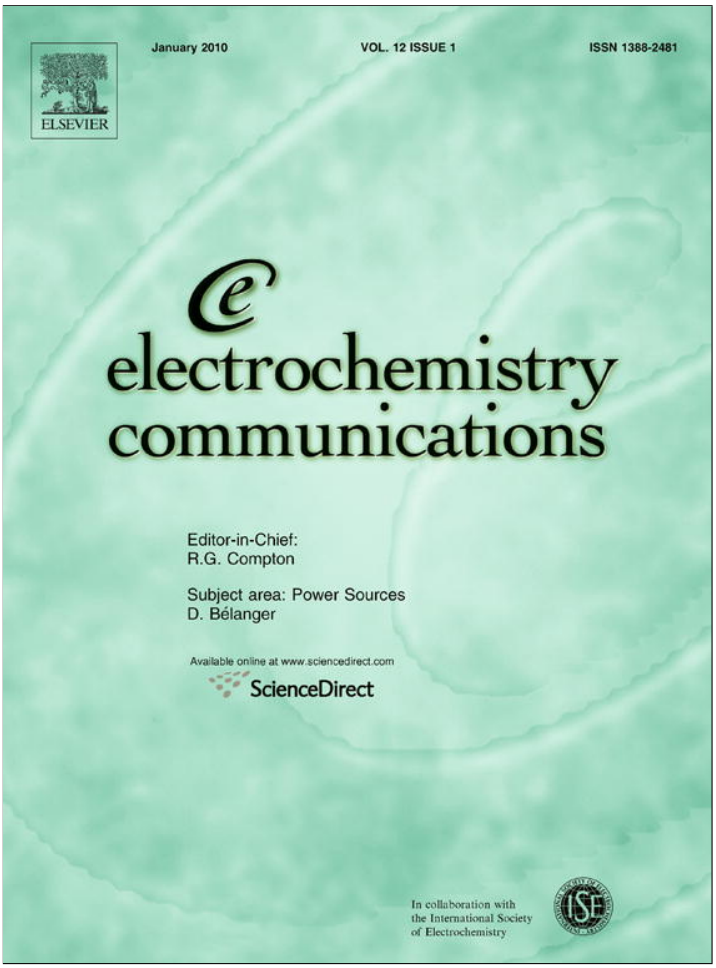

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Tracking protein electrodenaturation fronts in the electrochemical treatment of tumors

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ABSTRACT

Electrochemical reactions in the electrochemical treatment of tumors (EChT) induce extreme pH changes and, consequently, protein electrodenaturation fronts intimately related to tumor destruction. Here we introduce a new in vitro EChT collagen–macronutrient gel (CMG) model to study protein electrodenaturation fronts as a mean of assessing EChT effectiveness. Our CMG model shows that from an initial uniform condition two electrodenaturation fronts evolve expanding towards each other until collision. Moreover, electrodenaturation front tracking reveals that the front grows under a diffusion-controlled regime. Based on this evidence it is possible, in principle, to predict the time needed for tumor destruction without compromising healthy tissue. These results are consistent with those previously obtained with in vivo and in vitro EChT modeling. In contrast to previous simpler in vitro models, our CMG model represents a better structural and chemical approximation to a real tissue thus providing a better tool for validation of new in silico EChT models aimed at a more accurate prediction of tissue destruction level.

1. Introduction

In the electrochemical treatment of tumors (EChT) a direct electric current flows through cellular and interstitial tumor compartments. Tissue destruction, produced basically by strong pH gradients, has been reported in a wide range of solid tumors, with greater efficacy observed in skin, lung, liver and breast malignancies. Some of the advantages of EChT are its simplicity, effectiveness, low cost and negligible side effects. At present, there are several groups working in China (15,000 patients treated in the last 15 years), Australia, Cuba, Brasil, Japan, Sweden, Slovenia and USA [1–3]. In spite of the wide clinical experience available, the destructive mechanisms involved in EChT are still not well known thus the need to elucidate the fundamental mechanisms involved and to elaborate a reliable strategy for dosage optimization.

Any physicochemical factor changing outside physiological conditions may induce the loss of quaternary, tertiary or secondary proteic structures, with the change of spatial conformations and, in many cases, loss of biological function. This phenomenon is called denaturation; it also induces a change in light absorption characteristics of proteins and thus a color virage. Main denaturation agents are temperature, ionic strength and pH [4,5]. In general, tumors are more acidic than normal tissues, with median pH values of 7.0 and 7.5, respectively [6], making them tolerant to more acidic environments. This not witstanding, a sufficiently low pH (2–4), as well as high pH values, will lead to tumor cell death [7]. In EChT, strong acidic and basic fronts emerge from anode and cathode, respectively, reaching extreme pH values.

Mathematical or in silico EChT modeling validated with in vitro modeling appears to be a powerful tool in the search of the underlying destruction mechanisms behind EChT, as well as for the development of a dose planning strategy. Nilsson and coworkers [8] pioneered this approach in a series of papers in which they first modeled the tumor tissue as an ionic solution; further refinements of the model included buffering capacity, a non specific organic content, and considering the impact of chlorine evolution on the medium. They showed that the pH profiles obtained correlated with the necrotic area, thus suggesting that the model could be used for predicting the area of the tissue lesion induced by the EChT.

In a more recent work, Colombo et al. [9] validated their EChT in silico model with in vivo experiments and an in vitro model using a collagen gel with NaCl at physiological concentrations. They found that an initial condition with almost neutral pH evolves between electrodes into extreme cathodic alkaline and anodic acidic fronts moving towards each other, leaving the possible existence of a

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biological pH region between them; towards the periphery, the pH decays to its neutral values.

Avramov Ivić et al. [10] introduced an in vitro EChT model consisting in an agar–agar gel with ionic content and a basic pH indicator (phenolphtaleine). They claim their in vitro model to be a better approximation for in silico model validation than previous aqueous solution models.

Here we present a new in vitro gel model composed of a matrix of collagen into which sodium chloride (main salt present in tissues), $NaHCO_3$ (incorporating, together with CO_2, buffering capacity) and egg yolk (organic matter similar to tissue composition) were added. By adding fresh egg yolk which contains a protein mixture of different chemical properties (vitelin being the main one) to the collagen gel in the CMG model we are approximating to the lipid (basically, triglycerides and phospholipids), protein and carbohydrate natural composition of a tissue. Adding $NaHCO_3$ we get closer to a real buffered tissue. We call this in vitro model the collagen–macronutrient gel (CMG) model. As the previous agar–agar model [9], it is basically a porous and hydrated elastic gel structure but, while agar–agar is a carbohydrated polymer of vegetal origin, collagen is a proteic polymer of animal origin and a main component of the animal extracellular matrix.

The addition of physiological ionic strength, as well as pH buffering, turns the model into a better approximation to a real tissue and thus, yields better predictions of the pH changes induced during EChT. In our CMG model pH changes induce, among other effects, egg protein denaturation around the electrodes (here we have called it electrodenaturation because it is a consequence of the direct electric current applied) [11]. This phenomenon has already been described [12], can be easily followed by a color virage, and can be used for mimicking tumor tissue destruction by an EChT treatment. A somehow related method that allows rapid visualization of the entire pH distribution by using laser confocal scanning microscopy coupled to microelectrochemistry was recently presented in [13,14].

The main objective of EChT is tumor tissue destruction. Electrodenaturation fronts are an adequate measure of the extent of it. Here we propose the study of the CMG in vitro model and their protein electrodenaturation fronts as a mean of assessing EChT dosage.

2. Materials and methods

The collagen–macronutrient gel (CMG) model is based on a collagen I solution (30 g/L) with 5% v/v egg yolk raw fresh, 0.16 mol/dm^3 NaCl and 27 mmol/dm^3 $NaHCO_3$. The solution is placed in a 9 cm diameter Petri dish and is allowed to gelificate. Two platinum quasiconical electrodes were inserted to the bottom within the thickness of the gel (0.4 cm) and with a separation of 3.8 cm between each other. Each electrode has an exposed area of 0.060 cm^2. All experiments were conducted at room temperature; no significant changes were observed throughout the experiments. Video images were obtained with a digital camera Canon PowerShot SD1000, 7.5 megapixels. Image J was used for image capturing and processing [15]. A constant direct electric current was applied (power supply: Consort E835, Belgium). Electric current and voltage were continuously monitored by a standard multimeter (20 s sampling).

3. Results and discussion

Fig. 1 shows a sequence of snapshots of EChT applied to the CMG model. At the constant current of 10 mA used; the potential difference between both electrodes ranged between 5.1 V and 6.0 V. The images reveal the gradual appearance of circular halos or electrodenaturation fronts around both electrodes, causing the disruption of the initially homogeneous color of the media. This is an evidence of protein electrodenaturation that can be analyzed by front tracking. Fig. 1d shows that, upon collision of opposed pH halos, proton and anion fronts remain stand still, due to neutralization.

Fig. 2a presents the log–log plot of the tracking of the anodic (H^+) electrodenaturation front. Circles indicate the mean value of

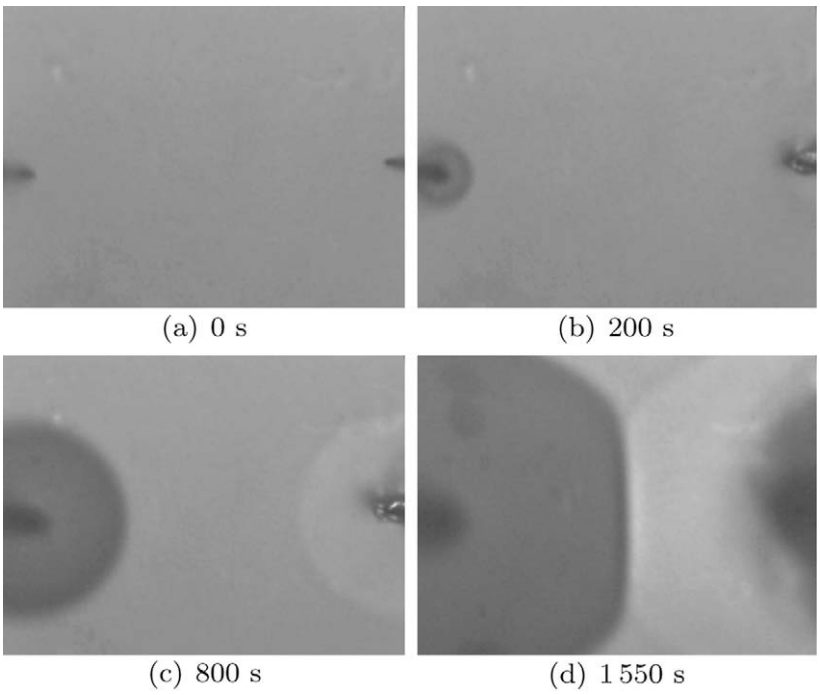

Fig. 1. Time (s) sequence of digital images during EChT applied to the CMG model with a constant current of 10 mA. Growth of electrodenaturation halos or fronts around both electrodes is clearly seen. Anode and cathode at the left and right sides of the gel, respectively.

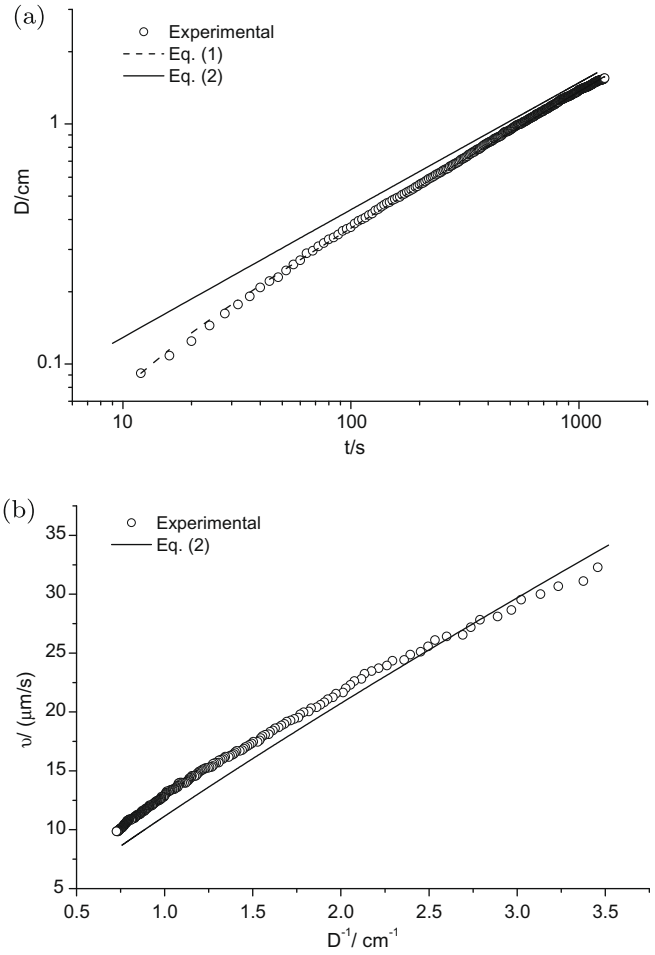

Fig. 2. Tracking of the anodic electrodenaturation front. (a) Log–log plot of distance from anode (cm) vs. time (s). (b) Electrodenaturation front velocity (μm/s) vs. the inverse of the distance (cm^{-1}) relative to the anode. Circles indicate experimental points; dashed line: fit to Eq. (1), continuous line: calculated with Eq. (2)

four experiments of the front tracking using a constant current of 10 mA. In this figure, the dashed line indicates the fitting according to

$$D = Kt^m \tag{1}$$

where D is the distance to the electrode and t the treatment time. A good fitting is obtained with $m = 0.56$. The continuous line shows the fitting according to the Eq. (2) described below.

This graph suggests that ion transport is governed by diffusion (typically, $t^{1/2}$) which can be explained observing that the medium presents a relatively high salt concentration (NaCl) which acts as supporting electrolyte thus migration is suppressed. The circular shapes of the electrodenaturation fronts or halos observed in Fig. 1 are also indicators of a diffusion-controlled regime. Though the possibility of some convective activity in the neighborhood of the electrodes cannot be completely disregarded [13], in the bulk region between electrodes the transport is diffusion-controlled as shown in our experiments.

The above results can be modeled in a semiquantitative way considering diffusion in one dimension. For electrode reactions such as (as for cathodic and anodic EChT reactions, respectively)

$$H_2O + e^- \rightarrow 1/2H_2 + OH^-$$

or

$$H_2O \rightarrow 1/2O_2 + 2H^+ + 2e^-$$

under galvanostatic conditions, the concentration profile of reaction products is given (neglecting initial H^+ and OH^- concentrations) by [16]

$$C(D,t) = C^0\left(\frac{D_p}{D_w}\right)^{(1/2)}\left(\frac{t}{\tau}\right)^{(1/2)}\left[\exp\left(-\frac{D^2}{4D_pt}\right) - \sqrt{\pi}\left(\frac{D}{2\sqrt{D_pt}}\right)\mathrm{erfc}\left(\frac{D}{2\sqrt{D_pt}}\right)\right] \tag{2}$$

where C^0 is the bulk water concentration, D_p the diffusion coefficient of the respective reaction product (H^+ or OH^-, with D_w for water) and τ the Sand time given by

$$\tau = \left(\frac{nFAD_w^{1/2}\pi^{1/2}C^0}{2i}\right)^2$$

Here, n is the number of electrons involved, F the Faraday's constant, A the electrode area and i the applied current.

If electrodenaturation (tissue destruction) occurs when H^+ or OH^- concentration rises above a critical value C_c, then the electrodenaturation front advance observed is given by the (D,t) points where such value is reached. Considering the anodic (H^+) front, taking $C_c = 10^{-4}$ mol/dm^3(pH 4), $D_p = 6.37 \times 10^{-5}$cm^2 s^{-1}, $D_w = 1.15 \times 10^{-5}$cm^2 s^{-1}, $i = 10$ mA, $n = 1$ and $A = 0.05$ cm^2, numerical evaluation of Eq. (2) gives the plot shown as a continuous line in Fig. 2a. Here we considered a medium similar to that of a 20% sucrose solution, having a viscosity nearly double than that of pure water and assuming valid the Stokes–Einstein equation. Thus, the diffusion coefficient of water was taken as 0.5 the value in pure water, and, for H^+, it was taken as 0.7 the corresponding one in water [17].

From this curve, evaluating the front velocity v (μm/s) as a function of D^{-1}, the plot in Fig. 2b (dotted line)) is obtained, showing an approximately linear relationship, as observed in the experiments (circles). Thus, taking v as a linear function of D^{-1}, the time needed for tumor destruction without compromising healthy tissue can be estimated knowing the geometry of the problem. The experimental behaviour is fairly well reproduced by Eq. (2), which is consistent with an essentially diffusive behaviour of the electrodenaturation process. This conclusion in principle is qualitative, because the actual diffusion coefficients in the CGM medium are not known. In particular, it should be noted that the cathodic front shows a similar behaviour (data not shown), with velocity close to that of the anodic front (as revealed by the fact that both fronts colide almost at the center). Greater model accuracy would require the knowledge of diffusion coefficients in the CGM and tumor tissues and a numerical solution of the problem in the real geometry. This is a subject of future work.

4. Conclusions

We introduced an in vitro collagen–macronutrient (proteins, lipids and carbohydrates) gel model (CMG model) under constant electric current conditions to study the impact of electrodenaturation in a tumor tissue as a mean of assessing effectiveness of an electrochemical treatment. Our CMG model shows that from an initial uniform condition two electrodenaturation fronts evolve expanding towards each other until collision. Electrodenaturation front tracking unveils that, before collision of opposing pH fronts, they grow as $t^{0.56}$, evidencing a diffusion-controlled regime, whereas v can be considered a linear function of D^{-1}, so that the time needed for tumor destruction can be evaluated knowing the geometry of the problem. This is essential for an optimal prediction of the time necessary for total tumor destruction without compromising healthy tissue. In contrast to previous in vitro models of

tissues subjected to EChT, our CMG model provides a more realistic approximation to the real tissue composition, thus greater accuracy to an EChT occurring in vivo and, consequently, a better tool for in silico model validation. We propose the CMG model as an aid in the future optimization of EChT operative conditions and dose planning.

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