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THERMAL STRESS MODELING OF THE FREEZING OF BIOLOGICAL TISSUE

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ABSTRACT

The extent of injury of biological tissues by freezing is influenced by many factors such as the cooling rate, the thawing rate, the minimal temperature achieved, the number of repeated freezing thawing cycles, and the presence of cryoprotectants. The mechanisms of cryo-destruction may generally be separated into two groups; the first is related to the freezing process within the phase transition temperature range (typically between 0 and -22°C), while the second group is related to further destruction after phase transition has completed. Destruction mechanisms of the first group are related to heat transfer, mass transfer, and chemical equilibrium in the intracellular and extracellular solutions. Destruction mechanisms after the phase transition has been completed are related to mechanical stresses in the frozen state. Mechanical stresses develop when changes in density occur nonuniformly in the tissue, a consequence of the presence of temperature gradients. The current presentation gives an up-to-date report on ongoing research to model the freezing of biological tissues and to measure their physical properties. The mechanical boundary condition at the freezing front is emphasized in this presentation, and examples for typical cases of cryosurgery and cryopreservation are discussed.

INTRODUCTION

It is well known that freezing biological tissues can introduce severe damage. Sometimes this damage is intentional and desired, as in cryosurgery. In other situations, such as cryopreservation, this damage is an undesired byproduct. Mechanical stresses that develop during the freezing of biological solutions and tissues have been identified as one cause of tissue damage. In attempting to simulate cryosurgery and cryopreservation, mechanical stress development has been analyzed by a number of researchers (Rubinsky et al., 1980; Rubinsky, 1982; Lin et al., 1990; Gao et al., 1995; Rabin and Steif, 1996). While mechanical stress is one mechanism of tissue destruction in cryobiology applications (Ishiguro and Rubinsky, 1994; Hunt et al., 1994; Gao et al., 1995; Rabin et al., 1996, 1997, 1998), there are other destruction mechanisms related to crystal growth and to mass transfer at the cellular level (Meryman, 1974; Mazur, 1984).

Unfortunately, predictions of mechanical stresses are inconsistent with one important observation of tissue destruction: in cryopreservation applications, severe fractures often form at the early stages of thawing and not as commonly expected during freezing (Pegg, 1996). Comparable observations in the context of cryosurgical applications have also been reported (Rabin and Steif, 1999). This inconsistency has prompted us to re-examine the assumptions underlying the models of freezing tissues presented to date. It is our belief, now, that behavior at the freezing front has not been properly modeled heretofore. Specifically, the deviatoric stress should be zero at an advancing freezing front. Any (volume preserving) strain that occurs while the material is still in the liquid state cannot contribute to the deviatoric stress. Therefore, material which has just solidified at an expanding freezing front, must start with zero deviatoric stress. We note that the zero deviatoric stress condition at the freezing front has been well appreciated by workers in the area of metal solidification and casting (Boley and Weiner, 1963; Zabaras et al., 1991).

Following this new approach for thermal stress modeling of freezing tissues, a typical cryopreservation procedure has recently been analyzed by simulating an inward freezing of a sphere, and a closed-form solution for this model has been derived. (Rabin and Steif, 1998). This analysis included the condition of zero deviatoric stresses at the freezing front; that condition, together with a proper accounting for the hydrostatic pressure that develops in the contained liquid region, resulted in dramatically different stress distributions during freezing than had previously been found.

The outward freezing problem simulative of cryosurgical procedures has also been analyzed, and a closed-form solution for this model has been derived. Results show that the stress distributions during the thawing stage are very different than those during freezing. Furthermore, it is shown that significant stresses remain in the frozen region during thawing, even in the absence of significant temperature gradients. The distributions are qualitatively consistent with limited cracking at the cryoprobe surface during freezing and large scale cracking during thawing, which were observed during validation testing of cryoprobes in water and gelatin solutions (Rabin and Steif 1999).

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MATHEMATICAL MODELING

The underlying assumption is that the temperature field is independent of the solid mechanics problem. Hence the bioheat transfer problem is solved first, the thermal strains are calculated next, and the resulted mechanical stresses are calculated last.

Heat Transfer Problem

Two cases are considered here, the first is typical for cryopreservation and the second is typical for cryosurgery. Heat transfer during cryosurgery is assumed to be governed by the classical bioheat equation:

$$\frac{\partial H}{\partial t} = \nabla(k\nabla T) + \dot{w}_b\left(T - T_b\right) \tag{1}$$

where T is the temperature, H is the volumetric enthalpy, k is thermal conductivity, $\dot{w}_b$ is the production of the blood perfusion and its volumetric specific heat, and T_b is the blood temperature. The initial temperature distribution is assumed uniform and equal to the blood perfusion temperature. In the cryopreservation case, heat transfer is assumed to be governed solely by conduction, where the blood perfusion term of Eq. (1) is set to zero.

The heat transfer problem is solved numerically in both cases for typical thermophysical properties of frozen biological tissues, using the numerical scheme and parameters presented by Rabin and Shitzer (1998).

Solid Mechanics Problem

The key physical quantities in the solid mechanics analysis, the displacements, strains and stresses, are functions of position and time, just as the temperature. We will be particularly careful in defining displacements and strains so as to permit a proper accounting for the stresses which develop in the solid region. Initially, the entire domain is liquid. This state, prior to cooling, is the initial state (at time $t = 0$) from which all displacements and strains are measured. Let a material point, which is initially at the point r, be located at the point r' at time t. Hence, r' is a function of r and of t. The radial displacement, u, is defined as the change of radial position of a material element, that is:

$$u(r,t) = r'(r,t) - r \tag{2}$$

Notice that all points are liquid at time $t = 0$, while a point may be either liquid or solid at time t.

Before defining the relations between stresses and strains (the constitutive relations), one important point needs to be made regarding the strains. Consider a point r which at time t' has become solid. For the purposes of defining the stresses, what is its strain? Say that this element remained liquid up to the time t^*, at which time it solidified. In the time period $0 < t < t^*$, this element has strained, yet as a fluid, this strain can occur without stresses. Or, more precisely, strains involving no volume change (deviatoric strains) occur without stress, while any change in volume will require a pressure change (inversely proportional to the compressibility of the fluid). Only the straining that occur in the period $t^* < t < t'$ is to be included in assessing the stress at t'. Hence, it will be necessary to keep track of the strain at which each element solidifies; only strain occurring thereafter contributes to deviatoric stress (the stress with the pressure subtracted off).

This background indicates the necessity of separating the stress components into two parts, the deviatoric stress S_{ij} and the hydrostatic pressure p, in the standard fashion:

$$\sigma_{ij} = S_{ij} - p\,\delta_{ij} \tag{3}$$

Likewise the strains must be split into deviatoric e_{ij} and hydrostatic e parts according to:

$$\varepsilon_{ij} = e_{ij} + \frac{1}{3} e\,\delta_{ij} \tag{4}$$

Since the strain-rate time the fluid viscosity is negligible compared to elastic stresses, the unfrozen region can sustain hydrostatic pressure only. This pressure has to be essential uniform, otherwise rapid flow would occur.

Consider now the solid region. As pointed out above, it is necessary to keep track of how much straining occurs after solidification. With that idea in hand, though, it is still necessary to choose an appropriate stress-strain relation for the solid. We take the tissue to behave elastically. From the above considerations, we can express the deviatoric stresses in terms of the deviatoric strains as follows:

$$S_{ij} = 2G\left(e_{ij} - e_{ij}^*\right) \tag{5}$$

where G is the shear modulus, and e_{ij}^* is the deviatoric strain of the material element just after solidification; e_{ij}^* is a function of the position r. Notice that the total strain at a point r may change with time as the procedure continues, while the quantity e_{ij}^* has a single value at the point r.

Notice that the material is assumed to be isotropic; consistent with this assumption, the thermal strains have zero deviatoric part (the thermal expansion is equal in all directions). The hydrostatic pressure in the solid is given by:

$$p = -\kappa(e - e_{th}) \tag{6}$$

where κ is the bulk modulus. The thermal strain, e_{th}, is the integral with respect to temperature of the linear thermal expansion coefficient.

Finally, the mechanical equilibrium equation in a quasi-steady state is applied:

$$\nabla \cdot \sigma = 0 \tag{7}$$

For the cryopreservation case, we assume there to be an upper limit to the range of elasticity. Thereafter, an elastic-perfectly plastic model is assumed, in the form of the Mises yield condition:

$$\left|\sigma_r - \sigma_\theta\right| = \sigma_y \tag{8}$$

The limited elasticity, or plasticity effect, is found to have a major role in the stress development in the case of cryopreservation. However, for the cryosurgery case, the solution is developed for a linear elastic material, where the plasticity was found to have a minor effect.

Applying the appropriate boundary conditions, closed-form solutions for both typical cases of cryopreservation and of cryosurgery have been generated based on Eqs. (2)-(8). More detail for the mathematical manipulations is given in Rabin and Steif (1998,1999).

MATERIAL PROPERTIES

There are at least 9 known different pure water ice phases (Fletcher, 1970). Ice I is the most relevant ice phase for the current study; this is the only phase in which water expands upon freezing. Solidification of ice I takes place between pressures of 5 kPa and 207 MPa, while the phase transition temperature decreases monotonically from 0.01°C to -22°C, respectively. At higher pressures water contracts upon freezing and the freezing temperature increases continually with pressure (up to at least 4.4 GPa and 440°C). Due to lack of relevant data for biological materials, the volume strain upon phase transition is assumed constant, and equal to that of water at standard conditions of temperature and pressure, that is, Δe= 0.0907. Other physical properties are assumed to be constant and uniformly distributed in each phase; relevant physical properties values for water, soft frozen biological tissues, and the chosen values for the current study, are listed in Table 1.

The assumption of equal bulk moduli in the liquid and solid phases is largely one of convenience. There is much uncertainty regarding the bulk modulus of ice, although if inferred from measured elastic moduli and a reasonable value of Poisson's ratio, it may be up to four times that of water at standard conditions. Choosing differing bulk moduli substantially complicates the constitutive description as well as the solution, since the strain difference at phase change $(\varepsilon_r-\varepsilon_\theta)^*$ will no longer be constant. Since rather strong qualitative conclusions are arrived at in this paper, we believed it unwise to introduce a second order effect based on rather uncertain physical properties.

Finally, we note that biological tissues are composite materials which may have different physical properties in different orientations. The simplified analysis presented here presumes a homogeneous material only, which can be identified with, say, the average property values. The modeling of a biological tissue as a composite material is beyond the scope of this presentation.

Table 1: Typical properties of polycrystalline ice water and soft frozen biological tissues

	Polycrystalline Ice Water @ 101.3 kPa	Frozen Biological Tissues	Current Study
Poisson's Ratio, v	0.31 - 0.36 (Fletcher, 1970)	-	0.33
Volume Strain of Phase Transition, Δe	0.0907 @ 0°C (Sohnel and Novotny, 1995)	-	0.0907
Thermal Expansion Coefficient: $\beta_1+\beta_2 T$ [1/°C]	$\beta_1=56.3\times10^{-6}$ $\beta_2=2.53\times10^{-7}$ -180<T<0°C (Powell, 1958)	$\beta_1=65\times10^{-6}$ $\beta_2=2.89\times10^{-7}$ -180<T<-20°C (Rabin et al., 1998)	$\beta_1=60\times10^{-6}$ $\beta_2=2.5\times10^{-7}$
Elastic Modulus, E [GPa]	8.9 - 9.9 @-5°C (Fletcher, 1970)	14 - 132 @ -196°C (Rabin et al., 1996)	10

RESULTS AND DISCUSSION

A Cryopreservation Case

Since the solution for the elastic response in the solid depends independently and linearly on the two "driving forces", that is, the thermal expansion in the solid state and the volume expansion due to phase transition, we consider these separately. Case A corresponds to neglecting the volume expansion due to phase transition and accounting for a temperature history which is the consequence of immersion in dry ice, i.e., a step-like temperature function is imposed at the sphere outer surface having a magnitude of -79°C (Fig. 1). Case B, which approximates a typical cryopreservation protocol, corresponds to maintaining the material at nearly the phase transition temperature and allowing the phase transition to occur, presuming the volume strain is that which occurs at standard atmospheric pressure, i.e., Δe = 0.0907 (Fig. 2).

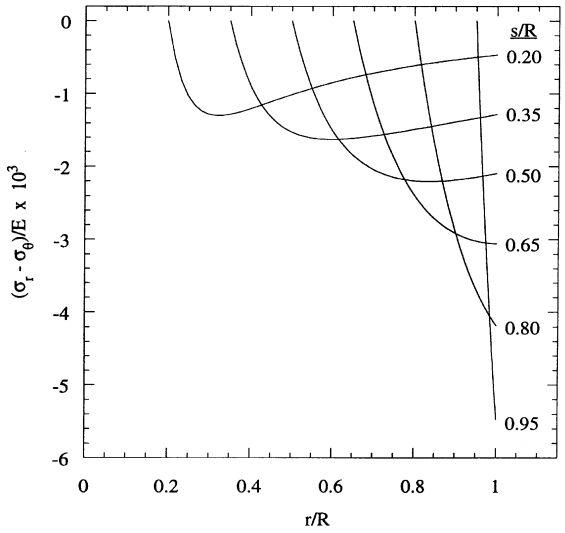

Figure 1: Deviatoric stress distribution for cryopreservation case A, where no phase transition volume changes are included (Δe=0; ΔT=79°C); where s is the freezing front and R is the sphere radius.

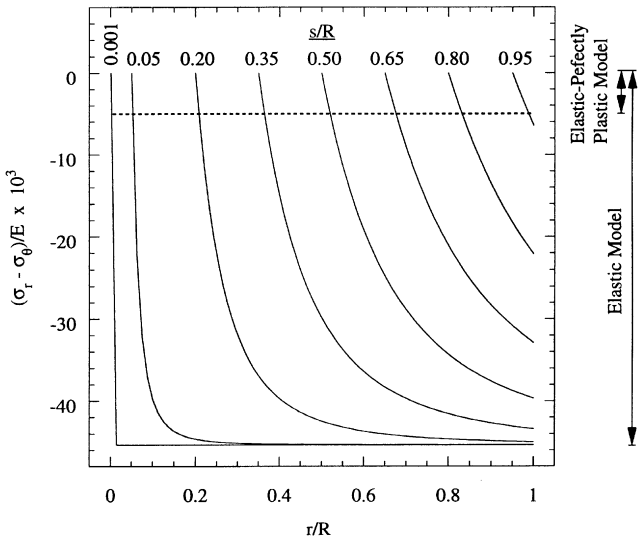

Figure 2: Deviatoric stress distribution for cryopreservation case B (Δe=0.0907; ΔT=0); where s is the freezing front and R is the sphere radius. The dashed line represents the extreme deviatoric stress in the case of an elastic-perfectly plastic model and a yield strain of 0.005.

For physical properties similar to those of water, it can be seen that the effect of volume expansion upon phase transition is much more significant than the effect of thermal expansion in generating stresses, in a typical cryopreservation protocol. Assuming a yield strain of 0.005 (Rabin et al., 1996) it can be seen that a plastic zone is likely to occupy most of the frozen region after the completion of the freezing process. It follows that the attendant potential for tissue destruction are unavoidable regardless of how slowly the freezing is carried out, provided there is a substantial expansion associated with phase transition. It is noted that the phase transition temperature may significantly decrease during the cryopreservation process, due to the elevated hydrostatic pressure in the unfrozen region.

A Cryosurgical Case

Parametric studies were performed for a spherical cryoprobe with radius 2.5 mm for a freezing period of up to 20 min. The cryoprobe was assumed to be stainless steel, having an average thermal expansion coefficient of 2×10^{-5} °C^{-1}. Typical thermophysical properties of the biological tissues were assumed (Rabin and Steif, 1999).

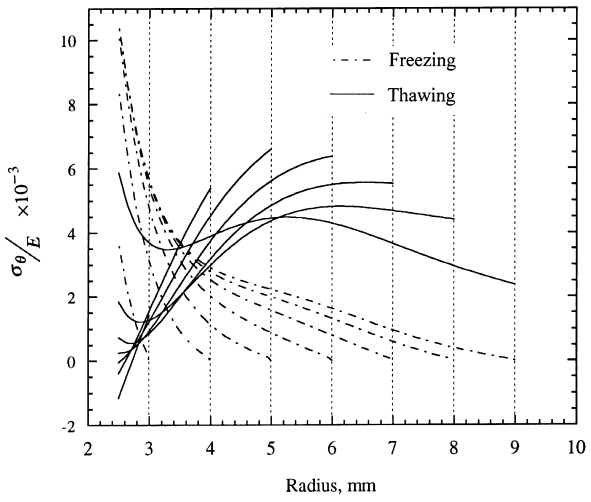

Figure 3: Circumferential strain at various time instants during freezing and thawing of a cryosurgical procedure, where the solidus front advances in 1 mm increment between every two consecutive time instants.

The distributions in the total circumferential stress (normalized by the elasticity modulus) are depicted in Fig. 3 for a number of positions of the solidus front. Similarly, the deviatoric stress components are shown in Fig. 4. The dashed curves correspond to freezing, and the solid curves correspond to thawing. There is a rather dramatic difference between freezing and thawing; the stress σ_θ increases nearly instantaneously from zero to the value of $2\times10^{-3}E$ after thawing commences, when the solidus front has reached 9 mm. These differences can be attributed to the now proper accounting for the strain that contributes to stress in the solid phase. As required by this theory, both stresses are zero at the solidus front during freezing. By contrast, only the radial stress, σ_r, is zero at the solidus front during thawing. The radial stress is always compressive, while the circumferential stress, σ_θ, can be compressive or tensile.

From these distributions one can draw only qualitative conclusions regarding failure, since departures from idealized elastic behavior will eventually occur. For example, the material can deform plastically with micro-cracks appearing (as found from compression testing of frozen tissues by Rabin et al., 1996; 1997); under tensile stresses, one expects macroscopic cracks to form. In either case, the stress distributions will depart from those predicted here once plasticity or cracking occur. With this caution in mind, one can see from the plot of σ_θ that cracks, should they appear during freezing, will do so near the probe. Since the tensile stress drops off rapidly with radius, these cracks should be confined to the probe region. This is observed qualitatively in our experiments. On the other hand, once thawing initiates there is a rapid rise in the tensile stress in the circumferential direction, which quickly becomes maximum near the solidus front. Since the tensile stresses do not drop off rapidly with radius (they are substantial over much of the frozen region, in contrast to freezing), one would not be surprised to find cracks throughout the frozen region; again, this was observed. The radial stress, however, is always compressive but its magnitude increases significantly during thawing. Note that the rapid change in deviatoric stress distribution at the beginning of thawing is associated with extremely high strain rates; the strength of the frozen material is expected to decrease with increase in the strain rate.

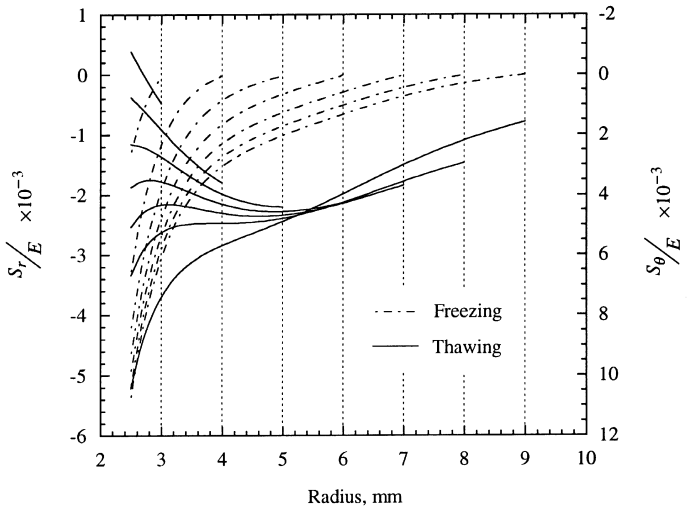

Figure 4: Deviatoric stress components during freezing and thawing at various time instants of a cryosurgical procedure, where the solidus front advances in 1 mm increment between every two consecutive time instants.

SUMMARY AND CONCLUSIONS

A new approach for thermal stress modeling of freezing biological tissues has been presented. The major difference between the new model and the previous ones is that strains involving no volume changes (deviatoric strains) are assumed to occur without stress prior to freezing, while any change in volume requires a pressure change (inversely proportional to the compressibility of the fluid). Hence, it is necessary to keep track of the strain at which each unit volume solidifies; only strain occurring thereafter contributes to deviatoric stress (the stress with the pressure subtracted off).

For physical properties similar to those of water and a typical cryopreservation protocol, parametric studies have shown that the effect of volume expansion upon phase transition is much more significant than the effect of thermal expansion in generating stresses. It has been shown that a plastic zone is likely to occupy most of the frozen region after the completion of the freezing process. It follows that the attendant potential for tissue destruction are unavoidable regardless of how slowly the freezing is carried out, provided there is a substantial expansion associated with phase transition and no opportunity for stresses to release.

A parametric study has been performed for typical parameters and conditions of cryosurgery around a spherical cryoprobe. Results have shown that the stress distributions during the thawing stage are very different than those during freezing. Furthermore, it is shown that significant stresses remain in the frozen region during thawing. The distributions are qualitatively consistent with limited cracking at the cryoprobe surface during freezing and large scale cracking during thawing.

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