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A Two-State Cell Damage Model Under Hyperthermic Conditions: Theory and In Vitro Experiments

Yusheng Feng,

Computational Bioengineering and Nanotechnology Laboratory, Department of Mechanical Engineering, The University of Texas at San Antonio, San Antonio, TX 78249

J. Tinsley Oden, and

Institute for Computational Engineering and Sciences, The University of Texas at Austin, Austin, TX 78712

Marissa Nichole Rylander

Department of Mechanical Engineering, and School of Biomedical Engineering and Sciences, Virginia Tech, Blacksburg, VA 24061

Yusheng Feng: yusheng.feng@utsa.edu; J. Tinsley Oden: oden@ices.utexas.edu; Marissa Nichole Rylander: mnr@vt.edu

Abstract

The ultimate goal of cancer treatment utilizing thermotherapy is to eradicate tumors and minimize damage to surrounding host tissues. To achieve this goal, it is important to develop an accurate cell damage model to characterize the population of cell death under various thermal conditions. The traditional Arrhenius model is often used to characterize the damaged cell population under the assumption that the rate of cell damage is proportional to $\exp(-E_a/RT)$, where E_a is the activation energy, R is the universal gas constant, and T is the absolute temperature. However, this model is unable to capture transition phenomena over the entire hyperthermia and ablation temperature range, particularly during the initial stage of heating. Inspired by classical statistical thermodynamic principles, we propose a general two-state model to characterize the entire cell population with two distinct and measurable subpopulations of cells, in which each cell is in one of the two microstates, viable (live) and damaged (dead), respectively. The resulting cell viability can be expressed as $C(\tau, T) = \exp(-\Phi(\tau, T)/kT) / (1 + \exp(-\Phi(\tau, T)/kT))$, where k is a constant. The in vitro cell viability experiments revealed that the function $\Phi(\tau, T)$ can be defined as a function that is linear in exposure time τ when the temperature T is fixed, and linear as well in terms of the reciprocal of temperature T when the variable τ is held as constant. To determine parameters in the function $\Phi(\tau, T)$, we use in vitro cell viability data from the experiments conducted with human prostate cancerous (PC3) and normal (RWPE-1) cells exposed to thermotherapeutic protocols to correlate with the proposed cell damage model. Very good agreement between experimental data and the derived damage model is obtained. In addition, the new two-state model has the advantage that is less sensitive and more robust due to its well behaved model parameters.

Keywords

cell viability; Arrhenius law; two-state model; cancer treatment; thermal therapy

1 Introduction

Thermotherapy—using laser, microwave, radio-frequency, or ultrasound energy sources—is a minimally invasive therapeutic modality that can be calibrated to deliver a lethal dose to the targeted tumor regions for cancer treatment [1,2]. The biological basis for thermotherapy is

that exposing cells to temperatures outside of their natural environment (i.e., under either hypo- or hyperthermic conditions) for certain periods of time can damage and even destroy the cancerous cells or sensitize them to follow-up treatments such as radiation. To achieve the optimal treatment outcome, one needs to address the fundamental issue of how to accurately characterize the cell viability under various therapeutic protocols in terms of temperature and exposure times.

Thermal damage processes in cells and tissues are usually quantified by kinetic models based on a first-order rate process to characterize the pathological transformation to specific states by observable alterations such as coagulation. While the Arrhenius law is commonly used to describe the rate of chemical reactions involving temperature [3,4], Henriques and Moritz proposed a model of this form in 1947 to quantify thermal damage [5,6]. The thermal damage associated with exposing cells to thermotherapeutic conditions is generally predicted using the Arrhenius law based on the assumption that the rate of cell damage is proportional to $\exp(-E_a/RT)$, where E_a is the activation energy (or the heat of activation), R is the universal gas constant, and T is the absolute temperature [7], with a few exceptions (e.g., Ref. [8]).

Although thermal damage models based on the traditional Arrhenius law are widely used, the model possesses two major inherent limitations: (1) its inability to fit all the cellular damage data over the entire thermotherapeutic temperature range and throughout the entire heating process, and (2) its sensitivity to small changes in parameters due to its double exponential function form. In general, several fundamental questions can be raised. How do cells essentially respond to temperature? Why does the rate of thermal damage follow the first-order unimolecular chemical reaction? What is the biophysical interpretation of both parameters in the traditional Arrhenius model? Some answers related to these questions may be found in Refs. [9–11]. However, further investigation is warranted due to the importance of these questions.

Experimental data reported in literature [12,13] suggest that there are at least two transitional temperatures (“break points”), around 43°C and 54°C in the temperature range from 39°C to 60°C. It is possible that a different damage mechanism may be initiated at each of these temperatures. Cells initially exhibit resistance to the thermal damage due to the induction of heat shock proteins by sublethal temperatures as autoregulatory mechanisms. This phenomenon can be observed in measured cell viability data in which the cell damage rate is initially slow (to form a so-called “shoulder region”) followed by a damage rate dominated by exponential decay [13]. Usually, the traditional Arrhenius model permits the fitting of data solely within the exponential decay region of the curve, where cell viability is plummeting due to extensive damage, but is not able to accommodate the shoulder region characterized by the sustained high cell viability encountered in the initial stages of the heating process for lower temperatures.

In general, although it depends on the cell line and the temperature, measured cell viability profiles in this temperature range initially exhibit a shoulder region where cell viability remains high until a threshold lethal thermal dose is achieved to initiate rapid declines in cell viability. The traditional Arrhenius model is capable of predicting the complete damage phenomenon for cells exposed to $T < 54^\circ\text{C}$ where the thermal dose is substantial at short exposure times causing rapid declines in cell viability immediately following thermal stress [13,14].

One solution may be to employ three sets of damage parameters for different temperature regimes ($T < 43^\circ\text{C}$, $43^\circ\text{C} \leq T \leq 54^\circ\text{C}$, and $T > 54^\circ\text{C}$) in order to permit accurate fitting of cellular damage data for the entire range of temperatures. However, it is not desirable from physical and mathematical modeling points of view where a unified formula is usually sought. An additional problem with the thermal damage model based on the traditional Arrhenius model

is the numerical sensitivity of the model parameters to small changes in measurement data. As a result, therapeutic outcomes could be difficult to control.

To overcome these limitations of the traditional Arrhenius model described above, various types of cell damage models have been proposed to model the thermal damage of cells and tissues. With a few exceptions [15], most of these models use chemical kinetics or empirical laws as a starting point [12,16–20] to derive their cell damage models. However, there are two related developments to the current study by Moussa et al. [21] and Jung [22,23], which are based on the statistics. In the work of Moussa et al., the concept of susceptibility to thermal damage was introduced, which is defined as inversely related to the exposure time. The number of cells with certain susceptibility is assumed to follow a normal (Gaussian) distribution. On the other hand, Jung assumed that the heat-induced cellular inactivation was dictated by a two-step process, which follows a Poisson distribution. The final cellular survival function with respect to time is a double exponential form with “shifted” and scaling parameters in comparison to the Arrhenius model. For a comprehensive discussion of various cell damage models, we refer to a review article by He and Bischof [7].

In this study, a general two-state model for cell damage under hyperthermic conditions is proposed. Instead of assuming statistical distributions for either of the cell populations as in Refs. [22,23], we only assume that the total cell population can be separated into two distinct and measurable states. Based on arguments motivated by classical statistical thermodynamics, the distribution of two subpopulations can be derived. Specifically, the proposed two-state model characterizes two subpopulations of viable (live) and damaged (dead) cells, which are the ensemble average of two distinct microstates. It leads to the conclusion that cell viability can be expressed as $C(\tau, T) = \exp(-\Phi(\tau, T)/kT) / (1 + \exp(-\Phi(\tau, T)/kT))$, or alternatively, $C(\tau, T) = 1/2 + 1/2 \tanh(-\Phi(\tau, T)/2kT)$, where k is a constant. Based on in vitro experimental data, we found that the two-state model correlates very well with the experimental data if $\Phi(\tau, T)$ is chosen to be a function that is linear in the exposure time τ while T is constant and linear with respect to $1/T$, if τ is holding as a constant. To determine the parameters in $\Phi(\tau, T)$, we use in vitro cell viability data for experiments conducted for human prostate cancerous (PC3) and normal (RWPE-1) cells to calibrate the two-state cell damage model. Very good agreement between experimental data and the proposed model is obtained through the least-squares regression.

As compared to the traditional Arrhenius model, the two-state model developed in this study captures the damage process more accurately over a wider thermotherapeutic temperature range, including the beginning phase (the shoulder region) when cells are first exposed to the heat shock. Also, the model successfully characterizes the sigmoidal phenomenon of the cell response.

2 Theory of Two-State Model and Its Application to Cell Damage

Consider a population of particles with two distinct states, $\mathcal{A}$ and $\mathcal{B}$, which are the ensemble average of all particles in particular microstates. The microstate is associated with certain measurable properties. To determine the distribution of these two sub-populations, each particle is measured by certain physical experiments to determine to which microstate it belongs. The sub-population of $\mathcal{A}$ consists of all the particles in Microstate $\hat{A}$. Likewise, the collection of all the particles in Microstate $\hat{B}$ forms the other subpopulation in Microstate $\mathcal{B}$. Obviously, the sum of the two subpopulations adds to the total population.

In the context of cell viability, we use the standard in vitro experimental protocol, described in Sec. 3.3, to determine whether a cell is alive (denoted Microstate $\hat{A}$) or dead (denoted Microstate $\hat{B}$). The goal is to determine the distribution of these two-state subpopulations under experimental conditions. To that end, classical arguments of statistical thermodynamics in

deriving the Boltzmann distribution law were employed to develop a two-state model for cell damage under thermotherapeutic conditions.

In an in vitro system of the fixed population of total n cells, we assume that there are only two species of cells in this population, dead and live cells. We denote by n_0 the total number of dead cells and by n_1 the number of live cells. The cell viability function $C(\tau, T)$ is a function of temperature T and the exposure time τ ; the cell damage function is $D(\tau, T) = 1 - C(\tau, T)$. Evidently,

$$C(\tau, T) = \frac{n_1}{n} = p_1, \quad D(\tau, T) = \frac{n_0}{n} = p_0 \quad (1)$$

Let us now consider a system of the fixed population of the total n cells with the following basic assumptions.

- i. There are only two species of cells in this population, dead and live cells, with

$$n = n_0 + n_1 \quad (2)$$

Each distribution of dead and live cells constitutes a microstate of the system in the sense of classical statistical thermodynamics.

- ii. The probability p_0 that a cell is dead or p_1 that a cell is alive is thus

$$p_0 = \frac{n_0}{n}, \quad p_1 = \frac{n_1}{n}$$

and

$$p_0 + p_1 = 1 \quad (3)$$

- iii. To fix the notation, we denote each dead cell in the Microstate $\hat{B}$ with index ε_0 and that of a live cell in Microstate $\hat{A}$ with ε_1 , where ε_0 and ε_1 are constant indices representing state variables that are independent of the temperature and the exposure time. However, the numbers of dead cells n_0 and live cells n_1 depend on the temperature and the exposure time, although the total number n is a constant for a closed in vitro system. Cell division is considered to be much slower than the exposure time. Thus, the state of the system can be represented by the following equation:

$$n_0(\tau, T)\varepsilon_0 + n_1(\tau, T)\varepsilon_1 = E(\tau, T) \quad (4)$$

where ε_0 and ε_1 are state variables for live and dead cells, respectively. For simplicity, we choose $\varepsilon_0 = 0$ and $\varepsilon_1 = 1$.

Thus, $E(\tau, T)$ is the global state function for the entire population.

$$p_0(\tau, T)\varepsilon_0 + p_1(\tau, T)\varepsilon_1 = \frac{E(\tau, T)}{n} = \bar{\varepsilon}(\tau, T) \quad (5)$$

where $\bar{\varepsilon}(\tau, T)$ is the statistical mean state.

- iv. The multiplicity function for the system, which gives the total number of possible combinations of microstates, is defined by the following:

$$\omega = \frac{n!}{n_0!n_1!} \quad (6)$$

Following classical arguments, the most likely distribution occurs when the multiplicity function ω is a maximum, subject to constraints Eqs. (3) and (4), or equivalently, when $\ln(\omega)/n$ is maximized subject to these constraints. Using Stirling's formula for large n , we obtain

$$\ln(\omega) = \ln(n!) - \ln(n_0!) - \ln(n_1!) \approx -n[p_0 \ln(p_0) + p_1 \ln(p_1)] \quad (7)$$

Now, our goal is to find the most probable distribution by maximizing the function

$$\Gamma(p_0, p_1) = \frac{\ln(\omega)}{n} = -[p_0 \ln(p_0) + p_1 \ln(p_1)] \quad (8)$$

subject to the constraints described earlier.

The following lemma relates the probability distribution of two populations to their respective states, $\mathcal{A}$ and $\mathcal{B}$.

Lemma 1

Given $\Gamma(p_0, p_1)$ defined in Eq. (8), and constraints in Eqs. (3) and (4), there exist unique distributions p_0 and p_1 such that $\Gamma(p_0, p_1)$ is maximized, given by

$$p_0 = \frac{e^{\lambda \varepsilon_0}}{e^{\lambda \varepsilon_0} + e^{\lambda \varepsilon_1}} \quad \text{and} \quad p_1 = \frac{e^{\lambda \varepsilon_1}}{e^{\lambda \varepsilon_0} + e^{\lambda \varepsilon_1}} \quad (9)$$

where

$$\lambda = \frac{1}{\varepsilon_0 - \varepsilon_1} \ln \left(\frac{\bar{\varepsilon} - \varepsilon_1}{\varepsilon_0 - \bar{\varepsilon}} \right) \quad \text{and} \quad \bar{\varepsilon} = E(T, t)/n \quad (10)$$

Proof—Let $\mathcal{L}(\hat{p}_0, \hat{p}_1, \hat{\lambda}, \hat{\mu})$ be the Lagrangian for $\Gamma(\hat{p}_0, \hat{p}_1)$ associated with the constraints

$$\mathcal{L} = \Gamma(\hat{p}_0, \hat{p}_1) + \hat{\lambda}[(\hat{p}_0 \varepsilon_0 + \hat{p}_1 \varepsilon_1) - \bar{\varepsilon}] + \hat{\mu}[\hat{p}_0 + \hat{p}_1 - 1]$$

where $\hat{\lambda}$ and $\hat{\mu}$ are Lagrange multipliers. To find critical values of $\hat{p}_0$ and $\hat{p}_1$ and associated Lagrange multipliers $\hat{\lambda}$ and $\hat{\mu}$ that maximize $\mathcal{L}$, we differentiate $\mathcal{L}$ to obtain the following:

$$d\mathcal{L} = \frac{\partial \mathcal{L}}{\partial \widehat{p}_0} d\widehat{p}_0 + \frac{\partial \mathcal{L}}{\partial \widehat{p}_1} d\widehat{p}_1 + \frac{\partial \mathcal{L}}{\partial \widehat{\lambda}} d\widehat{\lambda} + \frac{\partial \mathcal{L}}{\partial \widehat{\mu}} d\widehat{\mu} \\ = (\widehat{\lambda}\varepsilon_0 - \ln \widehat{p}_0 - 1 + \widehat{\mu}) d\widehat{p}_0 + (\widehat{\lambda}\varepsilon_1 - \ln \widehat{p}_1 - 1 + \widehat{\mu}) d\widehat{p}_1 + [(\widehat{p}_0\varepsilon_0 + \widehat{p}_1\varepsilon_1) - \bar{\varepsilon}] d\widehat{\lambda} + (\widehat{p}_0 + \widehat{p}_1 - 1) d\widehat{\mu} \stackrel{\text{set}}{=} 0$$

Setting $d\mathcal{L} = 0$, we have

$$\ln \widehat{p}_0 - \widehat{\lambda}\varepsilon_0 = \ln \widehat{p}_1 - \widehat{\lambda}\varepsilon_1 = \widehat{\mu} - 1$$

Equivalently,

$$\widehat{p}_0 = e^{\widehat{\lambda}\varepsilon_0 + \widehat{\mu} - 1} = e^{\widehat{\mu} - 1} e^{\widehat{\lambda}\varepsilon_0} \quad \text{and} \quad \widehat{p}_1 = e^{\widehat{\lambda}\varepsilon_1 + \widehat{\mu} - 1} = e^{\widehat{\mu} - 1} e^{\widehat{\lambda}\varepsilon_1}$$

Next, we substitute $\widehat{p}_0$ and $\widehat{p}_1$ into the constraint condition $\widehat{p}_0 + \widehat{p}_1 = 1$ to obtain

$$e^{\widehat{\mu} - 1} = \frac{1}{e^{\widehat{\lambda}\varepsilon_0} + e^{\widehat{\lambda}\varepsilon_1}}$$

Then, with the constraint

$$\varepsilon_1 \widehat{p}_1 + \varepsilon_0 \widehat{p}_0 = \varepsilon_1 e^{\widehat{\lambda}\varepsilon_1 + \widehat{\mu} - 1} + \varepsilon_0 e^{\widehat{\lambda}\varepsilon_0 + \widehat{\mu} - 1} = \bar{\varepsilon}$$

we find

$$\lambda = \frac{1}{\varepsilon_0 - \varepsilon_1} \ln \left(\frac{\bar{\varepsilon} - \varepsilon_1}{\varepsilon_0 - \bar{\varepsilon}} \right)$$

Finally, we have

$$p_0 = \frac{e^{\lambda\varepsilon_0}}{e^{\lambda\varepsilon_0} + e^{\lambda\varepsilon_1}} \quad \text{and} \quad p_1 = \frac{e^{\lambda\varepsilon_1}}{e^{\lambda\varepsilon_0} + e^{\lambda\varepsilon_1}}$$

where λ depends on T and τ through $\bar{\varepsilon}$. This completes the proof.

We now derive an intermediate result. By setting $\varepsilon_0 = 0$ and $\varepsilon_1 = 1$, we obtain the following.

Lemma 2

Let $\varepsilon_0 = 0$ and $\varepsilon_1 = 1$, and let $\lambda = -\Phi(\tau, T)/kT$ in Eq. (9), where $\Phi(\tau, T)$ is any function of temperature and exposure time, and k is a constant. Then, the following probability representations are equivalent:

$$\begin{aligned}
 \text{i.} \quad & p_0 = \frac{1}{1+e^{-\Phi(\tau,T)/kT}} \text{ and } p_1 = \frac{e^{-\Phi(\tau,T)/kT}}{1+e^{-\Phi(\tau,T)/kT}} \\
 \text{ii.} \quad & p_0 = \frac{1}{2} - \frac{1}{2} \tanh(-\Phi(\tau,T)/2kT) \text{ and } p_1 = \frac{1}{2} + \frac{1}{2} \tanh(-\Phi(\tau,T)/2kT)
 \end{aligned} \tag{11}$$

Proof—These results follow directly from applying Lemma 1 and basic algebraic operations.

Theorem

Let $C(\tau, T)$ denote the cell viability function and $D(\tau, T)$ denote the cell damage function, where T and t are the temperature and the exposure time, respectively. Let conditions of Lemma 2 hold. Then, we observe the following.

$$\text{i.} \quad C(\tau, T) = p_1 \text{ and } D(\tau, T) = p_0, \text{ i.e.,}$$

$$C(\tau, T) = \frac{e^{-\Phi(\tau,T)/kT}}{1+e^{-\Phi(\tau,T)/kT}} \text{ and } D(\tau, T) = \frac{1}{1+e^{-\Phi(\tau,T)/kT}} \tag{12}$$

or equivalently,

$$C(\tau, T) = \frac{1}{2} + \frac{1}{2} \tanh(-\Phi(\tau, T)/2kT)$$

$$D(\tau, T) = 1 - C(\tau, T) = \frac{1}{2} - \frac{1}{2} \tanh(-\Phi(\tau, T)/2kT)$$

$$\text{ii.} \quad \text{Suppose } \Phi(\tau, T) \text{ is of the form } \gamma - (\alpha + \beta(\tau))T, \text{ then } C(\tau, T) \text{ defined in Eq. (12) is a solution to the following partial differential equations:}$$

$$\begin{aligned}
 \frac{\partial C(\tau, T)}{\partial T} &= \frac{\gamma}{kT^2} \frac{C(\tau, T)}{1+e^{-\Phi(\tau,T)/kT}} \\
 \frac{\partial C(\tau, T)}{\partial \tau} &= \frac{\alpha}{k} \frac{C(\tau, T)}{1+e^{-\Phi(\tau,T)/kT}}
 \end{aligned} \tag{15}$$

where γ , α , and β are constants.

Proof

$$\begin{aligned}
 \text{i.} \quad & \text{Since } p_0 \text{ represents the ratio of the dead cells to the total cell population and } p_1 \text{ the ratio of the live cells to the total cell population, the results follow directly from Lemma 2.} \\
 \text{ii.} \quad & C(\tau, T) = e^{-\Phi(\tau,T)/kT} / (1+e^{-\Phi(\tau,T)/kT}) \text{ is a solution to Eq. (15) that can be proved by direct substitution.}
 \end{aligned}$$

Remark 1—If $e^{-\Phi(\tau,T)/kT}$ is very small, then $1+e^{-\Phi(\tau,T)/kT} \approx 1$. Equation (15) can be approximated by

$$\begin{aligned}
 \frac{\partial C(\tau, T)}{\partial T} &= \frac{\gamma}{kT^2} C(\tau, T) \\
 \frac{\partial C(\tau, T)}{\partial \tau} &= \frac{\alpha}{k} C(\tau, T)
 \end{aligned} \tag{16}$$

which reduces to the differential form of the Arrhenius model.

Remark 2—Although we only use two states (dead and live) for cell viability in this study, this model could be easily extended to include multiple states, as long as each state can be measured and distinguished.

3 In Vitro Measurement of Cell Response Under Hyperthermic Conditions

The in vitro experiments discussed in this section describe a process that closely follows the conceptual framework of the canonical ensemble in classical equilibrium statistical thermodynamics (e.g., Refs. [24,25]). The composite system consists of bodies $\mathcal{F}$ (flask) and $\mathcal{W}$ (water bath), which quickly reaches thermal equilibrium since $\mathcal{F}$ is relatively small compared to body $\mathcal{W}$. In the case of our experiments for cell viability, a flask that contains a population of cells cultured in liquid medium is immersed into a heated water bath at a fixed temperature T and, after a transition period, thermodynamic equilibrium of the composite system is achieved. At each fixed temperature, only a percentage of the original population remains viable. We expect this percentage, which is equivalent to the cell survival probability, to decrease with higher temperatures. We can thus apply this classical theory to model cell viability in vitro for the common thermotherapeutic protocol of constant temperature with various time durations, as we discussed in the previous section.

A sequence of experiments was performed to measure cell viability profiles using human prostate cancerous (PC3) cells and normal (RWPE-1) cells exposed to temperatures and exposure times typically encountered in thermotherapy. The cell viability data were employed for the development of the two-state cell damage model. The same set of data permits the determination of cell damage using the traditional Arrhenius model for comparison.

3.1 Cell Culture

PC3 cells (ATCC, CRL-1435, Manassas, VA) were cultured with HAMs F12 medium (ATCC, 30-2004, Manassas, VA) with 10% FBS (ATCC, 30-2020, Manassas, VA) and 1% penicillin/streptomycin (Gibco, 15140-122, Carlsbad, CA). RWPE-1 cells (ATCC, CRL-11609, Manassas, VA) were cultured with a keratinocyte serum free medium (GIBCO-BRL, 17005-042, Carlsbad, CA) supplemented with 5 ng/ml of human recombinant EGF and 0.05 mg/ml of bovine pituitary extract. Cultures were maintained in a 5% CO₂ incubator in 25 cm² phenolic culture flasks to prevent contamination from leakage during the heating process.

3.2 Hyperthermic Protocol

A constant temperature circulating water bath (NESLAB RTE-100, Thermo Electron Corporation, San Jose, CA) was employed as the heat source to produce a relatively short stabilization time within 4 s. The detailed experimental protocol and calibration process used are those described in Ref. [13]. Briefly, upon reaching confluence, the medium was removed and PBS at the desired temperature was added to the flask. Then, flasks were submerged in the water bath at the hyperthermic conditions with temperatures and durations in the ranges of 44–60°C for 1–30 min. The maximum experimental temperature caused complete cell death for the shortest exposure time (we use the term hyperthermic conditions in a broad sense that include temperatures as high as 60°C). Following heating, PBS was removed and the regular cell culture medium was replenished. The flasks were returned to a 37°C incubator for 72 h to permit the complete manifestation of damage; after which time cell death and viability were measured.

3.3 Cell Viability Measurement

Cell damage in response to thermotherapeutic conditions was measured via propidium iodide staining by quantifying the fraction of cells stained with this dye (PI only stains damaged cells) using a flow cytometer. Following 72 h postheating (shown to be an effective evaluation period for measuring the extent of cell death [13]), cells were trypsinized, pelleted, and resuspended in a 4 ml PBS solution. Propidium iodide (1:1000 dilution in PBS) was added to the cell suspension and the percentage of dead cells was measured with a flow cytometer (Beckman, Irvine, CA) utilizing an argon laser (wavelength = 480 nm). Histograms of propidium fluorescence intensity were generated and analyzed using WINMDI 2.8 software. Samples of unheated controls and cells necrosed by methanol treatment (70% methanol for 30 min) and by extreme heat shock (60°C, 5 min) were used to calibrate regions of the histogram denoting live and dead cell populations. The region of the histogram occupied by the control (unheated) sample was defined as the live cell population with low levels of propidium iodide staining. The dead cell population was defined as the region of the histogram occupied by the methanol-treated and severely heat-shocked sample, which also corresponded to the region excluding the control sample live population. In order to represent the dead cell population in terms of cell viability, the percentage of dead cells was converted to live cell values and normalized with the percentage of live cells for the control. The normalized percentage of live cells provided the value for cell viability characterized in the damage calculations. Cells were also counterstained with calcein AM to confirm cell viability and provide comparison to the converted live cell values from propidium iodide staining.

Figure 1 shows histograms for control, methanol-treated, severely heat-shocked (complete cell death), and a typical heated sample. The dead cell population was defined by the marker (M1) as the region of the histogram occupied by both the methanol-treated and severely heat-shocked samples (this region excluded the live population defined by the control sample). The events label on the y-axis corresponds to the cell number.

The cell viability values for PC3 cells at 72 h postheating are shown in Fig. 2(a). With increasing thermal stimulation temperature, damage uniformly increases and occurs more rapidly. The highest measured temperature of 60°C yielded less than 1% live cells for the shortest exposure time of 1 min, whereas the lowest temperature of 44°C with the longest duration of 30 min maintained a cell viability of 10%. The standard deviation in the cell viability measurement was in the range of 0.4–6.5% with the average standard deviation of ±3.5%.

The corresponding data for RWPE-1 cells are shown in Fig. 2(b). The standard deviation for the cell viability measurement was in the range of 0.4–6.3% with the average standard deviation of ±2.9%. RWPE-1 cells were slightly less sensitive to thermal stress than PC3 cells as evidenced by the higher viability for identical temperature histories.

4 Parameter Estimation

To determine $\Phi(\tau, T)$, defined as $\gamma - (\alpha + \beta\tau)T$ where T and τ are the temperature and the exposure time, respectively, we need to estimate the constant parameters α , β , and γ . Since the first equation in Eq. (12) can be written as

$$\frac{\Phi(\tau, T)}{kT} = \ln \left(\frac{1 - C(\tau, T)}{C(\tau, T)} \right) \quad (17)$$

by solving for $\Phi(\tau, T)/kT$. Furthermore,

$$\frac{\Phi(\tau, T)}{kT} = \left(\frac{\gamma}{k}\right) \frac{1}{T} - \left(\frac{\alpha}{k}\right) \tau - \frac{\beta}{k} \ln \left(\frac{1 - C(\tau, T)}{C(\tau, T)} \right) \quad (18)$$

For simplicity, we denote $\bar{\gamma} = \gamma/k$, $\bar{\alpha} = \alpha/k$, and $\bar{\beta} = \beta/k$. To estimate these three constants, we utilize a standard bilinear least-squares regression technique. Suppose that there are $m \times n$ experimental data points for cell viability $C(T_\ell, \tau_\ell)$, $\ell = 1, \dots, m \times n$, i.e., m temperature measurement points with n exposure time for each temperature. Denote by z the function $z = \ln[(1 - C(\tau, T))/C(\tau, T)]$, then the data points $(T_\ell, \tau_\ell, z_\ell)$, $\ell = 1, \dots, m \times n$ can be plotted in three-dimensional space with respect to $1/T$ and τ .

At each measurement point $(T_\ell, \tau_\ell, z_\ell)$, Eq. (18) can be rewritten as

$$\bar{\gamma} \left(\frac{1}{T_\ell} \right) - \bar{\alpha} \tau_\ell - \bar{\beta} z_\ell, \quad \ell = 1, \dots, m \times n \quad (19)$$

where parameters h , α , and β are to be determined by the standard least-squares regression using measurement data.

Tables 1 and 2 summarize the algorithmic steps of parameter estimation for both the traditional Arrhenius and the two-state models.

Since the range of $C(\tau, T)$ is in the interval $[0, 1]$, we need to exclude initial points $C(T_i, 0) = 1$, $i = 1, \dots, m$, in the least-squares regression process. This will not affect the final results because the initial conditions in the original form of Eq. (12) are automatically satisfied.

Figure 3 illustrates that the transformation by introducing a z -variable converts a curved surface representing the cell viability into a flat plane in three-dimensional space. The two-dimensional projections on the C - $1/T$ and C - τ planes are also illustrated in Fig. 3.

The cell damage index Ω is defined as usual

$$\Omega = \ln \left(\frac{C(T, 0)}{C(\tau, T)} \right) \quad (20)$$

When the cell viability function $C(\tau, T)$ is normalized and $C(T, 0)$ is set to 1, we have $\Omega = -\ln C(\tau, T)$. Recall that the traditional Arrhenius model assumes the cell damage rate as being proportional to the rate of reaction $r(T) = e^{-E_a/RT}$. Thus, the cell damage index based on the traditional Arrhenius model is

$$\Omega = \int_0^\tau A e^{-E_a/RT(\tau)} d\tau \quad (21)$$

where τ is the total exposure time and A is a constant that is often referred to as the frequency factor [10]. If temperature is kept constant during the entire exposure time τ , then

$$\Omega = A\tau e^{-E_a/RT} \quad (22)$$

In fact, cell viability $C(T, \tau)$ defined in the traditional Arrhenius model satisfies the following differential equations

$$\begin{aligned} \frac{\partial C(\tau, T)}{\partial T} &= -r(T)C(\tau, T) \\ \frac{\partial C(\tau, T)}{\partial \tau} &= -\left(r(T) \frac{E_a \tau}{R}\right) \left(\frac{C(\tau, T)}{T^2}\right) \end{aligned} \quad (23)$$

which is equivalent to Eq. (16) with proper choices of parameters. A more detailed account to compare the two models is provided in the next section.

5 Results

The model parameters for both the traditional Arrhenius and the two-state models are obtained by the algorithmic processes described in Tables 1 and 2 using PC3 and RWPE-1 cell viability data.

For PC3 cells, the model parameters $E_a = 2.318 \times 10^5 \text{ J mol}^{-1}$ and $A = 1.19 \times 10^{35} \text{ s}^{-1}$ in the traditional Arrhenius model are obtained using the algorithm described in Table 1. The model parameters $\bar{\gamma} = 70,031 \text{ K}$, $\bar{\alpha} = 0.0493 \text{ s}^{-1}$, and $\bar{\beta} = 215.64$ are obtained using the algorithm described in Table 2. We plotted cell viability for both the PC3 and RWPE-1 cells as a function of exposure time for the temperatures range of 44–56°C. Each plot depicts measured cell viability data and the predicted cell viability from both the two-state and the traditional Arrhenius model as a function of exposure time for a constant temperature (Figs. 4 and 5). The solid line denotes the cell viability predicted by the two-state model and the dashed line represents the cell viability predicted by the traditional Arrhenius model. Error bars indicate results over a range of three experiments for each data point. It is evident from these plots that the cell viability predicted by the two-state model corresponds more closely with the measured cell viability than the traditional Arrhenius model. In general, the traditional Arrhenius model has a much larger error margin (15–20%) than that of the two-state model (3–5%), although the predictions of both models are close at the higher temperature range ($T > 54^\circ\text{C}$).

6 Discussion

In general, the rate of cell viability decline is more rapid as the stress temperature is increased. However, PC3 cells exhibit a slightly greater sensitivity to thermal stress than the RWPE-1 cells, since a lower cell viability of PC3 cells was observed in vitro than that of RWPE-1 cells under the same thermal conditions. A larger difference in cell viability between the PC3 and RWPE-1 cells is expected in vivo due to the presence of the vascular network in which perfusion could affect the local temperature field during thermal therapies.

6.1 Two-State Model From a Kinetic Viewpoint

From a kinetic point of view, we rewrite the rate equations for the two-state model defined in Eq. (15) in terms of the following kinetic relations:

- i. $\partial C(\tau, T)/\partial \tau$ is proportional to $(1/(1 + e^{-\Phi(\tau, T)/kT}))C(\tau, T)$
- ii. $\partial C(\tau, T)/\partial T$ is proportional to $(1/(1 + e^{-\Phi(\tau, T)/kT}))C(\tau, T)/T^2$

Namely, the rate of change in cell viability in the two-state model with respect to exposure time τ , while holding temperature T constant, is proportional to $(1/(1 + e^{-\Phi(\tau,T)/kT})C(\tau,T)$. The rate of change in cell viability with respect to temperature T , while holding τ constant, T is proportional to $(1/(1 + e^{-\Phi(\tau,T)/kT})C(\tau,T)/T^2$.

If $e^{-\Phi(\tau,T)/kT}$ is very small, then these relations can be reduced to the kinetic relations used in the traditional Arrhenius model as shown below:

- i. $\partial C(\tau,T)/\partial \tau$ is proportional to $C(\tau,T)$
- ii. $\partial C(\tau,T)/\partial T$ is proportional to $C(\tau,T)/T^2$

That is, the rate of change of cell viability in the traditional Arrhenius model with respect to exposure time τ , while holding temperature T constant, is proportional to itself. The rate of change in cell viability with respect to temperature T , while holding τ constant, is proportional to itself divided by T^2 .

Therefore, it is important to realize that the factor $1/(1 + e^{-\Phi(\tau,T)/kT})$ reflects the key difference between the two-state model and the Arrhenius model, which mathematically enables the two-state model to capture the shoulder effect observed in experimental measurements of cell viability.

6.2 Numerical Sensitivity

One of the advantages of the proposed two-state model is that the prediction of the cell viability corresponds closely with the measured data and effectively captures the shoulder region present in the measured in vitro. Another advantage of this model is its numerical stability. The traditional Arrhenius model describes the cell viability in the double exponential form, which makes its model parameters (E_a and A) highly sensitive to variations in cell viability measurement data [26]. On the other hand, the cell viability function defined by the two-state model is well-behaved numerically, and is a coupled exponential form. In the case of heating with constant temperatures, the cell viability given by the traditional Arrhenius model can be written as

$$C(\tau, T) = e^{-A\tau e^{-E_a/RT}} \quad (24)$$

On the other hand, the cell viability defined by the two-state model can be defined as

$$C(\tau, T) = \frac{e^{-(h/T + \alpha\tau + \beta)}}{1 + e^{-(h/T + \alpha\tau + \beta)}} \quad (25)$$

The double exponential form (see Eq. (24)) creates a stringent requirement for cell viability data in order to obtain reliable Arrhenius parameters. Using the transformation defined by Eq. (18), the parameters ($\bar{\alpha}$, $\bar{\beta}$, and $\bar{\gamma}$) in the two-state model, which are much less sensitive to the measurement data, can be easily generated by least-squares regression following the process described in Table 2.

7 Conclusions

Experimental data demonstrate that the rate of cell viability declines rapidly with increasing temperature and exposure time. In our experimental study, PC3 cells exhibit a slightly greater sensitivity to thermal stress than RWPE-1 cells, as demonstrated by their lower cell viability

following heating. Cells exposed to $T < 54^{\circ}\text{C}$ experience high viability, initially, for a range of exposure times until a threshold thermal dose is achieved that initiates cellular damage and a corresponding decline in cell viability. Therefore, cell viability profiles in this temperature range exhibit a shoulder region where cell viability remains relatively constant followed by a gradual decline in cell viability. For cells exposed to $T > 54^{\circ}\text{C}$, the thermal dose is substantial at short exposure times causing rapid decline in cell viability immediately following thermal stress. Based on our in vitro experiments, it was found that the traditional Arrhenius model does not effectively capture the cellular damage phenomenon over the entire temperature range of $T = 44\text{--}60^{\circ}\text{C}$, particularly in the shoulder region for the lower part of this temperature range. We have developed a two-state cell damage model that is capable of predicting the cellular damage in close correspondence to the measured cell viability over the entire temperature range with a high level of numerical stability. Based on the measured cell viability data for PC3 and RWPE-1 cells, the two-state model provides a more accurate prediction of cellular damage than the traditional Arrhenius model over a temperature range of up to 60°C and can be ultimately used for more effective treatment planning for various thermotherapies.

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Fig. 1. Flow cytometric analysis of cell viability. (a) control (unheated), (b) methanol treated, (c) severely heat-shocked (52°C , 6 min), and (d) typical heated sample (44°C , 15 min).

Fig. 2. Experimental measured cell viability for (a) PC3 cells and (b) RWPE-1 cells under various hyperthermic protocols consisting of temperature ranges of 44–60°C and exposure durations of 1–30 min.

Fig. 3. Illustration of the transformation by introducing a z -variable that converts a curved surface for cell viability C to a flat plane for bilinear regression. (a) Three-dimensional surface plot of cell viability C in terms of $1/T$ and τ . (b) Three-dimensional surface plot of z -variable, which is defined as $\ln[(1-C)/C]$, in terms of $1/T$ and τ .

Fig. 4. Comparison of the two-state model with the traditional Arrhenius model at $T=44\text{--}56^{\circ}\text{C}$ for PC3 cells. The solid line represents the two-state model, the dashed line represents the traditional Arrhenius model, and the boxes with error bars indicate measurement data over three experiments.

Fig. 5.

Comparison of the two-state model with the traditional Arrhenius model at $T=44\text{--}56^{\circ}\text{C}$ for RWPE-1 cells. The solid line represents the two-state model, the dashed line represents the traditional Arrhenius model, and the boxes with error bars indicate measurement data over three experiments.

Table 1

Summary of algorithmic steps for Arrhenius model

Step	Description
(i)	Plot data points $C(T_i, \tau_j)$ versus time for all $i=1, \dots, m, j=1, \dots, n$.
(ii)	Determine time τ_i such that $C(T_i, \tau_i) = 36.8\%$ ($\Omega=1$), $i=1, \dots, m$.
(iii)	Plot data points $(\ln \tau_i, 1/T_i)$ on a 2D graph, $i=1, \dots, m$.
(iv)	Use linear regression to find the slope and $(\ln \tau)$ -intercept.
(v)	The resulting slope is E_a/R and $(\ln \tau)$ -intercept is $\ln A$.

Table 2

Summary of algorithmic steps for two-state model

Step	Description
(i)	Compute $z_\ell = \ln[(1 - C(T_i, \tau_j))/C(T_i, \tau_j)]$, $i=1, \dots, m$; $j=1, \dots, n$; $\ell=1, \dots, m \times n$.
(ii)	Plot data points z_ℓ versus time τ and $1/T$ on a 3D graph.
(iii)	Use bilinear regression to find the coefficients: $\bar{\gamma}$, $\bar{\alpha}$, and $\bar{\beta}$.