A Multi-scale Feedback Control System Model for Wound Healing Electrical Activity: Therapeutic Device/Protocol Implications

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Abstract— **Regulation, growth and healing in biological systems involve many interconnected and interdependent processes that include chemical and electrical mechanisms of action. Unfortunately, the significant contributions that electrical events provide are often overlooked; resulting in a poor transfer of knowledge from science, to engineering and finally to therapy. Wound site electrical processes can influence cell migration, fluid transport, cellular signaling events, gene expression, cell differentiation and cell proliferation; affecting both form and function at the cell, tissue and organ levels. Wound healing, and its interrelationships with transport, regeneration, and growth, cannot be understood or therapeutically assisted unless both chemical and electrical activities associated with the healing process are addressed.**

I. Introduction

The confusion and lack of understanding with certain facets of wound healing can be traced to a chemically dominated view of the wound healing process [1] at the molecular, cellular and tissue biology levels. Both plant and animal tissue display a variety of electrically regulated processes at wound sites. Physical injury to plant tissue produces electrical activity similar to that which occurs in wounded animal epithelial tissues; resulting in measurable 10 to 50 mV electric potential responses [2]. Human and animal wound healing could be better understood and wound healing methodology could be much more effective if the structurally dependent electrical activities associated with wound site tissues and cells are considered [1], [3], [4] along with chemical and mechanical mechanisms of action.

Using measured wound healing electrical responses, the electrical component of the wound healing system response, associated with chemical and mechanical wound healing mechanisms, will be developed and related to electrotherapeutic device and protocol design issues.

II. Wound Site Electrical Activity: Scientific Background and Basic Mechanisms of Action

For both naturally occurring endogenous wound site electrical activity, and exogenously applied therapeutic electric current; the links between immune response, tissue reconstruction and wound healing in general involve a wide spectrum of activity. For example, some papers discuss the bactericidal effects of direct current [5]. Others are concerned with wound site mechanisms-of-action and relationships involving the skin epidermal battery, cell lysis, pH gradients, and the Nernst equation [4], [6]–[9]; where the center of the wound site has a positive electrical potential compared to the uninjured tissue at the wound site periphery [7]–[9]. This positive potential at the center of the wound produces an electric field that attracts a variety of mobile cells to the wound site including those of the immune system [8]–[10]. In this case, cells with a negative surface charge density will be attracted to regions that are positively charged [8], [10], [11]. From the standpoint of cell mediated immune responses, when electrical characteristics of a wound site are considered, Coulomb's law complements chemotaxis. The more positively charged wound site will modulate immune response and inflammation by attracting white blood cells.

The electrically positive wound site attracts fibroblasts that produce and deliver tropocollagen to support extracellular matrix reconstruction. Keratinocytes are attracted to the electrically negative region of the wound periphery for wound reepithelialization [12]. The electric field between the positive wound center and the more electrically negative periphery influences electro-osmotic and electrophoretic activity at the molecular, cellular and tissue levels [10], [11].

As shown in Fig. 2, wound site electrical responses involve processes associated with molecular, cell and tissue biology in a multi-scale negative feedback system, with interconnected modular elements that regulate form and function. Negative feedback serves as a regulator or control in wound healing to maintain stability. Knowing form and functional characteristics associated with endogenous healing process can have positive influences on therapeutic device design [13].

III. Wound Healing Modeling and Device/Protocol Design

A. Mathematical Expressions Derived From Endogenous Wound Healing Data of Current Density vs. Time

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Plots of time varying endogenous wound healing current densities and electric fields can be used to synthesize block diagram representations of the wound healing process.

To address the wound healing model, current density over time (J(t)) obtained from [3], [14], and [15], can be coupled with the following fourth order differential equation (1). Coefficients are chosen so that J(t) will closely match the measured endogenous wound current density (dotted line in Fig. 1). When accumulated cell/tissue density from the wound healing feedback path (Fig. 2) subtracted from the input variable N(t) is zero; tissue cell density is in equilibrium. Variations in N(t) represent a wound.

$$a_4 \frac{d^4J(t)}{dt^4} + a_3 \frac{d^3J(t)}{dt^3} + a_2 \frac{d^2J(t)}{dt^2} + a_1 \frac{dJ(t)}{dt} + a_0 J(t) = b_3 \frac{d^3N(t)}{dt^3} + b_2 \frac{d^2N(t)}{dt^2} + b_1 \frac{dN(t)}{dt} \quad (1)$$

Equation (1) can be converted into a Laplace transform format, where the output J(t) and the input N(t) are represented by J(s) and N(s) in Laplace notation, which then defines the wound healing system structure (block diagram of Fig. 2). The resulting wound healing system transfer function is of the form:

$$\frac{J(s)}{N(s)} = \left(\frac{b_3}{a_4}\right) \frac{s\left(s^2 + \frac{b_2}{b_3}s + \frac{b_1}{b_3}\right)}{(s+c)(s+d)(s^2 + 2\omega_N \zeta s + \omega_N{}^2)} \quad (2)$$

where; $\mathbf{d \approx a_2 / 2\, a_4}$, $\mathbf{c < 1}$, $\omega_N{}^2 \approx b_1 / b_3$, $\zeta \approx b_2 / b_3$).

B. Wound Healing Simulation Results

For a unit step input, Fig. 1 shows the graph (solid line) defined by (1), (2) and (3) that approximates the measured wound healing current density. Root locus plots associated with (2) indicate that the wound healing system is operating with very large gain margins for stability. However, in the

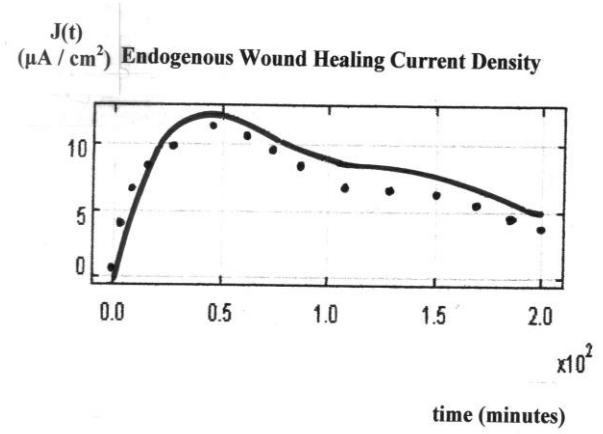

Figure 1. The dotted line in the graph represents a plot of current density vs. time obtained using a customized, non-invasive, tapered vibrating microelectrode probe to measure current flowing out of a murine corneal wound site [3], [14]. Even before wounding, there is a very small amount of current flow from the tissue. The simulation based on (1), (2) and (3) (solid line in the graph) follows closely the measured data for the wound healing current density. By varying simulation element gains, the simulation can duplicate wound healing current density plots for both murine corneal tissue and human skin tissue.

wound healing system feedback network, the cell signaling pathway consists of various interconnected first order equations based on chemical kinetics that can exhibit certain long term instabilities due to the effects of cross talk between pathways and nonlinearities [16].

Assuming N(s) is the Laplace input variable for N(t), representing a unit step function, and taking the inverse Laplace transform of (2):

$$J(t) \approx \frac{e^{-\zeta\omega_N \sqrt{1-\zeta^2}\, t}}{\omega_N{}^2 \sqrt{1-\zeta^2}} - \frac{e^{-c\,t}}{\omega_N{}^2} + \left(\frac{2b_2}{b_3}\right) \frac{e^{-\zeta\omega_N t}}{\omega_N{}^2 \sqrt{1-\zeta^2}} Sin\left(\omega_N{}^2 \sqrt{1-\zeta^2}\, t\right) - (2\,c) \frac{e^{-\zeta\omega_N t}}{\omega_N{}^2} Cos\left(\omega_N{}^2 \sqrt{1-\zeta^2}\, t\right) \quad (3)$$

For time and frequency scaling factors of approximately 100, very low values of ζ and c, and values of d that are approximately equal to the natural frequency multiplied by the damping ratio; (3) provides outputs that closely match the measured wound healing current density shown in Fig. 1. The block diagram of Fig. 2, synthesized from Fig. 1, (1) and (2), appears to provide a reasonably accurate representation of the wound healing system responses.

C. In Vitro Implications vs. In Vivo Reality: Impact of Frequency Response

Research at the in vitro level has shown that DC and time varying electric fields and electric currents can influence gene expression [17], cell differentiation and proliferation [18], [19], production/release of nucleotides [20], DNA synthesis [21], protein synthesis [20], [21] and protein transport [20]. The elements of the forward gain portion of the wound healing control system can respond to time varying high frequency inputs. However, the characteristics of the feedback elements limit the feedback path frequency response for exogenously applied electrical currents at frequencies beyond 0.5 Hz when the cells are part of a complete tissue system. In other words, for therapeutic applications involving exogenously applied currents at frequencies above 0.5 Hz; *in vitro* responses for cells in culture may not accurately reflect natural or relevant wound healing responses at different frequencies for those same cells when they are part of an organized tissue system.

D. The Ligand Concept

The interaction between electric fields or electric current density and cell receptors is often referred to as "ligandless" or "ligand-independent" receptor activation [22]. That view appears to need an adjustment. A ligand "binds." It may be a single molecule or ion. An accumulation of bound ions can be included in the ligand definition. An electric ligand model (involving ions bonded to receptors and molecules in the receptor neighborhood) can be structured based on data associated with wound site electric field and electric current density measurements. The influence of ion

accumulation, represented by an electric current integrator activating a cell signaling pathway, can then be described and evaluated.

The wound healing system block diagram derived from Fig. 1 and (1) and (2), indicates that the electrical equivalent of a ligand (or electric ligand) can be represented by a

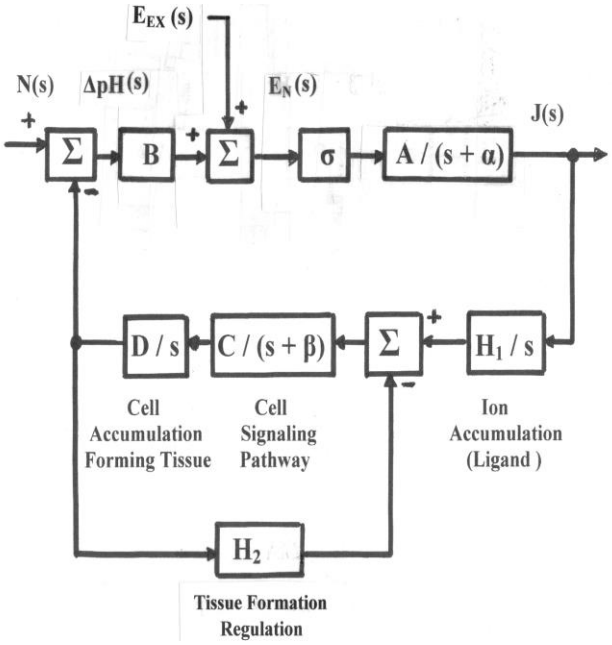

Figure 2. Using a Laplace transform format for the wound healing system block diagram, the tissue injury input involves N(s). The system response associated with wound healing current density is (J(s)). With no exogenous input, the wound current density (J(s)) is the product of wound tissue conductivity (σ) and the wound electric field intensity (E_N (s)), where E_N (s) is a function of ΔpH (s). The Laplace transform block diagram provides a convenient way to arrange system elements for simulations. In the Laplace format, a 1 / (s + α) block is a partial integrator producing a delayed output response. For therapeutic applications, the exogenous (therapeutic) electric field (E_{EX}(s)) derived from the output of a therapeutic device is shown. The electric ligand for this model consists of an accumulation and distribution of charge on cell receptors and the cell membrane due to the integration of the wound healing current density by the H_1 / s block.

mathematical integration of wound healing current density over time and area, and conceptualized by visualizing charged entities distributed over cell receptors and the cell receptor neighborhood (or surrounding membrane area).

From the standpoint of multi-scale form and function, as long as wound current continues to flow; the primary feedback loop integrator (with a gain of H_1) will continue contributing to cell proliferation and tissue increase until the cell/tissue accumulation output in the primary feedback loop reaches a completely healed value set by N(t). At that point, the desired level of N(t) and the primary feedback loop accumulated cellular output are equal. They, then, cancel each other out, which drives the wound healing current to zero (or to a very low off-set level). The secondary feedback loop integrator provides a regulating influence in case the cell signaling pathway behavior becomes aberrant. Reducing the gain term (H_2) deregulates the system, causing the entire wound healing system to become unstable. In this case, the wound does not heal and eventually becomes malignant.

From their chemical ligand model, DeLisi and Wiegel [23] provide a chemical ligand flux expression that, with a few minor adjustments, is compatible with an electric ligand concept. Assuming that the ligand flux (or current) impinging on one 5 nm receptor is independent of any other receptor, they provide an expression for total ligand flux into a certain proportion of the exposed receptors (N_C). In the expression, D represents the ion diffusion constant (electrically enhanced in this case); which when multiplied by the unit of charge (e) and receptor radius (s), can represent total current density response for an electric ligand:

$$J_+ \approx 4\, e\, D\, s\, N_C\, L \leq 4\ \mu A/cm^2 \qquad (4)$$

The wound current density calculated above is within the range of injury current densities that have been measured and reported [3], [6], [7], [14], [24]. The value obtained for J_+ assumes that, at any instant of time, and assuming some dissociation, the fractional cell receptor occupancy is less than 50% and the probability that the free ionic components of the electric ligand concentration will be intercepted by a receptor is less than or equal to 20% [23].

E. Mobile Ion Content in Wound Healing Current Density

Using ion sensitive electrodes, some papers describe wound healing currents in certain tissues as groups of mobile ions, such as Na^+, K^+ and Cl^-, with lesser contributions from H^+ [25]. However, at the cellular level, hydrogen ions are major contributors to current flow in epithelial cells, small neurons, and neutrophils. Some of these currents are "surprisingly large." [26], [27]. At the tissue level; major contributions to wound current and electro-osmosis from H^+ ions are described in wound healing models by Nordenström for tumors [10] and Callejón, Roa and Reina for epidermal cells [9]. Differences in ion transport mechanisms at various concentrations indicate that hydrogen ion contributions to wound site current flow are significant; but may be masked by extensive use of buffering agents.

F. Thermodynamic Constraints: Noise

Schwan and Foster [28] indicate that a cellular current density of approximately 1 mA / cm^2, coupled with membrane voltages of – 50 to – 70 mV are necessary to maintain the basic metabolic rate. Thermally induced fluctuations across the cell membrane are estimated to be approximately 1 μV. If a crude approximation is made for a one-to-one relationship between voltage and current density; as membrane voltage is scaled down, the thermal noise current density level associated with the 1 μV membrane noise voltage would be close to 20 nA / cm^2. Normal and malignant cell proliferation measurements indicate reproducible results down to current levels of 1 nA and current densities of 100 nA / cm^2 [29]. This sets a lower limit from the standpoints of both measurement capability and potential therapeutic benefit.

Burst noise levels reported for the ligand – receptor region of Fig. 2 appear to be "unusually large" [30] with statistical data indicating signal-to-noise ratios of 4.5 to 20 dB. Also, standard deviations for gene expression can be

approximately one half of the mean values [30], [31]. With the wound healing system modeled as a discrete level signal detection system, signal-to-noise levels for the cell signaling pathway and gene expression would be quite low (~ 6 dB), and the resulting gene expression error rate could be close to 1 error in 100. This error rate is not acceptable for transcription or translation, which require approximately 1 error in 10,000 or better. Various formatting, spatial and temporal signal processing techniques including encoding, interconnecting, integrating, and noise reduction feedback techniques appear to be operating at the molecular biology – cell signaling pathway level to compensate for the low signal-to-noise ratios [32].

IV. Discussion

The literature shows that there are significant inconsistencies with respect to the exogenous applications of electrotherapy in wound healing. The wound healing model simulation and published wound healing research results [7], [15], [10], [33] suggest that electrotherapeutic wound healing inconsistencies could be significantly reduced if the high mA current levels often administered to patients with chronic wounds are reduced to levels closer to naturally occurring currents and current densities. For better and more consistent results; response of severe wounds to electrotherapeutic intervention indicate that exogenously applied wound healing currents should be decreased from an initial short-term mA level, to μA [33], [34] and/or nA levels [35] over the long term; with total treatment times substantially increased. Considering the location of the exogenous electrotherapeutic input in Fig. 2, it makes sense that electrotherapeutic intervention for wound healing should incorporate the structure, intensities and time frame associated with naturally occurring wound healing current density and electric field waveforms [35], [34]. Section III. F indicates that from a signal-to-noise standpoint, electric currents specified for device design down to the 10 nA level are applicable for observable wound healing effects.

Many wound healing protocols call for applied voltage and current frequencies in the 1 to 100 Hz range and beyond. Electric fields and electric currents with frequencies in this range can influence cell proliferation, angiogenesis, cyclic AMP production, kinase activity, Ca^{++} influx into cellular cytoplasm, DNA replication and transcription. But Section III C indicates that the integrating function in the wound healing system primary feedback loop may filter out most of the therapeutic effects of frequencies above 0.5 Hz for cells accumulating and forming organized tissues. However, direct currents and naturally occurring low level wound healing current densities can also stimulate gene expression, enhance the production or release of ATP, enhance protein synthesis and have major effects on calcium regulation, the transport and incorporation of amino acids, and cell proliferation[16], [17], [19], [20], [21]. There is a noticeable dose-time effect with exogenously applied electrotherapeutic techniques. And what seems to work best for severe wounds often involves low levels of currents, with very low frequencies, applied each day over long periods of time [35].

The area of wound healing system noise response could benefit from more follow-on work. The block in Fig. 2 that appears to be the most susceptible to noise is the cell signaling pathway. However, preliminary results with noise simulations indicate that the wound healing system architecture itself provides significant protection from pulsed and burst noise levels that are up to ten times higher than the signal levels. Issues concerning the accuracy of that level of architecture induced noise protection need to be addressed further. It will be interesting to evaluate the parameters that affect the wound healing model's coefficients (such as decay rates, transport parameters, statistical distributions, etc.), incorporate the effect of those parameters on the model's coefficients, and determine their impact on the system's signal quality as noise levels increase.

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