MODIFIED AVRAMI KINETICS OF ICE PROPAGATION IN ONE-DIMENSIONAL TISSUES

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INTRODUCTION

Cryopreservation is a critical technology for bringing tissue-engineered biomedical products to market, whereas the shelf-life of mammalian cells is short (on the order of hours), and the need for inventory control, quality control, and product distribution requires storage of living biological materials for periods ranging from weeks to months or years. Freezing of tissues in which cells are in contact with each other (e.g., liver, skin, kidney) can result in significant damage due to the ability of deleterious intracellular ice to propagate from cell to cell via gap junctions or other intercellular structures [1]. Thus, towards the goal of minimizing damage associated with the cryopreservation process, we have developed theoretical models of intercellular ice propagation in confluent tissues.

Based on experimental observations in micropatterned tissue constructs with well-defined cell-cell interactions, we have developed a microscale model of the kinetics of ice propagation [1], allowing us to simulate ice formation in one-, two-, or three-dimensional tissues containing up to on the order of 10^6 cells (i.e., a 2D monolayer with area 1 cm^2, or a 3D tissue with volume 1 mm^3), using Monte Carlo techniques [2]. In order to overcome the prohibitive computational costs of simulating discrete cell freezing events in larger tissues, we have now developed a continuum approximation of intracellular ice formation (IIF) in confluent tissues. We have previously shown that the kinetics of IIF in tissue are governed by two processes—spontaneous IIF (e.g., by intracellular ice nucleation) and intercellular ice propagation—giving rise to the appearance of clusters of frozen cells within the tissue, which are initiated by a spontaneous IIF event and subsequently grow (via ice propagation) until they "impinge" on other clusters or on the tissue boundaries [2]. The phenomena observed during freezing of confluent tissue are thus analogous to the processes of nucleation, growth, and impingement of crystals from a supercooled melt. Here, we present an analysis of the kinetics of IIF in one-dimensional tissues using the well-known Johnson-Mehl-Avrami (JMA) theory of phase transformation kinetics [3]. The JMA equation was modified to account for the non-negligible size of the initial "nuclei" (i.e., the spontaneously frozen cells).

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THEORETICAL BACKGROUND

The JMA theory describes the kinetics of phase transformation using the equation

$$X(t) = 1 - \exp\left\{-kt^n\right\} \tag{1}$$

where $X(t)$ is the transformed volume fraction as a function of time; k and n depend on geometric factors and on the mechanisms of nucleation and crystal growth. The values of k and n can be estimated from experimental measurements by linear regression on a log-log plot of $\ln\{(1\text{-}X(t))^{-1}\}$.

In tissue freezing, the transformed fraction is equal to the fraction of frozen cells, i.e., the probability of IIF. To predict the kinetics of IIF, we define a non-dimensional time τ and a non-dimensional propagation rate α as previously [2]:

$$d\tau \equiv J_i \cdot dt \tag{2}$$

$$\alpha \equiv J_p / J_i \tag{3}$$

where J_i and J_p are the average rates of spontaneous and propagative IIF, respectively, per cell and per interface with a frozen neighbor. Thus, in a one dimensional tissue, the average number of frozen cells N in a transformed region at a time $\delta\tau$ after the spontaneous IIF event that initiated the transformation of this region, is given by

$$N = 2\alpha \cdot \delta\tau + 1 \tag{4}$$

if α is constant and J_i is nonzero, and the transformed region does not impinge on other clusters of frozen cells, or on the tissue boundaries. Using the customary Avrami approach for taking into account the formation and impingement of multiple transformed regions, it can be shown that the probability of IIF is

$$P_{IIF} = 1 - \exp\left\{-\left(\alpha \cdot \tau^2 + \tau\right)\right\} \quad (5)$$

where the second term in the exponent arises from the cells that freeze spontaneously. An analogous term is not present in Eq. (1) due to the fact that the size of the initial nucleus is neglected in conventional JMA theory. Thus, we will describe the kinetics of IIF using the transformation

$$\ln\left\{\ln\left(1 - P_{IIF}\right)^{-1} - \tau\right\} = n \cdot \ln\tau + \ln k \quad (6)$$

where $k=\alpha$ and $n=2$ for IIF in a one-dimensional tissue. Note that τ can be estimated from experimental data as previously described [1], so Eq. (6) should prove useful for analyzing experimental data.

RESULTS AND DISCUSSION

To test our method for analyzing the kinetics of IIF in tissues, we simulated intracellular ice nucleation and intercellular ice propagation using Monte Carlo techniques, as previously described [2]. Figure 1 shows the probability of IIF in one-dimensional tissues (each consisting of 1,000 cells), transformed using Eq. (6), for various values of the propagation rate α. As seen, the transformed curves are approximately linear, except for deviations and random scatter during the initial stages of IIF. A less pronounced deviation can sometimes also be observed near the end of the transformation. These deviations are expected, whereas the continuum approximation will not be valid when there are only a small number of frozen cells (or unfrozen cells). Linearity improves for larger tissues, or if data from an ensemble of multiple identical tissues are aggregated (data not shown).

Aggregate IIF data for linear cell arrays comprising a total of 10^5 cells were obtained by numerical simulations at various values of α and various tissue sizes. The Avrami coefficient k and exponent n were obtained by linear regression to the data transformed using Eq. (6). As shown in Fig. 2, the Avrami coefficient was approximately equal to the expected value of $n=2$, for $\alpha \geq 1$, for all tissue sizes. Similarly, Fig. 3 shows that the Avrami coefficient measured in the numerical experiments was approximately equal to the expected value $k=\alpha$. For $\alpha<1$, the value of n appears to decrease with decreasing α, which is consistent the theoretical result that $n=1$ for the limiting case $\alpha=0$.

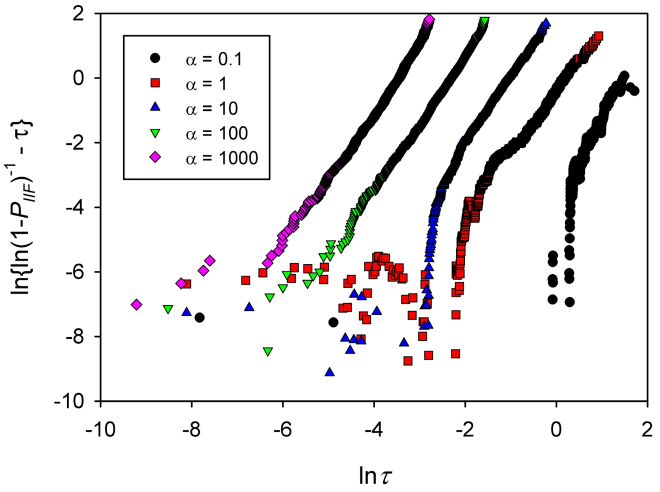

Figure 1. Kinetics of IIF in a one-dimensional tissue of 1,000, transformed using a modified Avrami equation (Eq. 6).

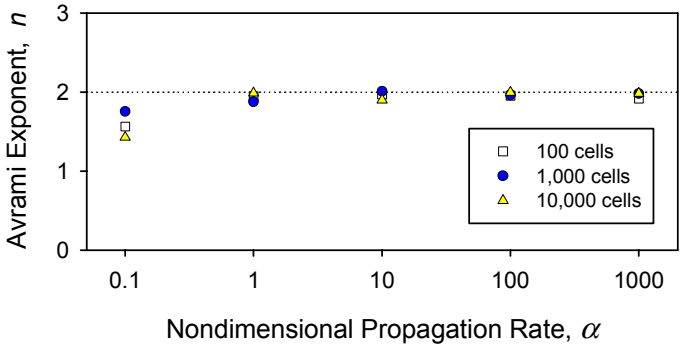

Figure 2. Avrami exponent for IIF in one-dimensional tissues of various sizes. The dotted line represents the theoretical result $n=2$.

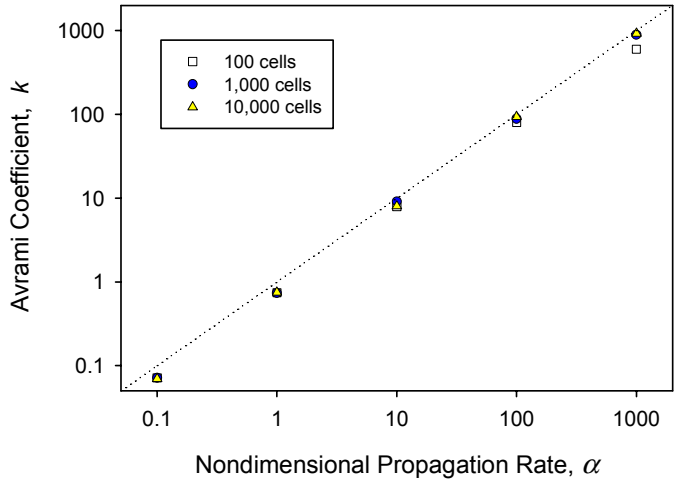

Figure 3. Avrami coefficient for IIF in one-dimensional tissues of various sizes. The dotted line represents the theoretical result $k=\alpha$.

CONCLUSION

A modified Avrami theory which takes into account the non-negligible contribution of nuclei to the transformed fraction can be used as a continuum approximation for ice formation and propagation within confluent tissues. By extending the present analysis to two- and three-dimensional tissues, models will be obtained that can be used to simulate IIF in macroscale tissues, as well as to estimate the magnitudes of J_i and J_p by fitting model predictions to experimental observations.

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