate. We believe much more can be done with the multivariable form of the hyperbolastic models, and we hope to use these models to investigate the impact of various factors on wound healing. In particular we are very interested to model wound healing as a function of different levels of one or more growth factors.

In summary, the hyperbolastic models prove to be highly effective in modelling the time course of the healing of wounds, and furthermore their generalization to a multivariable form presents researchers an additional tool to model the roles of additional variables.

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Wound Healing: Altered Expression and Therapeutic Potential" of Cianfarani *et al*. (2006). Use of this data allows us to demonstrate the hyperbolastic models also accurately model data from diabetic wounds, an important area within the field of wound healing.

We would also like to express our thanks to the referees for the paper whose many helpful comments have improved the form and presentation of the paper.

Abbreviations: ECM, extra-cellular matrix; MgOH, magnesium hydroxide; AMP, adenosine monophosphate; ATPase, enzymes catalyzing adenosine triphosphate; MMPs, matrix metalloproteinases, TIMPs tissue inhibitor of metalloproteinases; AGEs, advanced glycation endproducts; IGF-1, insulin-like growth factor 1; TGF(beta), transforming growth factor beta; KGF, kerationcyte growth factor; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; PlGF, placental growth factor; mRNA, messenger ribonucleic acid; NF(kappa)B, nuclear factor kappa B; IL-1(beta), interleukin-1 beta; TNF(alpha), tumor necrosis factor alpha

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