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THE DYNAMICS OF THE INFLAMMATORY PHASE OF SKIN WOUND HEALING IN RATS

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Abstract

The skin wound healing is a complex process divided into three overlapping and interdependent phases (inflammatory, proliferative and remodelling). The inflammatory response must occur rapidly to avoid chronic inflammation and it depends on biochemical, molecular and cellular events. The effective crosstalk between leukocytes and cytokines (proinflammatory and anti-inflammatory) lead to correct healing of the lesions. We considered a system of ordinary differential equation to model the skin wound healing process under *Copaifera langsdorffii* oleoresin treatment. In order to verify the treatment efficiency we compared the results of the oleoresin against Lanette cream (the base of oleoresin). Thus, we analysed the roles among the main leukocytes (neutrophils and macrophages), present in the inflammatory phase, and the proinflamatory cytokine (interleukin 6). The model can exhibit two stable steady states corresponding to healthy or unhealthy skin. The model solution reproduced the dynamics of the neutrophils and macrophages during inflamamatory phase, however there was a delay between numeric and biological results and it fitted better to Lanette cream than to oleoresin, therefore suggesting the necessity to improve the model. One possible strategy to enhance this model is to consider the interaction with the anti-inflammatory cytokine in the wound healing process.

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Introduction

Considering all the stages of wound closure, inflammation has a great importance in the final result. Whether harmful agents persist in inflammation the healing process does not reach the next stage (ploriferative) and it may result in chronic wounds. We present a simple model that represents the inflammatory phase of a wound through the interaction between cells which act in this process, macrophages and neutrophils, considering the pivotal role they play in the inflammatory process [2]. The modelling was made on two biological treatment: Lanette cream (LC) and *Copaifera langsdorffii* oleoresin (OR 10%), both of them applied on the dorsal skin of the rats [3].

Mathematical Model

We consider two populations of neutrophils, the population of macrophages, a proinflammatory cytokine - interleukin 6 (IL-6), and their interactions. The variables and the parameters description, with their units of measurement, are given in Table 1. According to [2] this process can be modelled by the system of ordinary differential equation (ODE):

$$\begin{cases} \dfrac{dn}{dt} &= c - \nu\, n, \\ \dfrac{da}{dt} &= \nu\, n - \gamma_a\, a - \phi\, m\, a, \\ \dfrac{dm}{dt} &= c - \gamma_m\, m, \\ \dfrac{dc}{dt} &= \alpha\, f(t) + \gamma_a \left(\dfrac{a^2}{\beta_a^2 + a^2} \right) - c, \end{cases}$$

where the function $f(t) = H\left(A\,\pi - t\right)\,\sin^2\left(t\right)$ represents the effects of physical injury to skin and H is the Heaviside function. In the instant that occurs the injury, $t = 0$, there are not cells and IL-6 inside the wound then the initial conditions are $n(t) = a(t) = m(t) = c(t) = 0$.

Table 1: Variables and the parameters used in the mathematical model [2].

Variable	**Description**	**Unit**
t	Time	day
n	Density of active neutrophils	cell mm^{-3}
a	Density of apoptotic neutrophils	cell mm^{-3}
m	Density of active macrophages	cell mm^{-3}
c	Concentration of pro-inflammatory mediators (Cytokine IL-6)	pg mm^{-3}
Parameter	**Definition**	**Unit**
χ_n	Maximal rate of neutrophils influx	cell pg^{-1} day^{-1}
χ_m	Maximal rate of macrophage influx	cell pg^{-1} day^{-1}
ν	Rate of neutrophils apoptosis	day^{-1}
ϕ	Rate at which macrophages engulf neutrophils	cell^{-1} mm^{-3} day^{-1}
γ_a	Rate of secondary necrosis	day^{-1}
γ_m	Rate that macrophages leave the tissue	day^{-1}
γ_c	Rate of decay of mediator c	day^{-1}
α	Rate of production of mediator c from initial damage	pg mm^{-3} day^{-1}
k_a	Concentration of (c) produced in response to damage	pg mm^{-3}
β_a	Saturation constant	cell mm^{-3}

Inflammation can have one of two distinct outcomes: healthy or unhealthy (chronic inflammmation) [2]. Whether the neutrophils and macrophages leave the wound tissue inflammatory mediators decrease and the subsequent healing phases are reached thus the skin becomes healthy, otherwise chronic inflammation will occur. This outcomes are represented by two correspondent stable steady states and they are found assuming that the initial stimulus of damage has been removed ($\alpha = 0$).

The model has up to three steady states, if $\frac{1}{4} > \left(\frac{\beta_a \gamma_c}{k_a \chi_n}\right)^2 \left(1 + \frac{k_a \phi \chi_m}{\gamma_m}\right)$: the trivial steady state $P_h = (0, 0, 0, 0)$ or non-trivial steady state $P_{uh} = (\bar{n}, \bar{a}, \bar{m}, \bar{c})$ given by

$$\bar{a} = \frac{\frac{k_a \chi_n}{\gamma_c}\left(1 \pm \sqrt{1 - 4\left(\frac{\beta_a \gamma_c}{k_a \chi_n}\right)^2 \left(1 + \frac{k_a \phi \chi_m}{\gamma_m}\right)}\right)}{2\left(1 + \frac{k_a \phi \chi_m}{\gamma_m}\right)},$$

$$\bar{c} = \frac{k_a\, \gamma_a}{\gamma_c}\left(\frac{\bar{a}^2}{\beta_a^2 + \bar{a}^2}\right), \qquad \bar{n} = \frac{\chi_n}{\nu}\,\bar{c} \qquad \text{and} \qquad \bar{m} = \frac{\chi_m}{\gamma_m}\,\bar{c}.$$

Results and Conclusions

The model was resolved numerically using *ode23s*, a Stiff ODE solver within Matlab, concatenated with *lsqnonlin*, a nonlinear least square tool of Matlab, to determine the best parameter set. The initial conditions were estipulated in the following way: α and k_a were estimated from biological datas, the others parameters were based on [2] and we set $\chi_n = 6\,\chi_m$ based on [4, 5]. The wound was considered healed when the total cell density has reached less than 40% of maximun total cell density, that is, the total density must to come to the basal levels of the skin. The results are presented in the Table 2 and illustrated in the Figures 1-4.

Table 2: Parameters values used in the mathematical model.

Parameter	Lanette Healed	Lanette Unhealed	OR 10% Healed	OR 10% Unhealed
χ_n	1.3407	2.1551	0.0861	0.8671
χ_m	0.2386	0.2616	0.6399	0.3002
ν	1.3939	1.9738	5.1461	9.1446
ϕ	0,0234	0.0187	3.0221	0.0086
γ_a	2.2842	2.1585	4.9084	1.7332
γ_m	0.0399	0.4765	0.3961	0.3693
γ_c	0.4699	0.8975	0.5764	0.4414
α	1756.38	1756.38	3659.91	332.55
k_a	8091.18	8091.18	24399.33	4582.96
β_a	329.99	599.99	3.0455	432.01

The model solution reproduced the dynamics of the neutrophils and macrophages during inflammatory phase. The model fitted better the datas of healed LC case, ilustrate in the Figure 1, than to healed OR 10% case, ilustrated in the Figure 3. The numeric results of LC showed the necessity of 23 days to heal the wound, this is in agreement with it was observed in laboratory, nevertheless this time was not determined biologically. The numerical solution of OR 10% presented an 'advance' compared to biological results, the healed time found by numeric result was 9 days whereas the biological experiment was 14 days, suggesting the necessity to improve the model. One possible strategy to enhance this model is to consider the interaction with the anti-inflammatory cytokine in the wound healing process.

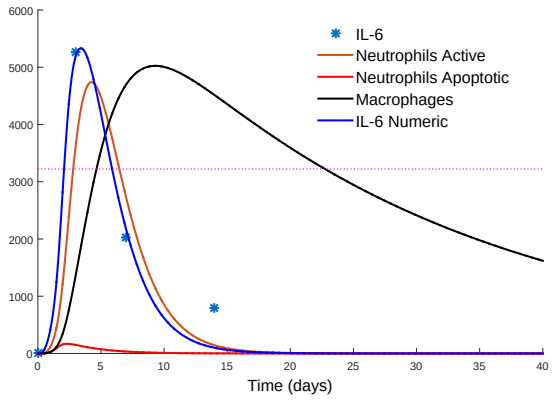

Figure 1: Healed skin: Lanette cream.

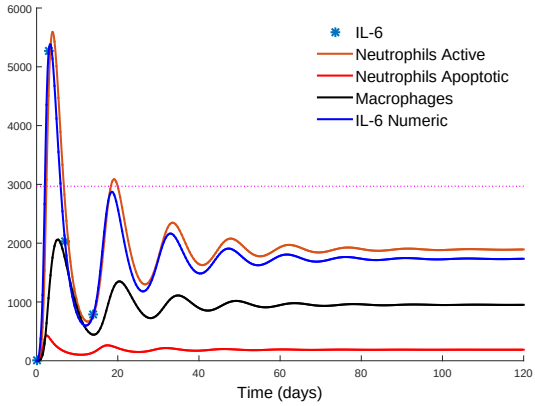

Figure 2: Unhealed skin: Lanette cream.

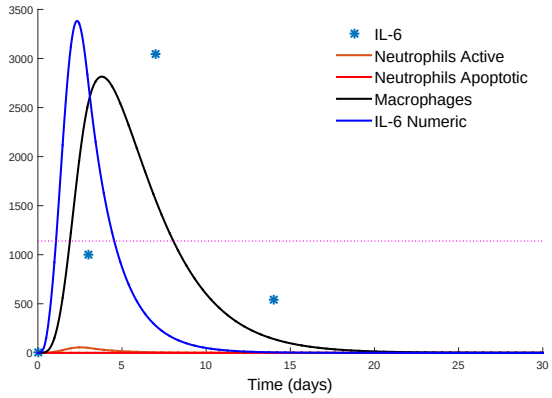

Figure 3: Healed skin: Oleoresin 10%.

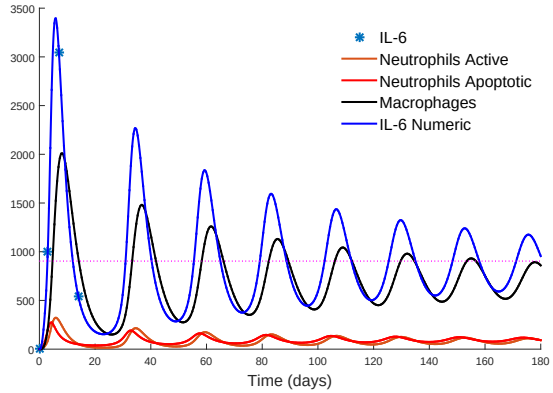

Figure 4: Unhealed skin: Oleoresin 10%.

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