SIMULATING THE EFFECTS OF INTERCELLULAR ICE PROPAGATION DURING CRYOSURGERY

Shannon L. Stott , Jens O.M. Karlsson

Woodruff School of Mechanical Engineering
Georgia Institute of Technology
Atlanta, GA

INTRODUCTION

Tumor ablation through cryosurgery requires the maximization of lethal intracellular ice formation (IIF) in the tumor cells, while minimizing the damage to the healthy cells in the periphery of the tumor. Although much is known about the mechanisms and kinetics of IIF in isolated cells [1], few models exist for ice formation in tissues [2], hampering the development of optimal protocols for cryosurgery. Our previous observations in micropatterned tissue constructs have shown that two distinct mechanisms are responsible for IIF in confluent tissues: ice can form by spontaneous nucleation inside cells, but ice can also propagate between cells that are in contact [3]. Furthermore, we have demonstrated that intercellular ice propagation is mediated in part by gap junctions [3]. We have recently developed a microscale model of the kinetics of ice propagation, allowing us to simulate IIF in tissues, using Monte Carlo techniques [3, 4].

The present study explores the kinetics if IIF in a model system relevant to cryosurgical tumor ablation. Whereas gap junctional intercellular communication is known to be down-regulated in tumor cells [5], we hypothesize that the rate of intercellular ice propagation will be lower in a tumor than in the surrounding tissue. This hypothesis is supported by the fact that cancer cells appear to be more resistant to freeze injury than healthy cells [6]. Thus, we have simulated IIF in a tissue containing a tumor, by assigning a different average rate of ice propagation in healthy and cancerous tissue. A parametric analysis is presented, demonstrating the effects of the two propagation rates on the kinetics and spatial distribution of cell injury by IIF.

THEORETICAL BACKGROUND

Spontaneous ice nucleation events in normal and tumor cells were assumed to occur at comparable rates (J_i). Thus, defining a non-dimensional time

$$\tau = \int_0^t J_i dt \qquad (1)$$

The probability of spontaneous IIF in a given cell within a small time interval $\Delta\tau$ can be shown to be:

$$p_i = \Delta\tau \qquad (2)$$

The probability of intracellular freezing due to ice propagation is

$$p_p = \left(k_h \cdot \alpha_h + k_t \cdot \alpha_t\right) \cdot \Delta\tau \qquad (3)$$

where k_h and k_t are the number of adjoining frozen healthy or tumor cells, respectively; α_h and α_t, the nondimensional rates of intercellular ice propagation, respectively. The propagation rates are normalized with respect to J_i. For convenience, a ratio of the two propagation rates, $\beta \equiv \alpha_t / \alpha_h$, is also defined.

Equations 2 and 3 were used in Monte Carlo simulations of IIF in two-dimensional tissues with six nearest neighbors per cell, as previously described [4].

RESULTS AND DISCUSSION

To investigate the kinetics of ice formation during cryosurgery, a two-dimensional composite tissue that contains both healthy and malignant cells was considered. A mass of tumor cells was simulated by centering a square of 9 by 9 tumor cells (82 tumor cells) inside a matrix of 30 by 30 cells (900 total cells). The spatial distribution of IIF (and thus, cell injury) was evaluated at the median time of IIF for healthy cells (τ_{50}).

Figure 1 shows the probability of IIF in tumor cells at τ_{50}, as a function of the ratio of propagation rates, β, for various values of α_h. For each experimental condition, the data from 1,000 simulations were aggregated. Three distinct regimes can be identified, depending on the value for α_h. For low propagation rates in the healthy tissue ($0<\alpha_h \leq 5$), a weak linear dependence exists between the probability of IIF in the tumor cells and β. In this regime, if $\alpha_t = \alpha_h$, approximately 50% of tumor cells are frozen when the healthy tissue is 50% frozen.

For intermediate propagation rates ($5 < \alpha_h \leq 100$), the probability of IIF in the tumor is approximately proportional to β (for $\beta \leq 1$). In this regime, if $\alpha_t = \alpha_h$, the probability of IIF is higher in the tumor than in the healthy tissue. Finally, for very large propagation rates (α_h>100), the dependence between β and the probability of IIF in the tumor is nonlinear, with the fraction of frozen cells being slightly larger in the tumor than in the healthy tissue, except for small values of β.

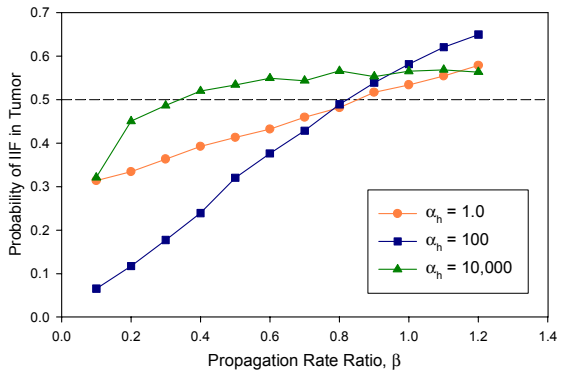

Figure 1. Probability of IIF in tumor cells when 50% of healthy cells are frozen.

A qualitative illustration of the mechanisms of ice formation in the three different regimes is presented in Fig. 2, showing the state of the tissue at τ_{50} for a representative experiment. As shown in Fig. 2A, the low propagation rate regime (α_h = 0.1) is characterized by an even distribution of IIF from both spontaneous and propagative mechanisms. Figure 2B is representative of intermediate propagation rate regime (α_h = 50), showing spatial gradients of IIF due to propagation. It has previously been shown that cells near the center of the tissue freeze faster than the cells on the periphery, and that this effect is most pronounced for intermediate propagation rates [4]. Fig. 2A shows the observed behavior for high propagation rates (α_h=10,000), in which the entire tissue freezes rapidly after the first nucleation event, and the average probability of IIF in the healthy tissue is not spatially dependent.

The observations in Fig. 2 can shed some light on the trends shown in Fig. 1. For example, at intermediate values of α_h, the number of frozen healthy cells surrounding the tumor will be larger than for the other regimes, due to the spatial distribution of IIF. Thus, the probability of tumor freezing in this regime will be dependent on propagation of ice from the healthy tissue into the tumor, explaining the high sensitivity to β, and the high probability of tumor damage for large β. The nonlinear effects in the high-α_h regime may be due to the fact that for low β, the value of α_t may fall in the intermediate regime, such that the average probability of IIF has spatial gradients in the tumor, but is spatially uniform in the healthy tissue.

In summary, our simulations show that if the average rate of ice propagation is lower in a tumor than in healthy tissue, the probability of IIF (and hence cell damage) will typically be lower in the tumor than in the surrounding tissue. Parametric analysis revealed three qualitatively distinct regimes, depending on the rate of ice propagation in the healthy tissue.

Future directions of this work include coupling the ice propagation model to a model of cell dehydration, incorporating the

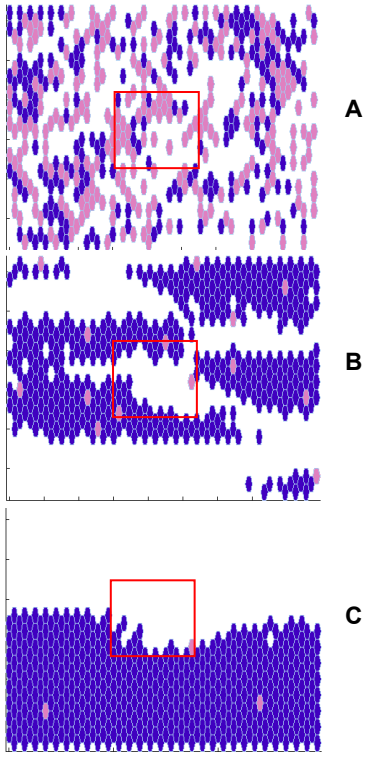

Figure 2. The state of IIF in a tissue at τ_{50} for representative experiments with β = 0.4 and α_h=0.1 (A); α_h=50 (B); α_h=10,000 (C). The location of the tumor is indicated by a rectangle. Cells are colored dark if frozen by propagation, light if frozen by spontaneous nucleation. Unfrozen cells are not shown.

temperature dependence of the rates of ice propagation, and allowing for cases in which the rate of spontaneous nucleation is different in healthy and malignant cells. Our goal is to use our models to identify regimes in which tumor cells can be destroyed while preserving the surrounding healthy tissue.

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