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The interaction between plasma filaments in dielectric barrier discharges and liquid covered wounds: electric fields delivered to model platelets and cells

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Abstract

The treatment of wounds by atmospheric pressure plasmas in the context of plasma medicine typically proceeds through a liquid layer covering exposed cells. The wounds and their liquid covering often have irregular shapes with electrical properties (i.e. conductivity and permittivities) that may differ not only from wound-to-wound but also for a single wound as healing proceeds. The differing shapes and electrical properties extend into the liquid within the wound that typically contains cellular materials such as blood platelets. The plasma, wound, liquid and intra-liquid cellular components represent an interacting system of mutual dependence. In this paper, we discuss the results from a computational investigation of the treatment of small, liquid-covered wounds by filamentary dielectric barrier discharges. The sizes of the wounds are of the order of the plasma filaments and the liquid within the wound, an approximation of blood serum, contains idealized blood platelets. We find that the electrical properties of a wound can have significant effects on the spreading of the plasma on its surface by virtue of the deformation of the vacuum electric fields due to the shape, the effective capacitance of the wound and the discontinuities in electrical permittivity. This in turn effects the penetration of the electric field to cells under the liquid. The orientation and permittivity of the platelets relative to the liquid determines the electric fields that may stimulate the platelets.

Keywords: dielectric barrier discharge, plasma medicine, wound, electroporation, blood

(Some figures may appear in colour only in the online journal)

1. Introduction

Non-equilibrium, atmospheric pressure plasma (APP) treatment of living tissue is being used in a variety of processes collectively called plasma medicine [1, 2]. These processes may involve the direct contact of non-equilibrium plasmas with biological cells resulting in sterilization or the production of therapeutic effects. The therapeutic and sterilizing effects of plasmas, and those produced by dielectric barrier discharges (DBDs) in particular, may be attributed to several processes the fluxes of radicals and charged species, the production of energetic fluxes of ions and photons, the activation of overlying liquids and the generation of surface and intracellular electric fields [1–7]. One favoured type of plasma applicator is the floating electrode DBD where the tissue being treated acts as the unpowered electrode [8,9].

One application of DBDs in plasma medicine is wound healing in which the plasma is in physical contact with the wound. In most such treatments, the wound is covered by or filled with a biological liquid, for example some variation

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of blood plasma (BP). BP consists of about 92% water with the remainder consisting of large organic molecules, such as proteins and clotting factors and cell fragments, often referred to as platelets [10]. Human blood platelets are anucleated cells, typically discoid in shape with dimensions of 2–4 μ m by about 0.5 μ m. Platelets are multifunctional and are critical to blood clotting through the release of platelet-derived growth factors (PDGF) which are known to play an important role in wound healing [11–22]. It is known that APPs can induce blood coagulation, which naturally occurs via a complex cascade of events that start with platelet activation and the triggering of clotting factors to form fibrin [8].

A related and likely important effect in the plasma treatment of wounds is the generation of intra-wound electric fields. Nanosecond pulsed electric field stimulation of a cell produces a variety of physiological responses, such as apoptosis, stimulation of calcium (Ca₂⁺) fluxes and changes in membrane potential. Platelets can be externally activated to release PDGF. For example, Zhang *et al* [11] applied 300 ns pulses of 30 kV cm⁻¹ to platelet-rich BP and observed platelet aggregation, an effect attributed to the electric field increasing Ca₂⁺ concentrations. High intensity electric pulses permit Ca₂⁺ entry into the platelet through the formation of nanopores [12, 19, 20]. Extremely high fields can result in irreversible platelet responses and even stimulate platelet apoptosis [21].

The interaction of plasmas, and DBDs in particular, with wounds is complicated by the wound not being a passive participant. The wound has electrical properties, permittivity and conductivity, which influence the distribution of electric fields. The wounds are also not simply flat homogeneous structures-they have different shapes and sizes which vary from patient to patient-which perturb or shape the vacuum electric fields. The properties (shape, conductivity, permittivity) of a single wound will evolve during its healing process. The perturbed vacuum electric fields then influence the formation and propagation of the DBD filaments. This in turn determines the flux of reactive species onto the surface of the wound and subsequent penetration of electric field into the wound. The activation of platelets to, for example, emit growth factors that may have important roles in wound healing may depend on these electric fields. The platelets are themselves not passive components as their shape and permittivity determine the degree of electric enhancement at their borders and penetration of the electric field to their interior.

In this paper, we discuss results from a numerical investigation of DBD treatment of liquid-covered wounds in humid air. The goal of this study is to provide insights to the manner in which the DBD filaments electrically interact with the wound, the BP covering the wound and platelets within the BP. These unique interactions dominantly occur during the few nanoseconds when the filament intersects and spreads on the wound during individual discharge pulses. In this investigation, we examined only single discharge pulses onto small wounds that have sizes commensurate with the plasma filament of the DBD. As such, we are not addressing the longer term and cumulative effects of the plasma-induced chemistry within the liquid. We found that the electrical properties of the wound can have significant effects on the spreading of DBD filaments on the surface of the wound by virtue of the deformation of the vacuum electric fields by the shape and by the effective capacitance of the wound. This in turn effects the penetration of the electric field into the liquid of the wound, and to the cells below the liquid. The shape and permittivity of the platelets then in turn determine the local electric fields to which the platelets respond.

The model used in this investigation is described in section 2. The dynamics of the DBDs over large, medium and small wounds are discussed in section 3. In section 4, we discuss the consequences of the wound-filling liquid on the electric fields that may stimulate platelets and cells below the liquid. Our concluding remarks are in section 5.

2. Description of the model and its geometry

The model used in the investigation, nonPDPSIM, is a twodimensional simulator in which Poisson's equation for the electric potential and transport equations for charged and neutral species are solved [23]. The electron temperature, $T_{\rm e}$, is obtained by solving an electron energy conservation equation with transport and rate coefficients coming from the local solutions of Boltzmann's equation. Radiation transport and photoionization are included by implementing a Green's function propagator [23]. In this study, the model was applied to the investigation of the propagation of plasma filaments in a DBD, their intersection with wounds filled with liquid, the generation of electric fields inside the wound-filling liquid and near platelets in the liquid. The liquid, platelets and tissue are modelled as non-plasma materials with specified dielectric and conductive properties. In most regions of the computational mesh, the tissue is treated with material-averaged electrical properties. In a sub-region of the tissue, individual cells are resolved with appropriate permittivities and conductivities to represent, for example, cytoplasm and cell membranes. Poisson's equation is solved throughout the computational domain, including the gas phase and the solid tissue. The gas phase reaction mechanism for 1 atm of humid air and other computational algorithms are the same as those described in [23]. The numerical grid uses an unstructured mesh with triangular elements and refinement regions to resolve the details of the plasma filaments, cell interior and nuclei. The mesh consists of approximately 11 200 nodes, of which about 5000 are in the plasma region to resolve plasma filaments and the majority of the remainder are expended in resolving the cellular structure described below.

To investigate the possible synergetic effects and the generation of electric fields in the wound, we use the geometries shown in figure 1. These geometries are intended to represent a conventional DBD with a powered electrode at the top covered by a dielectric material ($\varepsilon/\varepsilon_0 = 3$). The dielectric material which represents tissue is placed on a grounded electrode. The tissue in this case is represented as layers of epidermis, dermis and a subcutaneous layer that are not resolved at the cellular level except for a small patch in the dermis. This patch resolves a few layers of collagen and elastic



Figure 1. Parallel DBD geometry with a model of a wound through the epidermis and dermis. The upper (powered electrode) is covered by a dielectric. The bottom surface is grounded. The tissue is represented by the epidermis, dermis and subcutaneous layer with average conductivities and permittivities. (*a*), (*b*) and (*c*) show concave small (0.3 mm), medium (0.75 mm) and large (1.30 mm) wounds, respectively. Insets in the frames show flat wounds. (*d*) An enlargement of the medium wound with platelets inside the blood serum. (*e*) Cell structure typical for the dermis.

fibre cells under the wound. We resolve nuclei and the cell interior while membranes are represented as edges outlining the cells. The typical dimensions of filaments in atmospheric pressure DBDs are 0.4–0.6 mm. We expect that the manner of interaction of the filaments with wounds will depend on the relative sizes of the plasma filament and the wound. We therefore investigated the interaction of filaments with small

Table 1. Permittivities and conductivities used in the model and dielectric relaxation times [23, 24].

	Cell membrane	Cytoplasm	Nuclear envelope
$\frac{\varepsilon/\varepsilon_0}{\sigma \ (\Omega^{-1} \ \mathrm{cm}^{-1})} \\ \tau \ (\mathrm{s})$	$\begin{array}{l} 5.8 \\ 8.7 \times 10^{-8} \\ 5.9 \times 10^{-6} \end{array}$	$\begin{array}{c} 30 \\ 4.8 \times 10^{-3} \\ 5.5 \times 10^{-10} \end{array}$	$\begin{array}{c} 20\\ 3.0\times 10^{-5}\\ 5.9\times 10^{-8} \end{array}$

(0.3 mm), medium (0.75 mm) and large (1.30 mm) wounds (shown in figures 1(a)-(c)). This range of sizes spans from the wound being commensurate with the size of the plasma filament (small wound) to the wound being larger than the plasma filament (large wound). Concave and flat wounds are considered.

The nature of the interaction of electric fields with tissue is determined by the dielectric properties of the biological material. The values available in the literature for the dielectric constant of BP and components of cells vary over large ranges [24–27]. In this paper, we have adopted the parameters for cell structures recommended by Ermolina *et al* [25] and Feldman *et al* [26], and by Cook [24] for a BP. Based on these works, typical values for ε_r for the plasma membrane of mammalian cells are about 6, and σ is about 10^{-7} S cm⁻¹. For the cytoplasm, ε_r is between 30 and that of water, 80, and σ is typically one-fifth that of seawater, 0.005 S cm⁻¹. The dielectric constant of BP is $\varepsilon_r = 56$ –60, but can be as low as 7 depending on environmental and patient parameters, and the stage of wound healing. For demonstration purposes, we simply artificially alter ε_r of the wound fluid.

The wounds we investigated are filled with a liquid and platelets. An enlargement of the medium wound, platelets and the cellular structure are shown in figures 1(d) and (e). Since the electrical properties of the liquid in the wound can significantly vary during the wound-healing process, we chose two dielectric constants for the liquid, $\varepsilon_r = \varepsilon/\varepsilon_0 = 60$, intended to represent the early part of wound healing when the liquid in the wound is water-like, and $\varepsilon_r = 7$, intended to represent latter stages of wound healing when the liquid in the wound is dominated by proteins. The conductivity of the liquid is $10^{-4} \Omega^{-1} \text{ cm}^{-1}$. Platelets have a dielectric constant of $\varepsilon_r = 20$ and conductivity of $10^{-7} \Omega^{-1} \text{ cm}^{-1}$. Other electrical parameters for the cells are specified in table 1. The applied voltage is -30 kV with a rise time of 0.1 ns.

The permittivity of blood platelets is typically different from the permittivity of the BP in which they are immersed. As a result, there is a natural deformation of the electric field that occurs at its boundaries. For ideally spherical platelets, if $\varepsilon_1 > \varepsilon_2$ (platelet permittivity larger than the fluid), the electric field at the poles is increased and the electric field at the equator is decreased relative to the electric field in the bulk fluid [28]. The maximum electric field at the pole relative to the external electric field is $E/E_0 = 1+2(\varepsilon_1 - \varepsilon_2)/(\varepsilon_1 + 2\varepsilon_2)$. If $\varepsilon_1 < \varepsilon_2$, the electric field at the poles is decreased and at the equator increased relative to the bulk fluid. More complex electric field distributions are produced for nonspherical particles immersed in a liquid, however the same general scaling laws hold.

3. Filament dynamics over wounds

The electric fields and potentials prior to formation of the plasma are shown in figure 2 for the small, medium and large wounds for low ($\varepsilon_r = 7$) and high ($\varepsilon_r = 60$) permittivities of the wound-filling liquid. The cases of flat and concave surfaces of the wound are shown. In a stack of materials with different permittivities, electric field lines are excluded, on a relative basis, from the high permittivity material and into the low permittivity material. Since the liquid has the highest $\varepsilon_{\rm r}$, there is a reduced electric field in the wound and electric field enhancement near the surface. This occurs even for the wounds with flat surfaces since the tissue around the wound has a different ε_r . The wounds with concave surfaces have an additional electric field enhancement resulting from the non-perpendicular intersection of the electric field line and the surface, particularly at the edges of the wound (the conductivity of the liquid is $10^{-4} \,\mathrm{S \, cm^{-1}}$). The potential and electric field lines are perturbed above the wound a distance approximately equal to their diameters. The details of the distribution of the initial electric field depend on the size, curvature and permittivity of the fluid in the wound. The perturbed electric field in turn affects the streamer dynamics and its intersection with the wound.

Electron densities for plasma filaments approaching and intersecting the large wound are shown in figure 3 for the wound-filling liquid with $\varepsilon_r = 7$ and 60. Results are shown for flat and concave wounds. The avalanche of the streamer above the $\varepsilon_r = 60$ wound is marginally faster and produces a higher plasma density compared to the $\varepsilon_r = 7$ wound. Crossing the 1 mm gap requires only a few tenths of a nanosecond and spreading across the wounds takes at most 1 ns. In the middle of the gap, the electron density for the $\varepsilon_r = 7$ wound is 8.3×10^{14} cm⁻³, and for the $\varepsilon_r = 60$ wound is 9.5×10^{14} cm⁻³. There is more voltage in the gap above the high ε_r wound because of the expulsion of electric field from the wound and the larger capacitance produces a longer dwell time to charge the surface.

The spreading of plasma on the surfaces of DBDs is a wellknown phenomenon [29, 30] and this spreading occurs on the top dielectric, producing electron densities in the boundary layer of nearly 10^{16} cm⁻³. The spreading is in the form of a surface ionization wave (SIW) whose speed is determined by the rate of charging of the underlying capacitance of the dielectric. A similar spreading of the plasma occurs on the surface of the flat wound. The spreading of the plasma on surface of the flat wound is slower than on the top dielectric due to the larger capacitance of the wound which requires a longer dwell time to charge the surface compared to the top dielectric. For the same reason, the spreading of the plasma on the $\varepsilon_r = 7$ wound is faster than on the $\varepsilon_r = 60$ wound due to its lower capacitance. When the SIW reaches the boundary of the wound and spreads onto the skin, the speed of spreading increases as the underlying skin has a lower capacitance.

The intersection of the plasma filaments with the concave wounds follow similar trends as for the flat wounds. Since the effective gas-gap length is marginally larger due to the concavity of the wound, the electric field is smaller compared



Figure 2. Electric potential (lines) and electric field stream vectors for -30 kV applied voltage for low ($\varepsilon_r = 7$) and high ($\varepsilon_r = 60$) permittivity wound-filling liquids for the small, medium and large wounds. (*a*) Flat wound and (*b*) concave wound. Potential lines are drawn in every 1 kV in the air and every 0.5 kV inside the wound and tissue. For the high permittivity liquid, electric field lines are expelled from the wound. The effect is more pronounced for the concave wound.

to the flat wound and so the electron density is smaller and the streamer slightly delayed. The capacitance of the wound is larger (thinner and smaller radius of curvature) and so the rate of spreading is slower.



Figure 3. Electron density (2 dec, log scale) at different times for the large wound with low ($\varepsilon_r = 7$) and high ($\varepsilon_r = 60$) dielectric constant wound-filling fluid. (*a*) Flat wound and (*b*) concave wound. The maximum value is shown for each set of frames.

The dynamics of the SIW crossing the wound and the implications for electric fields within the wound are determined by the charging of the surface and electrical fields produced parallel to the surface. These are in turn sensitive functions of the topology of the wound and its electrical characteristics, such a permittivity and conductivity. For example, the spreading of the plasma on the flat wound and curved wound for $\varepsilon_r = 7$ proceeds somewhat continuously across the wound-skin boundary. The change in dielectric constant between the wound and skin is not so severe as to create significant discontinuities in the electric field (see figure 2). However, for the $\varepsilon_r = 60$ wound, the discontinuities are severe enough that the spreading of the plasma is significantly perturbed at the edge of the wound, as shown in figure 3. To further illustrate



Figure 4. Time evolution of plasma and electrical properties around and in the large, concave wound with a filling liquid with $\varepsilon_r = 60$. (*a*) Electron density, (*b*) electron impact ionization source, (*c*) electric potential and (*d*) electric field. The maximum value or range of values is shown for each frame or set of frames. Arrows show the direction of propagation of the local SIW.

these points, the electron density, electric potential, electron impact ionization source, S_e , and electric field are shown in figure 4 in the vicinity of the large concave wound with $\varepsilon_r = 60$. S_e indicates the location of the head of the SIW. Arrows show the direction of propagation of the SIW. The time sequence begins when the plasma filament intersects the wound.

With the diameter of the filament being smaller than the width of the wound, the incident filament is initially confined within the wound (0.24 ns). Upon intersection with the wound, a conductive channel extends from the top electrode to the surface of the liquid, translating the applied potential to the wound. This compresses the electric potential below the surface of the wound (0.33 ns). A momentary sheath is formed at the surface at the site of incidence having a momentary electric field $E > 400 \,\mathrm{kV \, cm^{-1}}$. The vacuum electric fields are already a bit warped with a net outwardly pointing electric field due to the curvature of the surface of the wound-filling liquid. The conductive filament which brings the cathode potential to the middle of the wound reinforces these lateral electric fields. The incident filament that negatively charges the central surface of the liquid also reinforces the lateral electric fields. These lateral electric fields, up to $200 \,\mathrm{kV \, cm^{-1}}$, produce the ionization source, S_e, that sustains the outwardly moving negative SIW (0.33 ns). Since the wound has the higher ε_r the electric field within the liquid is smaller, up to 80 kV cm⁻¹. This expulsion of electric field from the wound-filling liquid results in more of the applied potential being dropped in the tissue underneath the liquid, where $E \approx 280 \, \text{kV} \, \text{cm}^{-1}$ (0.47 ns).

The outwardly propagating SIWs extend the conductive plasma towards the edge of the wound, which compresses the potential contours and increases the electric field at the This occurs while photoionization seeds edge (0.47 ns).electrons above the skin on the other side of the region of high electric field at the edge of the wound. These seed electrons then initiate a positive SIW which propagates back towards the wound while the negative SIW from the interior of the wound is still propagating outwards (0.47 ns). The outwardly propagating negative SIW and the inwardly propagating positive SIW intersect at the edge of the wound (0.56–0.58 ns). The surviving SIW is the positive inward wave, which continues towards the centre of the wound, much like the restrike of a spark. (The spreading of plasma on the surface of the dielectric in a conventional DBD is usually in a single direction.) The plasma formed by the inward SIW is accelerated upwards at the edge of the wound due to the exclusion of potential from the high permittivity liquid that produces an upwardly pointing electric field. For these conditions, the plasma does not always spill over the edge of the wound, but may be newly produced beyond the edge with subsequent spreading over the epidermis.

The ionization source and electron density in the vicinity of the large flat wound with $\varepsilon_r = 60$ are shown in figure 5. The trends are similar to those for the concave wound. Electric field enhancement occurs at the edge of the wound due to the discontinuity in ε_r while the electric potential is compressed due to the spreading of the plasma from the centre of the wound. Both inward and outward SIWs co-exist. The ionization source and electron density in the vicinity of the concave wound with $\varepsilon_r = 7$ are also shown in figure 5. Here the spreading of the plasma is continuous in a single direction, though there is some slowing of the SIW near the edge of the wound. Although there certainly is a sensitivity of the dynamics of spreading of the plasma on the wound based on the concavity of the surface, it appears that the value of ε_r is the dominating factor in these dynamics.

The width of the plasma filament is about half the size of the medium wound considered here. So there is still some confinement of the filament within the medium wound. The electron density in the vicinity of the flat and concave medium sized wounds for $\varepsilon_r = 7$ and 60 are shown in figure 6. The trends

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Figure 5. Time evolution of the electron impact ionization source and electron density for the large wound. (*a*) Concave wound with the liquid with $\varepsilon_r = 7$. (*b*) Flat wound with the liquid with $\varepsilon_r = 60$. The maximum value or range of values is shown for each frame or set of frames. Arrows show the direction of propagation of the local SIW. The SIW for the low ε_r liquid propagates uniformly outwards, whereas the SIW has inward and outward components for the large ε_r liquid.

are similar to the larger wound, albeit on a shorter timescale. For $\varepsilon_r = 60$, SIWs inwardly propagate from the edge of the wound where electric field enhancement is largest. When these SIWs propagate towards the initial filament in the centre of the wound, the electric field is shorted out in the wound, while the plasma continues to spread outwardly. However, the surface coverage of the wound is less than for the large wound. There are regions near the edge of the wound for both the flat and concave wounds that are not covered by dense plasma. This lack of coverage is more severe in the concave wound due to the curvature of the wound that produces more electric



Figure 6. Time history of the electron density for flat (left frames) and concave (right frames) surfaces for the medium wound with (a) $\varepsilon_r = 7$ and (b) $\varepsilon_r = 60$. The maximum value is shown for each set of frames. Arrows show the direction of propagation of the local SIW.

field enhancement at the edge of the wound. Once plasma is formed, the electric field is then shorted out for the remainder of the wound that then prevents the outwardly moving SIW from fully covering the surface. As in the case of the large wound, the SIW continuously propagates outward for $\varepsilon_r = 7$, and the surface of the wound is uniformly covered by plasma.

The width of the plasma filament is comparable to the size of the small wound and so there is little confinement of the filament within the wound. The electron density in the vicinity of the flat and concave small wound for $\varepsilon_r = 7$ and 60 are shown in figure 7. Even though the wound is smaller, there are some indications that for $\varepsilon_r = 60$ the surface coverage by the plasma is still not uniform. Due to the closer proximity of the edge of the wound to photoionizing radiation, the inwardly propagating SIWs are launched earlier. The resulting production of plasma at the edge of the wound then shorts out the electric field before the initial filament is able to spread. On the other hand, for the wound filled with a lower ε_r liquid, the smaller size of the wound produces a smaller capacitance. The spreading of the plasma is barely perturbed by the wound.

The results just discussed are for a filament striking near the centre of the wound. There is a geometrical dependence on plasma coverage across the wound based on where the filament strikes the wound. For example, the electron density is shown in figure 8 for the large, concave wound with $\varepsilon_r = 60$ when the filament is offset from the centre of the wound by 260 and



(a)

(b)

Figure 7. Time history of the electron density for flat (left frames) and concave (right frames) surfaces for the small wound with (a) $\varepsilon_r = 7$ and (b) $\varepsilon_r = 60$. The maximum value is shown for each set of frames.

Surfaces

430 μ m. First, note the steering of the filament by the curvature of the vacuum electric fields towards the edge of the wound. As the filament is offset towards the edge of the wound there is a smaller tendency to produce the counter-propagating SIWs. This trend results from the highly conductive filament shorting out the electric field enhancement due to the discontinuity in ε_r that occurs at the edge of the wound. The spreading of the plasma on the outside of the wound, other than starting earlier, is not particularly affected by the shift in the location of the filament. However, the spreading of the filament over the wound is affected. When the filament strikes on the centre of the wound, the SIW propagates in both directions out of the wound and onto the skin. When the filament strikes the edge of the wound, the SIW stalls inside the wound and does not reach the skin on the other side. The capacitance of the wound is sufficiently large that the spreading plasma is unable to provide enough current to charge the capacitance and continue to propagate. For example, the capacitance of the large wound is $\approx 500 \,\text{pF}\,\text{cm}^2$ which requires $\approx 5 \times 10^{13} \,\text{cm}^{-2}$ net charge to fully charge the surface. The electron density near the tip of the SIW avalanche front is 1.5×10^{15} cm⁻³ and the thickness of the surface discharge is $50-100 \,\mu$ m. So there is insufficient charge in the SIW to both charge the capacitance and to continue to propagate the SIW across the wound.



Figure 8. Electron density for the large concave wound with $\varepsilon_r = 60$ when the filament is offset from the centre by (a) 260 μ m and (b) 430 μ m.

4. Electric fields within and below the wound

A potentially important effect in wound healing is the electrical stimulation of platelets within the liquid covering the wound and in cells under the liquid. To investigate the consequences of wound properties on these stimulated electric fields, we approximated blood platelets as otherwise identical elliptical particles oriented either horizontally or vertically. The platelets were distributed at the base of the wound along the perimeter of the cells as shown in figure 1(*e*). The major and minor radii of the platelets are 3.5 and 1.5 μ m, somewhat larger than actual platelets [13, 15] to enable us to resolve their internal structure with our numerical mesh. The platelets have $\varepsilon_r = 20$ and a conductivity of $10^{-7} \Omega^{-1} \text{ cm}^{-1}$.

The magnitudes of the electric field are shown in figure 9 in the vicinity of the platelets and the top layer of cells for liquids with $\varepsilon_r = 7$ (smaller than ε_r for the platelets) and $\varepsilon_r = 60$ (larger than ε_r for the platelets). For the $\varepsilon_r = 7$ liquid, there is a significant electric field inside the liquid. With the ε_r of the platelets larger than that of the fluid, the electric field is intensified at the vertices of vertically oriented platelets and the equators of the horizontally oriented platelets. At these sites, the electric fields can be momentarily as large as 380 kV cm^{-1} . The electric fields within the cells are smaller, up to 120 kV cm^{-1} , due to their higher permittivity.

For the $\varepsilon_r = 60$ liquid, there is a proportionately smaller electric field inside the liquid compared to the case where the liquid has a lower ε_r . Some of the excluded potential is







Figure 9. Electric fields inside the wound-filling liquid and the top cells under the liquid at different times as the surface charges. Platelets ($\varepsilon_r = 20$) are oriented either vertically or horizontally. Fields are shown for wound-filling liquids with (*a*) $\varepsilon_r = 7$ and (*b*) $\varepsilon_r = 60$. Electric field enhancement occurs around the platelets depending on the ε_r of the liquid.



Figure 10. Time dependence of the electric field inside a platelet above the tissue. (*a*) Location of the platelet, nucleus, cytoplasm and membrane (red dots) for which electric fields are shown. Electric fields are shown in the platelets for the liquids with (*b*) $\varepsilon_r = 7$ and (*c*) $\varepsilon_r = 60$. E_0 is the average electric field in the surrounding liquid.

redistributed into the cells below the liquid. With the ε_r of the platelets now being smaller than that of the liquid, the electric field is intensified at the equator of vertically oriented platelets and at the vertices of the horizontally oriented platelets. At these locations, the electric fields are $<100 \text{ kV cm}^{-1}$ while those in the cells are increased up to 160 kV cm^{-1} .

The electric fields inside a platelet about 30 μ m above the tissue for the $\varepsilon_r = 7$ and 60 liquids are shown in figure 10 as a function of time (the location of the platelet is shown in figure 10(*a*)). E_0 is the average electric field of the background. The initial linear rise in the electric field in the platelets is due to the rise time of the voltage pulse. The electric field then remains constant while the filament begins to avalanche. The rise in the electric field results from the compression of voltage ahead of the avalanche front and the charging of the surface of the wound. After the wound charges, the electric field in the liquid is nearly constant, with some variation due to the reconfiguration of charge on the top surface of the wound as the SIW spreads. The electric field will be maintained at this plateau value for tens to hundreds of nanoseconds until the wound discharges (we did not simulate for this length of time).

For the $\varepsilon_r = 7$ liquid, the ε_r of the platelet is larger than that of the liquid and so on a relative basis, electric field is

excluded from the interior of the platelet in favour of the surrounding liquid. The electric field inside the platelet reaches 200 kV cm^{-1} whereas in the surrounding liquid the average electric field exceeds 300 kV cm^{-1} . For the $\varepsilon_r = 60$ liquid, the ε_r of the platelet is smaller than that of the liquid and so on a relative basis, electric field is excluded from the liquid in favour of the interior of the platelet. The electric field inside the platelet reaches 90 kV cm^{-1} whereas in the surrounding liquid the average electric field is near 60 kV cm^{-1} . Even though the electric field inside the platelet exceeds that of the liquid, the electric field inside the liquid is smaller due to its larger ε_r , which then reduces the electric field inside the platelets.

Electric fields were similarly calculated for different wound geometries and sizes. Although there are systematic changes in the electric fields inside the platelets, the most sensitive parameter in determining the electric field inside the platelet was the ε_r of the liquid and whether the ε_r of the platelet was larger or smaller than that of the liquid. In experiments by Zhang *et al* [11] platelets were stimulated by nanosecond pulses which generated intense electric fields. When plateletrich BP was exposed to one 300 ns pulse with an electric field of 30 kV cm⁻¹, platelets aggregated and a platelet gel was produced. Platelet aggregation was observed with pulses as low as 7 kV cm^{-1} . The amount of Ca⁺₂ released by the platelets increased with increasing electric field up to 30 kV cm⁻¹ and increases with an increase in the number of voltage pulses. Our simulations show that much larger electric fields can be produced inside and around the platelets, and DBDs typically operate at repetition rates of many kHz. The cumulative effects on platelets due to exposure to successive pulsed electric fields is likely to be important in DBD treatment of wounds.

The time dependence of the electric field at the membrane, cytoplasm and nucleus of a top cell of the tissue is shown in figure 11 for the $\varepsilon_r = 7$ and $\varepsilon_r = 60$ liquids. These locations are shown in figure 10(a). Due to the high conductivity of the cytoplasm, the electric field in the interior of the cell is typically lower than for the medium-conductivity nucleus and lowconductivity membrane. The electric field for the $\varepsilon_r = 7$ liquid is about 95 kV cm^{-1} for the cytoplasm, 110 kV cm^{-1} for the nucleus and 140 kV cm⁻¹ for the cell membrane. For the $\varepsilon_r =$ 60 liquid, there is less potential dissipated across the liquid resulting in more electric field inside the tissue. The resulting electric fields in the tissue are $140 \,\mathrm{kV} \,\mathrm{cm}^{-1}$ for the cytoplasm, $160 \,\text{kV} \,\text{cm}^{-1}$ for the nucleus and $220 \,\text{kV} \,\text{cm}^{-1}$ for the cell membrane. The voltage drop across the membrane calculated for the actual thickness of a lipid membrane (5-10 nm) is about 0.15 V, a value exceeding the lower limit of cell electroporation for long pulses [31]. So the transient electric fields induced beneath the liquid of a small wound by DBD filaments may be sufficient to produce electroporation. The details of the process depend on the electric properties of the overlying liquid.

5. Concluding remarks

In this paper, we have discussed results from a computational investigation of the interaction of plasma filaments produced in an air DBD over small wounds filled with liquid. The relative permittivity and size of the wound are important parameters



Figure 11. Time dependence of the electric field inside a cell near the top of the tissue under the liquid for the cytoplasm, nucleus and membrane (locations are shown in figure 10(a)). Electric fields in the cell are shown for the liquids with (a) $\varepsilon_r = 7$ and (b) $\varepsilon_r = 60$. With the larger ε_r liquid, a greater electric field is delegated to the underlying tissue.

in determining the character of the interaction of the plasma filament with the surface and resulting surface ionization wave (SIW). First, simply the geometry of the interface between the liquid and air, and the ε_r of the liquid, determine the magnitude and orientation of the vacuum electric fields applied to the wound. Once the filament strikes the wound, the value of ε_r determines the character of the spreading of the plasma. This is particularly the case at the edge of the wound where there is a discontinuity in ε_r that produces electric field enhancement. For large values of ε_r , this discontinuity may produce both outwardly and inwardly propagating SIWs. The location on the wound where the filament strikes is important in the surface coverage of the SIW. Even though the SIW may propagate for many millimetres along the surface of the skin that has a lower ε_r , the SIW may stall when propagating across the higher ε_r wound. This stalling is due to the higher capacitance of the wound and the unfavourable orientation of both the vacuum and plasma perturbed electric fields. As such, there is significant variability in the propagation of the SIW across the wound depending on the location of incidence, the ε_r of the liquid and the orientation of the surface.

The ε_r of the liquid has a first-order effect on the electric fields induced in and around platelets in the wound-filling liquid and in the tissue below the liquid. If the ε_r of the liquid is larger than that of the platelet, the electric fields inside the platelet are larger than those in the surrounding liquid, and vice versa. For typical operating conditions, the induced electric field inside the platelets can be up to 100 kV cm^{-1} , which is larger than the experimentally observed threshold for platelet stimulation [11]. The ε_r of the liquid also has a first-order effect on the electric fields induced in the underlying tissue. A larger ε_r of the liquid produces larger relative electric fields in the underlying tissue, with values exceeding 100 kV cm⁻¹. These electric fields approach and exceed the limit for cell electroporation [31].

In actual DBD treatment of wounds, the area density of plasma filaments may be tens to hundreds per cm² and repetition rates may be many kHz to 10 kHz. The randomness of the formation of the filaments and the unavoidable small motion of the applicator will produce average exposures across the wound. The details of how the plasma filaments interact on a pulse-by-pulse basis and on a time average basis with the surface of the wound depend on these details, as well as the shape and electrical properties of the wound-filling liquid. The shape of the wound and the permittivity of the woundfilling liquids are not passive participants in these interactions. These properties likely have first-order effects on the electrical stimulation of cells and cell fragments in the liquid, and in the tissue beneath the liquid.

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