3 The Role of Maggots in Wound Healing

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3.1 Introduction

3.1.1 Chronic wounds

Chronic or intransigent wounds are wounds which, left to themselves, do not heal, nor do they respond well to clinical treatment. Venous ulcers, diabetic ulcers and pressure sores are the most common occurring chronic wounds and they all share an underlying physiological condition which promotes chronicity: a reduced blood flow to the wound site.

The wounds are localised areas of damaged tissue which are bordered by living cells. The wounds tend to range in size from about 5 - 40 cm^2. Importantly, the damaged tissue is typically infected by bacteria which makes the wound more difficult to treat. The bacteria in the chronic wound exist in biofilms rather than the planktonic state (planktonic bacteria being flushed by the wound exudate and more easily dealt with by the immune response), which makes them harder to remove. They prevent wound healing through the release of toxins and add to the health risks of the patient.

3.1.2 The use of maggots

Since the early 1990's there has been a resurgence in the use of maggot (or larval) therapy for chronic wounds. Up to 1000 (but typically a few hundred) sterile larvae of the green bottle fly *Lucilia sericata* are applied to to the wound for one to three days. The maggots promote wound healing in three ways:

Wound debridement

The larvae feed on the necrotic tissue of the wound. Proteolytic enzymes secreted by the maggots degrade dead material into a nutrient-rich liquid which is then ingested by the maggots. The action of maggots crawling about the wound and probing into dead tissue is also likely to aid debridement.

Antimicrobial activity

Larval secretions are known to actively kill bacteria. Furthermore they have shown to be active against bacterial biofilms by degrading the polysaccharide slime which makes up the film. This is important since biofilms are highly resistant to other treatments, protecting the contained bacteria from antibiotics and host immune responses.

Growth promoting activity

As well as removing the dead tissue and bacterial infection which was inhibiting wound repair, larval secretions directly stimulate the wound healing process. Probably the most significant effect is the proteolytic lysis of fibrin cuffs which are associated with the reduced blow flow at the site of the wound. Furthermore, released fibronectin fragments

and the larval proteases themselves directly induce the activity of human cells involved in the wound healing process.

3.2 Problem proposal and study group aims

The proposal put to the study group was to develop mathematical models to help interpret and quantify the interactions between the maggots, their secretions and the wound healing process. Ultimately the objective would be to help develop treatment protocols which only used (artificial) maggot secretions thus removing the difficulties and stigma associated with the use of live maggots.

3.3 Mathematical model

To construct a mathematical model of the effects of maggots on wound healing we need to make many simplifying assumptions. Firstly we assume that we can model all aspects of the problem as a continuum. This assumption is least appropriate for the maggots, but given the relatively high density of application (about 10 maggots per cm^2) it seems a reasonable first approximation.

We consider wound geometries similar to that sketched in Figure 1. The following variables are defined to describe the wound:

$h(x,t)$: healthy host cell density
$n(x,t)$: necrotic cell density
$s(x,t)$: sessile or biofilm bacterial density
$T(x,t)$: bacterial toxin concentration
$m(x,t)$: maggot density

where all the above quantities are cross-sectional and assumed to be averaged over depth.

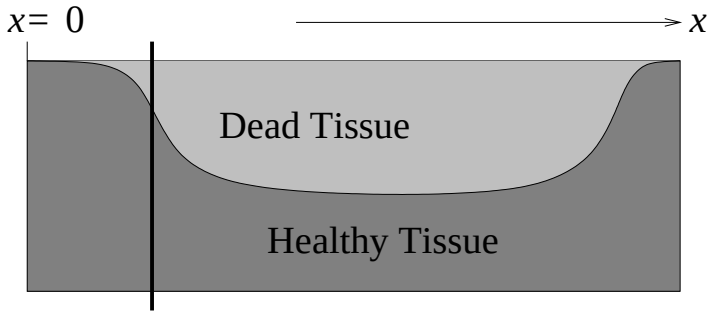

Figure 1: A schematic of the wound geometry. All quantities are assumed averaged in a vertical sense as indicated by the thick line.

We make the following assumptions about what the key processes in the wound are and how they act:

1. healthy cells and sessile bacteria grow logistically.

2. sessile bacteria disperses slowly in the wound (through shedding planktonic bacteria or growing into available space) but this is impeded by the presence of healthy tissue.

3. bacteria produce toxins which freely diffuse and kill healthy cells (yielding dead cells).

4. necrotic tissue, bacteria and toxins all decay due to natural breakdown and/or flushing by wound exudate.

5. bacteria are killed by the host immune response in the presence of healthy tissue.

6. maggots consume necrotic tissue to grow but they can also die.

7. maggots diffuse due to random motion but are also attracted to regions of high dead-cell density.

8. maggot secretions kill biofilm bacteria but also damage healthy tissue to some extent.

The corresponding system of partial differential equations can be written as:

$$\frac{\partial h}{\partial t} = k_h h\left(1-\frac{h}{h_0}\right) - k_{hn}Th - k_{hm}hm \tag{3.1}$$

$$\frac{\partial n}{\partial t} = k_{hn}Th - k_n n - k_g mn \tag{3.2}$$

$$\frac{\partial T}{\partial t} = D_T\frac{\partial^2 T}{\partial x^2} - \lambda_T T - k_{hn}Th + k_T s \tag{3.3}$$

$$\frac{\partial s}{\partial t} = k_s s\left(1-\frac{s}{s_0}\right) + D_s\frac{\partial}{\partial x}\left(\left(1-\frac{h}{h_0}\right)\frac{\partial s}{\partial x}\right) - k_f s - k_i hs - k_m ms \tag{3.4}$$

$$\frac{\partial m}{\partial t} = k_g mn - \mu m + D_m\frac{\partial^2 m}{\partial x^2} - \frac{\partial}{\partial x}\left(\chi mH(n-\bar{n})\frac{\partial n}{\partial x}\right) \tag{3.5}$$

We apply zero flux boundary conditions at both ends ($x = 0, L$) of the domain.

We non-dimensionalise the equations via the following scalings:

$$\widehat{h} = \frac{h}{h_0} \quad \widehat{s} = \frac{s}{s_0} \quad \widehat{t} = k_s t \quad \widehat{x} = \frac{x}{L} \quad \widehat{T} = \frac{\lambda_T + k_{hn}h_0}{k_T s_0}T \quad \widehat{n} = \frac{k_n(\lambda_T + k_{hn}h_0)}{k_{hn}h_0 k_T s_0}n \quad \widehat{m} = \frac{k_g}{k_s}m$$

so that (dropping the hats) the equations become

$$\frac{\partial h}{\partial t} = rh(1-h) - k_1 Th - k_2 hm \tag{3.6}$$

$$\frac{\partial n}{\partial t} = k_3(Th - n) - mn \tag{3.7}$$

$$\frac{\partial T}{\partial t} = D_1\frac{\partial^2 T}{\partial x^2} - k_4 T - k_5 Th + k_6 s \tag{3.8}$$

$$\frac{\partial s}{\partial t} = s(1-s) + D_2\frac{\partial}{\partial x}\left((1-h)\frac{\partial s}{\partial x}\right) - k_7 s - k_8 hs - k_9 ms \tag{3.9}$$

$$\frac{\partial m}{\partial t} = k_{10}mn - \bar{\mu}m + D_3\frac{\partial^2 m}{\partial x^2} - \frac{\partial}{\partial x}\left(\bar{\chi}mH(n-\bar{n})\frac{\partial n}{\partial x}\right) \tag{3.10}$$

where,

$$r = \frac{k_h}{k_s}, \quad k_1 = \frac{k_{hn}k_T s_0}{k_s(\lambda_T + k_{hn}h_0)}, \quad k_2 = \frac{k_{hm}}{k_g}, \quad k_3 = \frac{k_n}{k_s}, \quad k_4 = \frac{\lambda_T}{k_s}, \quad k_5 = \frac{k_h n h_0}{k_s}$$

$$k_6 = \frac{\lambda_T + k_{hn} h_0}{k_s}, \quad k_7 = \frac{k_f}{k_s} k_8 = \frac{k_i h_0}{k_s}, \quad k_9 = \frac{k_m}{k_g}, \quad k_{10} = \frac{k_g k_{hn} k_T h_0 s_0}{k_s k_n (\lambda_T + k_h n h_0)}$$

$$\bar{\mu} = \frac{\mu}{k_s}, \quad \bar{\chi} = \frac{k_h n k_T h_0 s_0}{k_s k_n (\lambda_T + k_{hn} h_0) L^2}, \quad D_1 = \frac{D_T}{k_s L^2}, \quad D_2 = \frac{D_s}{k_s L^2}, \quad D_3 = \frac{D_m}{k_s L^2}.$$

3.4 Steady-state analysis: maggot free case

Before discussing the role of maggots in wound healing, we first examine the maggot free case, in particular to determine parameter regimes for which a wound could heal, become chronic or to continually worsen due to bacteria presence. To do this we assume spatially uniform distributions of T and s and examine the existence and stability of steady-state solutions of the following system

$$\begin{aligned} \frac{d\,h}{d\,t} &= r\,h\,(1\,-\,h)\,-\,k_1\,T\,h, \\ \frac{d\,T}{d\,t} &= -T\,(k_4\,+\,k_5\,h)\,+\,k_6\,s, \\ \frac{d\,s}{d\,t} &= s\,(1\,-\,s\,-\,k_7\,s\,-\,k_8\,hs)\,. \end{aligned}$$

We denote the following classifications for the large time solutions

$$\begin{aligned} \text{Healed wound:} \qquad & (h, T, s) \;\to\; (1, 0, 0), \\ \text{Continually worsening wound:} \qquad & (h, T, s) \;\to\; (0, T^*, s^*), \\ \text{Chronic wound:} \qquad & (h, T, s) \;\to\; (h^*, T^*, s^*), \end{aligned}$$

as $t \to \infty$, for some constants $h^*, T^*, s^* > 0$.

3.4.1 Steady-states

The steady-state solutions for (h, T, s) are as follows

$$\text{i)}\;\; (0,\,0,\,0), \quad \text{ii)}\;\; (1,\,0,\,0), \quad \text{iii)}\;\; (0,\,k_6/(k_4(1+k_7),\,1/(1+k_7))\,, \quad \text{iv)}\;\; (h^*,\,T(h^*),\,s(h^*))\,,$$

where cases ii), iii) and iv) represent healed, continually worsening and chronic wound states, respectively. For case iv) we have

$$T(h^*) \;=\; \frac{r}{k_1}(1\,-\,h^*), \quad s(h^*) \;=\; \frac{1}{1\,+\,k_7\,+\,k_8\,h^*},$$

where $h^* \in (0, 1)$ (for non-negativity of $T(h^*)$), which satisfies $F(h^*) = 0$, $F(h)$ being the the cubic

$$F(h) \;=\; h^3 \;+\; c_2\,h^2 \;+\; c_1\,h \;+\; c_0,$$

where

$$c_2 \;=\; \frac{1+k_7}{k_8}\left(1-\frac{k_4}{k_5}\right), \quad c_1 \;=\; -\left(1-\frac{k_4}{k_5}\right)\frac{1+k_7}{k_8} \;-\; \frac{k_4}{k_5}, \quad c_0 \;=\; -\frac{k_4(1+k_7)}{k_8 k_5 r}\,\Psi,$$

and

$$\Psi \;=\; r \;-\; \frac{k_1\, k_6}{k_4\,(1+k_7)}.$$

It can be shown after some tedious analysis based on Routh-Hurwitz conditions that for $\Psi > 0$ (so that $F(0) < 0$ and $F(1) > 0$)) there is one and only one real solution for h^* in the required range of $0 < h^* < 1$. For $\Psi < 0$ there can be zero or two solutions for $h^* \in (0,1)$ provided that $c_1 < 0$, else if $c_1 \geq 0$ there are no physical chronic wound steady-state solutions.

3.4.2 Linear stability analysis

Examination of the linear stability for each of the steady-state cases i), ii) and iii) is straightforward and we can deduce that

i) saddle point,
ii) $1 - k_7 - k_8 < 0 \Rightarrow$ stable node,
iii) $\Psi < 0 \Rightarrow$ stable node.

Linear stability analysis for case iv), the chronic case, is much more difficult. However, we anticipate that if $\Psi > 0$, for which $h^* \in (0,1)$ exists, then the chronic state solution is stable. For the case $\Psi < 0$ and $c_1 < 0$ the stability of the two (possible) chronic -state solutions is uncertain; however by virtue of case iii) being stable for this case, at least one of the chronic states is expected to be unstable.

3.4.3 Conclusions

The analysis shows that for $\Psi < 0$ for which host tissue recovery rate is too slow in comparison to toxin production rate, the prognosis is bad indicating a bacterial "victory". For $\Psi > 0$ there is the possibility of co-existence of host cells and bacteria, which is the essence of a chronic wound. However, if $1 - k_7 - k_8 < 0$ then, depending on the initial conditions, a healed wound may result. In the context of this model, we need both $1 - k_7 - k_8 < 0$ and $\Psi > 0$ for maggot therapy to be effective, whereby an application of maggots (for finite time) may shift the balance of h, s and T from a chronic state into a basin of attraction for the healed steady-state. If $1 - k_7 - k_8 > 0$ then the bacterial growth capacity is too strong for the immune system to effectively deal with the infection, and healing is not possible without sustained maggot therapy.

3.5 Numerical Results

To obtain the numerical results presented here we require values for all of the parameters in our model. Some of these we can make educated guesses at, the rest we have chosen such that the results clearly demonstrate the ability of the maggots in our model to shift the wound from a worsening to a healing state.

For reference, we take $k_s \approx k_h \approx 0.1$ hr^{-1}, $s_0 \approx 10^{10}$ cells/ml and $h_0 \approx 10^8$ cells/ml. The particular non-dimensional parameter values used in the simulations are:

$r = 1$	$k_1 = 2$	$k_2 = 0.01$	$k_3 = 1$	$k_4 = 0.2$	$k_5 = 0.3$	$k_6 = 1$	$k_7 = 0.2$
$k_8 = 2$	$k_9 = 1$	$k_{10} = 1$	$\bar{\mu} = 0.01$	$\bar{\chi} = 0.1$	$D_1 = 0.01$	$D_2 = 0.001$	$D_3 = 0.001$

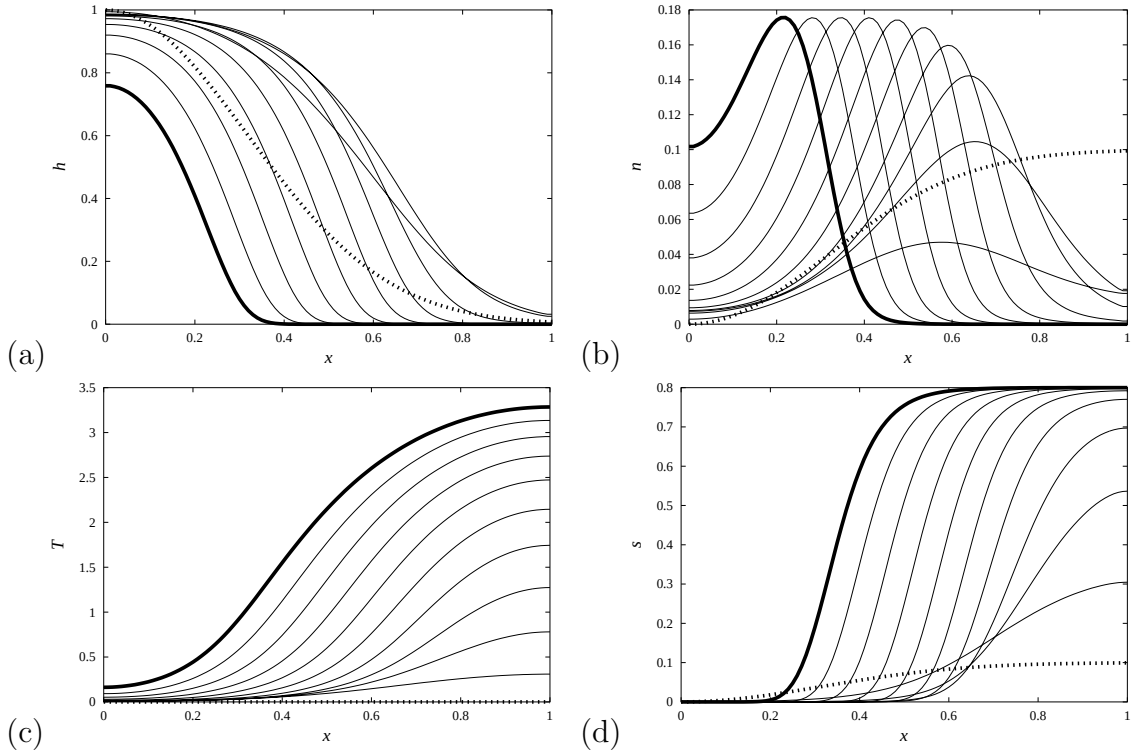

Figure 2: The evolution of a wound without maggot therapy from initial state (dotted line) to final state (thick line) with intermediate curves evenly spaced in time. (a) Healthy tissue density (b) Necrotic tissue density (c) Toxin concentration (d) Biofilm bacteria density

The two figure panels (Figures 2 and 3) below show the evolution of the wound without and with maggots respectively. The initial conditions are shown by the dotted lines and the subsequent curves are equally spaced in time with the final (non-dimensional time $t = 10$) curve being extra thick to help clarify the progression.

In Figure 2(a) we see the evolution of healthy tissue without maggots. The wound briefly starts to heal but at the same time the bacterial load, shown in Figure 2(d), is able to increase unchecked and the build up of toxins (Figure 2(c)) destroys the healthy tissue, making the wound larger. The retreating area of healthy tissue is preceded by a band of necrotic tissue (Figure 2(b)).

Figure 3(a) shows the progression of healthy tissue density for exactly the same wound as in Figure panel 2 but now with maggots added for the whole time (Figure 3(d)). The initial maggot distribution is uniform across the whole wound but as time progresses they tend to clump around high necrotic tissue density. In this case the wound completely heals and the bacteria (Figure 3(c)) and dead tissue (Figure 3(b)) are completely removed.

Figures 4(a) and 4(b) show the evolution of the healthy cell density for a short and longer maggot application respectively. The same maggot density is applied evenly across the wound at $t = 0$ and then removed at $t = 2$ (Figure 4(a)) and $t = 3$ (Figure 4(b)). In the first case the maggots slow, but do not stop, the infection and the wound gets worse. In the second case the maggots have been applied long enough, and cleared enough

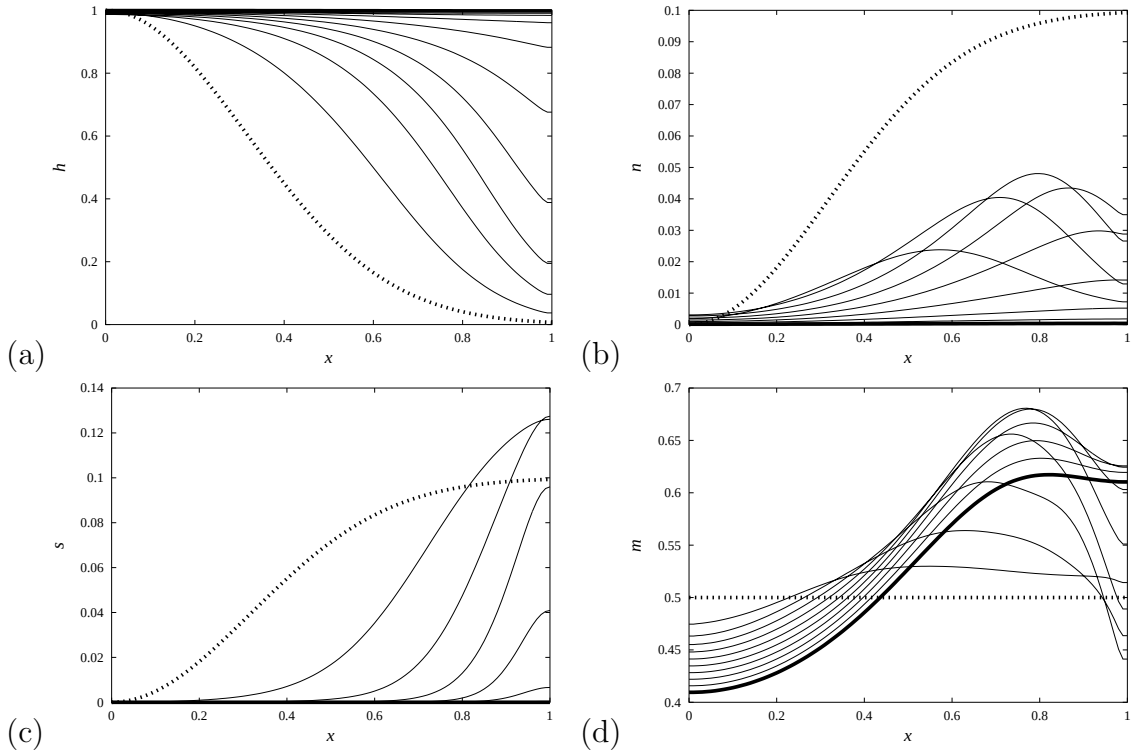

Figure 3: The evolution of a wound with maggots from initial state (dotted line) to final state (thick line). (a) Healthy tissue density (b) Necrotic tissue density (c) Biofilm bacteria density (d) Maggot density

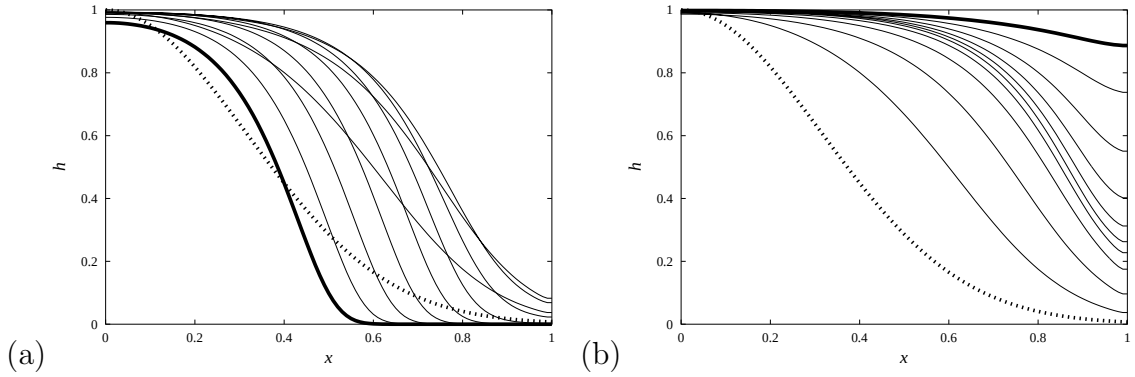

Figure 4: The evolution of healthy tissue density for two different maggot application durations. The curves are evenly spaced in time from initial condition (dotted lines) to final state at $t = 10$ (thick lines) (a) Maggots removed at $t = 2$ (b) Maggots removed at $t = 3$. In (b) the maggots have shifted the balance so that the wound is able to continue healing on its own after the maggots have been removed.

necrotic tissue and bacteria, to allow the wound to continue to heal on its own.

3.6 An alternate depth-based model

Here we introduce an alternate model conceived on the last day of the study group. Instead of modelling the horizontal extent of the wound and considering a "depth average" as in the previous model, here we explicitly model the depth structure of the wound while assuming the wound to be infinite in horizontal extent. This model will therefore be most applicable to the centre of a wound, away from the edges, but the model could also be extended into further dimensions. A sketch of the model wound is provided by Figure 5.

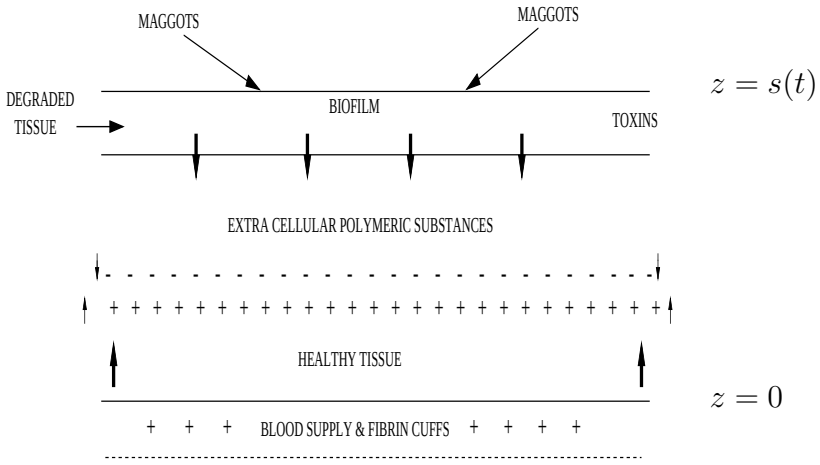

Figure 5: A sketch of the chronic wound

3.6.1 The model equations

Let the concentration of healthy cells be represented by $h(z,t)$, that of the biofilm by $b(z,t)$, the dead material ("gunk") by $g(z,t)$, the enzymes produced by the maggots by $m(z,t)$ and the internal velocity by $v(z,t)$. The equations governing these quantities are then

$$\frac{\partial h}{\partial t} + \frac{\partial}{\partial z}(vh) = k_1 h - k_2 hT + k_9 hm \tag{3.11}$$

$$\frac{\partial g}{\partial t} + \frac{\partial}{\partial z}(vg) = k_2 hT + k_3 b - k_4 g - k_8 gm \tag{3.12}$$

$$\frac{\partial b}{\partial t} + \frac{\partial}{\partial z}(vb) = k_5 b - k_3 b - k_7 bm \tag{3.13}$$

so that the rate of change of healthy cells in addition to the rate the cells spread out due to convection is equal to the birth rate by division, the effect of maggot enzymes and the death rate due to cells being killed by toxins. Similarly the dead material is produced by the action of toxins upon healthy tissue and from the biofilm while it is consumed by the maggot enzymes and also decays naturally. The growth of the biofilm depends upon the birth rate due to cell division, the natural death rate, and the added death rate due to the killing action of maggot enzymes. In this formulation we have implicitly assumed that maggot enzymes stimulate the growth of healthy cells, while in the previous model we had assumed that the enzymes broke down healthy cells. There is evidence for both of these effects and we can compare the two by changing the sign of k_9.

In our sketch of the wound (Figure 5) we have explicitly depicted the presence of fibrin cuffs on the blood supply, but we have not explicitly modelled the ability of maggot enzymes to dissolve fibrin (and hence restore the blood supply) here. This is a topic for future research.

The wound is a continuum of healthy tissue, biofilm and dead material so the sum of these must always be equal to one.

$$h + b + g = 1 \tag{3.14}$$

so it follows from (3.11)-(3.13) that the velocity field satisfies

$$\frac{\partial v}{\partial z} = k_1 h - k_4 g + k_5 b + k_9 hm - k_8 gm - k_7 bm \tag{3.15}$$

The toxins $T(z,t)$ produced by the biofilm diffuse into the wound so that

$$\frac{\partial T}{\partial t} + \frac{\partial vT}{\partial z} = D_T \frac{\partial^2 T}{\partial z^2} + k_6 b \tag{3.16}$$

where D_T is the diffusion coefficient. The enzymes produced by the maggots $m(z,t)$ also diffuse through the wound

$$\frac{\partial m}{\partial t} + \frac{\partial}{\partial z}(vm) = D_m \frac{\partial^2 m}{\partial z^2} - k_7 bm - k_8 gm - k_9 hm \tag{3.17}$$

The initial conditions are such that the wound consists of healthy tissue with a layer of biofilm at the surface. There are no toxins or dead material initially i.e. at $t = 0$

$$\begin{aligned} &h = 0, \quad b = 1, \quad T = g = 0 \quad 0.6 < z < s(0) \\ &h = 1, \quad b = 0, \quad T = g = 0 \quad 0 < z < 0.6 \end{aligned} \tag{3.18}$$

The concentration of maggot enzymes is $m = 1$ on the surface $z = s(0)$ and $m = 0$ elsewhere.

The boundary conditions are given as follows

$$\begin{aligned} \text{at } z = 0 \qquad & v = 0, \quad T = 0, \quad \frac{\partial m}{\partial z} = 0, \\ \text{at } z = s(t) \qquad & v = \frac{ds}{dt}, \quad \frac{\partial T}{\partial z} = 0, \quad m = 1. \end{aligned}$$

3.6.2 Numerical solution

We present a numerical solution to the model equations given by (3.11) - (3.17). The following values for the parameters k_1 - k_9 are chosen:

$$k_1 = k_3 = k_6 = 1, \quad k_2 = k_4 = 5, \quad k_5 = 1.5, \quad k_7 = 2, \quad k_8 = k_9 = 0, \quad D_m = D_T = 0.5.$$

A finite difference method is used with the domain being mapped onto a unit interval.

No maggots

We first consider the case before the maggots have been introduced to the wound. The initial conditions for h, g, b and T are shown in Figure 6.

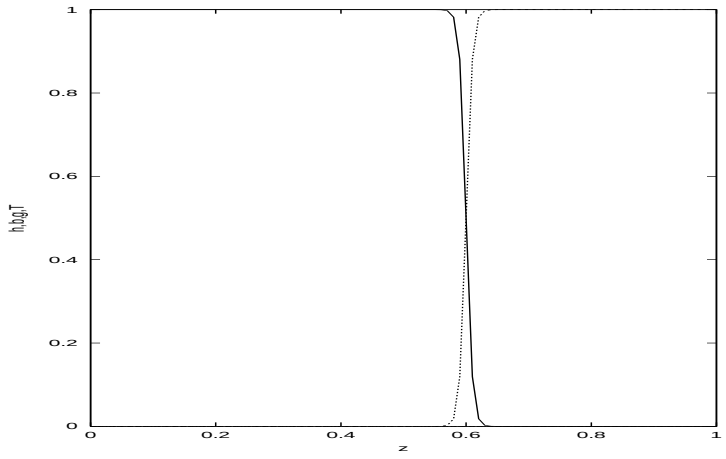

Figure 6: The initial conditions for h (solid line), b (dotted line), $g = T = 0$.

The wound initially consists of healthy tissue with a layer of biofilm above it. As we can observe from the following figure (Figure 7), as time progresses, the biofilm advances into the wound pushing back the healthy tissue. There is a build up of toxins at the surface of the wound and there is a small amount of dead material.

Maggots

At this time ($t = 10$) we introduce the maggots to the surface of the wound so that initially $m = 1$ on the surface $z = 1$.

After time $t = 10$ we observe that the enzymes produced by the maggots have diffused into the wound, destroying the biofilm, dead material and toxins allowing the healthy

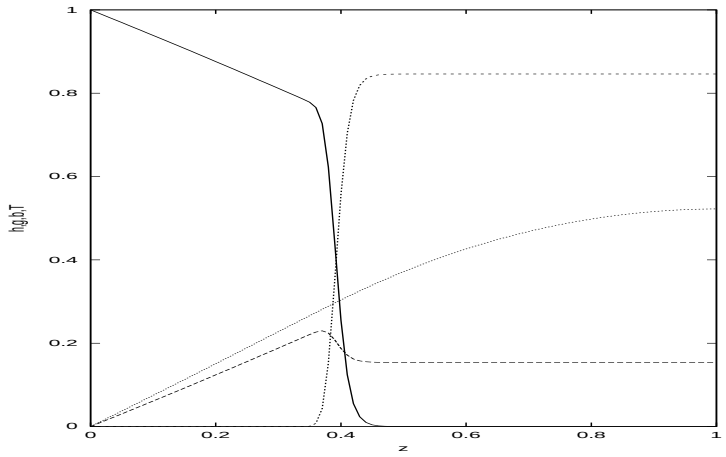

Figure 7: Concentration profiles for h (solid line), g (large dashed line) b (small dashed line), T (dotted line) at time $t = 10$.

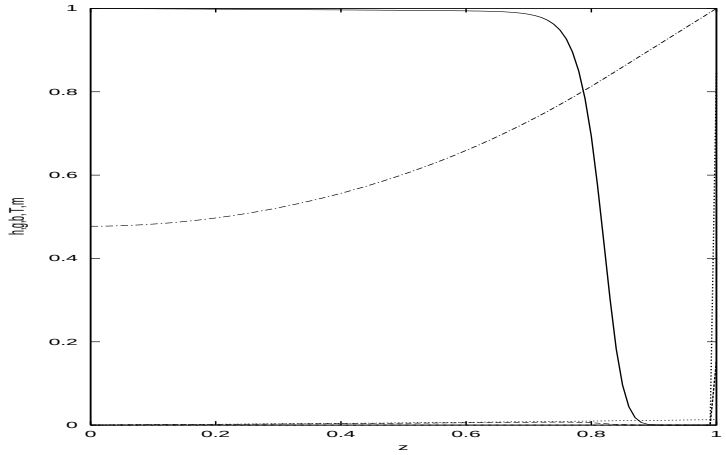

Figure 8: Concentration profiles for h (solid line), g (large dashed line) b (small dashed line), T (large dotted line), m (small dotted line) at time $t = 10$ after the maggots have been added.

tissue to grow and for the wound to be almost repaired (Figure 8). This repair continues with time until the wound is fully healed.

3.7 Conclusions

During discussion in study group between the "biologists" (pharmacologists / microbiologists / clinicians) and the mathematicians we have developed two models to describe the effect of maggot therapy upon chronic wounds. We have focused our modelling on the presence of bacteria in the wound; their inhibitory effect on wound healing and the importance of the bactericidal effect of maggots. Both of the models presented clearly demonstrate that an application of maggots can shift a wound from a chronic state to a healing state.

The results presented here are qualitative rather than quantitative and more exper-

imental data is required to estimate parameters and check the simulations. However, before, or at the same time as, these parameters are sought, the mathematical models require further refinement to include more detail of the biology. For example we could explicitly include the degradative effect of maggot secretions upon fibrin and the resulting increased blood flow to the wound site, as well as the stimulatory effect of fibrin fragments on the body's tissue repair mechanisms. Collaborative work will continue in the future, and we hope that mathematical simulations of the problem will eventually be able to help construct and test clinical chronic wound treatment protocols which utilize maggot secretions.

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