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Thermal problems in biomechanics – a review. Part III. Cryosurgery, cryopreservation and cryotherapy

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The aim of this paper is to review available results pertaining to various heat transfer problems of biomechanics. The present part is concerned with low-temperature phenomena, namely the issues of cryosurgery, cryopreservation and cryotherapy. The description of damage mechanisms is also discussed along with its possible applications.

Key words: heat transfer phenomena, cryosurgery, cryopreservation, cryotherapy, damage mechanics

1. Introduction

In the two previous parts of the present paper, various conditions leading to temperature rise in the tissue were considered [38], [39]. The problems of cryotherapy, cryosurgery and cryopreservation (Greek *kryos* – cold, freezing) arise from substantial temperature drop within the tissue.

In order to gain a deeper insight into the low-temperature biomechanics, in the present paper we propose to distinguish three classes of problems:

- **Cryosurgery** – low temperature (significantly below freezing point) is introduced to induce irreversible damage to undesirable tissue. The cooling device (cryoprobe) is usually brought into direct contact with the target region and causes localized freezing and destruction. This technique is used mainly in restricting tumor growth. Damage caused by the treatment should be restricted to the target region and maximal within that region.

- **Cryotherapy –** it is the act of controlled exposure of living tissue to low temperature, but nevertheless well above the freezing point. The purpose of the

cryotherapy is to induce physiological reactions and consequently to reduce pain, swelling etc. Some researchers do not distinguish between cryotherapy and cryosurgery and use both terms to describe the act of freezing and destruction of undesired tissues [4]. We propose that the distinction should be preserved because both the mathematical modelling and the clinical application of the cryotherapy and cryosurgery are different. No tissue damage due to low temperature is desired during the cryotherapy protocol.

• **Cryopreservation** – biological tissues (an organ prepared for transplantation, bone allograft, food products, specimen to be used in an experiment) are exposed to low temperature in order to preserve their properties. The requirements that the cryopreservation protocol must meet are quite different, depending on the subject of the treatment. In case of an organ, one requires that the cryopreservation does not change its physiological capabilities and that it can resume its functions after transplantation. In the case of specimens undergoing further mechanical tests, one wishes that the mechanical properties of the specimen remain unchanged after cryopreservation. Quite different requirements are needed for the storage of food products. Again, no tissue damage is desired in the case of cryopreservation.

The use of low temperature in medical treatments was invented long time ago. In 3500 B.C., Egyptians used ice as local anesthetic by applying it to wounds. The introduction of more modern techniques took place in 1851. Dr. James Arnott, a British physician, used a container filled with –24 °C ice-brine mixture, bringing it to a direct contact with skin cancers [6]. This proved to be helpful in restricting the tumor growth.

In 1961, the first cryoprobe was invented and used for the treatment of the Parkinson's disease. Since then, the cryosurgery became an established medical procedure [6]. During the treatment the cryoprobe is brought in direct contact with the diseased tissue. The design of the cryoprobe, duration of the treatment and temperature are dependent on the specific protocol [6].

Temperature drop is accompanied by the heat transfer, phase transition (freezing of the intracellular and extracellular water) and other phenomena often leading to tissue damage. The damage is the desired only in the case of cryosurgical treatment and, even then, its extent needs to be controlled.

A topic of fundamental importance in bone mechanics is the following one: how accurately the mechanical properties of bone, measured in some postmortem, *ex vivo* state, represent bone tissue as it exists in the living body? The central questions here are: 1. How do the mechanical properties of bone tissue change when its cells die and/or it is removed from the body? 2. How do various means of preserving bone tissue against postmortem changes alter its *in vivo* mechanical properties? These questions manifest themselves in two broad areas of orthopaedic research. First, they are of obvious concern to those experimentalists who wish to understand structure–function relationships in bone and others, who wish to define the changes in mechanical properties caused by the genetic variability, aging, disease, medical

treatments and so forth. Second, they are of interest to the surgeon in the context of bone allografts. The storing of bone for these purposes raises questions analogous to these encountered by the experimentalists.

In addition, the treatment of allografts to prevent immune responses or the transmission of infections may also affect mechanical properties. MARTIN and SHARKEY [18] reviewed the relevant literature concerning these interrelated and important questions. Particularly, the mechanical effects of preserving bone have been discussed. The available literature covers the range of temperature from –20 °C down to –196 °C and the mechanical tests such as compression, bending, pin pullout, torsion, indentation etc.

Freeze-drying, also known as lyophilization, is accomplished by deep-freezing the bone (e.g. –80 °C), introducing a high vacuum and gradually raising the temperature at the frozen water sublimates but still keeping the temperature well below the freezing. The end point of the process is usually defined as occurring when the residual water content of the specimen is less than 5%. The freeze-dried bone may be stored for 4 to 5 years at room temperature as long as it is sealed against the moisture. MARTIN and SHARKEY [18] have also summarized the effects of various treatments relevant to allograft bone on mechanical properties. For instance, several studies have shown that freeze-drying has a more detrimental effect on the mechanical behaviour of cortical bone than does freezing; the effects of freeze-drying on trabecular bone appear to be less severe. BALDERSON et al. have found that freeze-drying reduces the Young modulus by an average of eight percent, while other mechanical properties were not negatively affected [1].

Another field, where one needs to consider the freezing of tissues, are cryotechniques of fixation used during the mechanical testing of soft tissues. Many conventional techniques are available but they have shortcomings. Most important drawback is that they introduce nonuniformities in the loading pattern and uneven fibre recruitment under tension. In cryofixation, one can distinguish two approaches: *cryoclamps* – where the specimen is frozen prior to mechanical fixation, and *cryofixation* – where the ends of the sample are frozen into ice blocks thus avoiding direct mechanical contact with the testing machine. The development of such a cryofixation device is described in [31].

2. Temperature distribution

The mathematical prototype of the phase-change and free boundary problems is the well known Stefan problem introduced by STEFAN in [40]. There are hundreds of papers and books on this problem, see e.g. [5], [44]. The reader is advised to consult the book by FRÉMOND [10] who presented a unified thermomechanical approach to many free surface problems, including the Stefan problem. Here we give only a brief introduction and present a one-phase Stefan problem after RODRIGUES [32]. The entire

solid region is assumed to remain at the phase-change temperature, i.e. no temperature gradients exist in the solid phase. The phase change takes place at the temperature T_0. To simplify the notation we define $\Theta = T - T_0$.

In the usual energy conservation equation:

$$\rho \frac{\partial e}{\partial t} = \lambda \nabla^2 \Theta , \tag{1}$$

where λ is the thermal conductivity, the internal energy e is given by

$$e = cT + LH(\Theta) . \tag{2}$$

Here c stands specific heat, L is the latent heat of phase change and H denotes the Heaviside function defined by:

$$H(x) = \begin{cases} 1 & \text{if } x > 0, \\ 0 & \text{if } x \le 0. \end{cases}$$

Combining equations (1), (2) one obtains (assuming that c and L are constants)

$$\rho c \frac{\partial \Theta}{\partial t} + \rho L \frac{\partial H(\Theta)}{\partial t} = \lambda \nabla^2 \Theta \tag{3}$$

Apparently the last term on the left-hand side of this equation is singular whenever solidification or melting takes place. The Baiocchi–Duvaut transformation given by:

$$u = \int_0^t \Theta(\mathbf{x}, \tau) d\tau$$

yields a new variable u, called *the freezing index*, cf. also [10]. Integration of equation (3) yields

$$\rho c \left(\frac{\partial u}{\partial t} - \Theta(\mathbf{x},0) \right) + \rho L(H(\Theta(\mathbf{x},t)) - H(\Theta(\mathbf{x},0))) = \lambda \nabla^2 u(\mathbf{x},t) . \tag{4}$$

We now set

$$f(\mathbf{x}) = \rho c \Theta(\mathbf{x},0) - \rho L(1 - H(\Theta(\mathbf{x},t))) ,$$

so we can rewrite equation (4) as follows

$$\rho c \frac{\partial u}{\partial t} = \lambda \nabla^2 u(\mathbf{x},t) + f(\mathbf{x}) + \rho L(1 - H(\Theta(\mathbf{x},t))) . \tag{5}$$

We observe that in the one-phase Stefan problem, the solid phase is always at the melting temperature, therefore

$$u(\mathbf{x},t)(1-H(\Theta(\mathbf{x},t))=0\,.$$

So one obtains

$$u\geq 0,\quad \rho c\frac{\partial u}{\partial t}-\nabla\cdot(\lambda\nabla u)-f\geq 0\quad\text{and}\quad u\left(\rho c\frac{\partial u}{\partial t}-\nabla\cdot(\lambda\nabla u)\right)=0\,.$$

This form is suitable for development of the variational inequality.

The Stefan problem presented above is somewhat idealised. To obtain realistic solutions (temperature distribution, heat flow rates etc.) during the cryosurgery or cryopreservation protocol the tissue has to be treated as a nonideal material, whose properties are temperature-dependent and phase transition occurs over a temperature range [2], [9].

Realistic formulation of tissue freezing, incorporating "mushy region", was presented by DEVIREDDY et al. [8]. Instead of defining latent heat release in terms of the Heaviside function as in equation (2) they defined the energy in the following manner

$$e=cT+L\Lambda\,, \tag{6}$$

where Λ is the mass fraction of the tissue that has not yet released latent heat during freezing. It can be further subdivided into intra- and extracellular components

$$\Lambda=f_{ec}\Lambda_{ec}+f_{ic}\Lambda_{ic}\,,$$

where f_{ec} and f_{ic} are the mass of extracellular and intracellular water, respectively, divided by the mass of the tissue. Obviously Λ_{ec} and Λ_{ic} are unfrozen extracellular and intracellular water mass fractions.

Since the tissue is not composed entirely of water, $f_{ec}+f_{ic}<1$. DEVIREDDY et al. [8] defined unfrozen water fractions as:

$$\Lambda_{ec}=\begin{cases}1 & \text{if } T\geq 272.62\ \text{K},\\ \dfrac{0.53}{273.15-T} & \text{if } T<272.62\ \text{K}.\end{cases}$$

This formula corresponds to the behaviour of the NaCl solution, see [8] and references therein.

For the intracellular latent heat fraction the following formula is proposed [8]

$$\Lambda_{ic}(V(T,t),P(T,t))=\int_{V_0}^{V}(1-P)\frac{dV}{V_0-V_b}-\int_0^P\frac{V-V_b}{V_0-V_b}dP\,. \tag{7}$$

Here V_0 is the initial cell volume (before freezing begins), V_b is the osmotically inactive volume of the cell and V represents the momentary volume, P is the probability of intracellular ice formation. The first term on the R.H.S of the equation (7) corresponds to intracellular water that is transported out of the cell and freezes

outside. The second one represents contribution of the water that freezes inside the cell.

The model needs to be supplemented by the constitutive equations for cell volume V and probability of intracellular ice formation P. The first of these laws will be provided later on in equations (17), (18). For P the following expression has been proposed by TONER et al. [42]

$$P(T,t) = 1 - \exp\left[- \int_{T_{\text{seed}}}^{T} A\Omega_0 \sqrt{\frac{T}{T_{f0}}} \frac{\eta_0}{\eta} \frac{A}{A_0} \exp\left[\frac{-\kappa_0 \left(\dfrac{T_f}{T_{f0}} \right)^{0,25}}{\left(T - T_f \right)^2 T^3} \right] dt \right], \tag{8}$$

where T_{seed} is the temperature at which ice is seeded in the extracellular space, T_f is the temperature of equilibrium phase change of the intracellular water, η is the intracellular solution viscosity and κ and Ω are the model constants. The subscript 0 denotes the value of the parameter under isotonic conditions. For the numerical values of the parameters the reader is referred to [8] and [42].

The effects of blood perfusion and metabolic heat generation in unfrozen regions sometimes have to be accounted for (see part I of the present paper for appropriate formulations). In the table, some selected material properties of tissues at low temperature are presented along with the water properties, after [24].

Table. Thermophysical properties related to freezing of typical soft tissue, angioma and water, after [24]

Thermophysical properties	Soft tissue	Angioma	Water
Upper limit of phase transition	(–1) –0 °C	(–1) –0 °C	0 °C
Lower limit of phase transition	(–22)–(–8) °C	(–22)–(–8) °C	–
Thermal conductivity in unfrozen state [W/(m·K)]	0.5	0.56	0.6
Thermal conductivity in frozen state [W/(m·K)]	2	2.22	2.25
Specific heat in unfrozen state [(M·J)/(m^3·K)]	3.6	3.89	4.19
Specific heat in frozen state [(M·J)/(m^3·K)]	1.8	2.01	1.13
Latent heat [(M·J)/m^3]	250	250	331.7

The values of lower limit of phase transition given in the table refer to quasi-static freezing/thawing process. RABIN et al. [30] reported that they may be as low as –45 °C in non-equilibrium conditions and when sources of nucleation are absent.

The volumetric specific heat of soft tissue in the temperature range between lower and upper limits of phase transition is usually modelled in terms of the effective

property. Experimental data suggests piecewise linear approximation with two linear functions starting with intrinsic property values at phase change region boundaries, intersecting at the "peak temperature" (–3 °C for the phase transition in the range from –8 to –1 °C). The slopes of these functions are chosen in such a manner that the integral of the function representing the effective specific heat over the phase transition temperature range equals transition enthalpy changes, see [24]. The following theoretical justification for this assumption can be provided, see [17], [20].

Let us introduce the heat equation with an additional freezing/thawing term:

$$c(T)\frac{\partial T}{\partial \tau} = \nabla\left(\boldsymbol{\lambda}(T)\nabla T\right) + L\frac{\partial \Lambda}{\partial \tau}. \tag{9}$$

Assuming that Λ is a function of temperature in phase transition temperature range one may write:

$$L\frac{\partial \Lambda}{\partial \tau} = L\frac{\partial \Lambda}{\partial T}\frac{\partial T}{\partial \tau}.$$

Then equation (9) can be reduced to the standard Fourier–Kirchhoff equation by introducing the effective specific heat c_{eff}:

$$c_{\text{eff}}(T) = c(T) - L\frac{\partial \Lambda}{\partial T}. \tag{10}$$

For the practical reasons the function $\Lambda(T)$ is often assumed linear. However, this is only an approximation. In figure 1, examples of this function for three different water solutions of NaCl are given after [9].

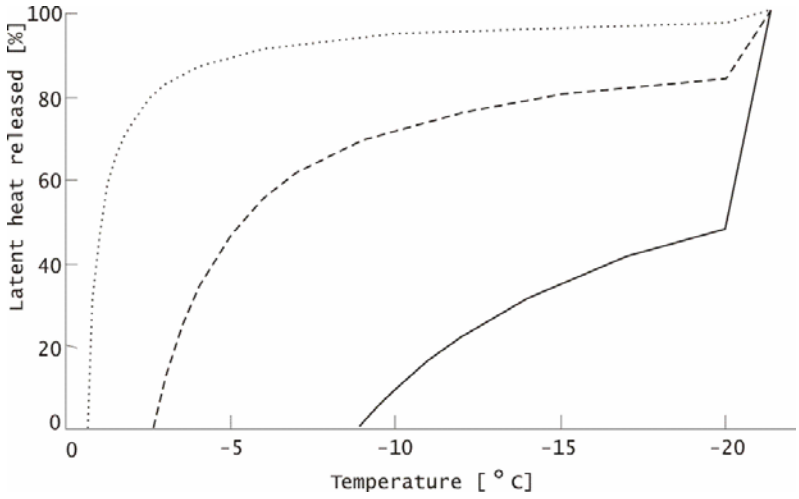

Fig. 1. Release of the latent heat as function of temperature during the equilibrium solidification of three different water solutions of NaCl, after [9]

The solution of practical problem of damage distribution in tissue requires that the appropriate heat transfer problem is solved in advance. Cryogenic damage criteria are formulated in terms of temperatures and cooling rates so these need to be obtained. The appropriate mathematical formulation should incorporate:

- moving phase boundary (freezing front),
- blood perfusion in the unfrozen tissue, usually modelled by the Pennes equation,
- metabolic heat generation in the unfrozen tissue.

The assumption that the specific heat of both frozen and unfrozen phases is negligible, when compared to the latent heat of fusion, is sometimes used.

The rates of blood perfusion and metabolic heat generation are usually assumed to remain constant throughout the freezing period or to decay linearly to zero at the freezing point. The former hypothesis is supported by the experimental investigations, cf. [34] and the references therein. Because of relatively high cooling rates applied during cryosurgery the blood vessels have no time to contract and flow continues at the same rate until blood freezes.

RUBINSKY and SHITZER [34], who investigated one-dimensional freezing problem, indicate that the effect of metabolic heat generation is small and may be neglected. They report, however, that the blood perfusion effects are significant and affect the freezing front velocity and the tissue temperature distribution.

On the other hand, HOFFMANN and BISCHOF [12], who considered, both experimentally and theoretically, the freezing in the cylindrical geometry of Skin Flap Chamber setup, report that neglecting the blood flow completely did not affect the solution significantly even when compared to results obtained for highly vascularized tumor tissue.

Another important property of the freezing tissue is the fact that living tissue – being an inhomogeneous solid, freezes over a temperature range and thus two moving fronts should be modelled rather than one. The Kirchhoff transformation method [12], [43] is particularly suitable for such problems. Let us pass onto its brief presentation.

The heat transfer problem is first formulated (here the Pennes bioheat equation):

$$\rho c \frac{\partial T}{\partial \tau} = \nabla \cdot (\boldsymbol{\lambda} \nabla T) + w_{bl} c_{bl} (T_a - T) + q_v \,, \tag{11}$$

where: c_{bl} is the blood perfusion rate, T_a is the blood arterial temperature and q_v is the metabolic heat generation rate. In the region of interest, one can distinguish frozen (solid) tissue, unfrozen tissue and the "mushy zone". The temperature of the frozen tissue lies below the lower limit of phase transition, the unfrozen tissue has the temperature that is above the upper limit of the phase transition and the mushy zone is distinguished by the temperature taking value that is between those limits. In the frozen and mushy zones, it is assumed that $w_{bl} = 0$ and $q_v = 0$.

The following variable transformation is introduced (Kirchhoff transformation):

$$\boldsymbol{\Psi} = \begin{cases} \int_{T_0}^{T} \boldsymbol{\lambda}_f(\phi)d\phi & \text{if } T < T_{m1}, \\ \int_{T_0}^{T_{m1}} \boldsymbol{\lambda}_f(\phi)d\phi + \int_{T_{m1}}^{T} \left[\boldsymbol{\lambda}_f(\phi) - (\boldsymbol{\lambda}_f(\phi) - \boldsymbol{\lambda}_u)\left(\dfrac{\phi - T_{m1}}{T_{m2} - T_{m1}} \right) \right] d\phi & \text{if } T_{m1} \le T \le T_{m2}, \\ \int_{T_0}^{T_{m1}} \boldsymbol{\lambda}_f(\phi)d\phi + \int_{T_{m1}}^{T_{m2}} \left[\boldsymbol{\lambda}_f(\phi) - (\boldsymbol{\lambda}_f(\phi) - \boldsymbol{\lambda}_u)\left(\dfrac{\phi - T_{m1}}{T_{m2} - T_{m1}} \right) \right] d\phi + \int_{Y_{m2}}^{T} \boldsymbol{\lambda}_u d\phi & \text{if } T > T_{m2}, \end{cases} \quad (12)$$

where T_{m2} and T_{m1} denote the onset and end of freezing, respectively, and T_0 is the reference temperature, $T_0 << T_{m1}$. The subscripts f and u denote frozen and unfrozen tissues, respectively. The Kirchhoff transformed temperature $\boldsymbol{\Psi}$ is a continuous function of the temperature T. The above definition is equivalent to

$$\boldsymbol{\Psi} = \int_{T_0}^{T} \boldsymbol{\lambda}(\phi)d\phi \,,$$

provided that the frozen phase accumulates linearly with dropping temperature and that the conductivity in the mushy zone is expressed by:

$$\boldsymbol{\lambda}(\phi) = f(\phi)\boldsymbol{\lambda}_f(\phi) + (1 - f(\phi))\boldsymbol{\lambda}_u \,.$$

The enthalpy of the tissue is defined as follows:

$$H = \begin{cases} \int_{T_{m1}}^{T} c_f(\phi)d\phi & \text{if } T < T_{m1}, \\ \dfrac{T - T_{m1}}{T_{m2} - T_{m1}} Q & \text{if } T_{m1} \le T \le T_{m2}, \\ Q + \int_{T_{m2}}^{T} c_u(\phi)d\phi & \text{if } T > T_{m2}, \end{cases} \quad (13)$$

where Q is the enthalpy change across the mushy zone which includes both the effects of the latent and the sensible heat and is defined by:

$$Q = L + \int_{T_{m1}}^{T_{m2}} \left(c_f(\phi) - \left(\frac{\phi - T_{m1}}{T_{m2} - T_{m1}} \right) \left(c_f(\phi) - c_u(\phi) \right) \right) d\phi \,. \quad (14)$$

Here L is the latent heat of fusion for the tissue.

Using equations (12)–(14) one can express equation (11) in the form:

$$\rho \frac{\partial H}{\partial \tau} = \nabla \cdot (\nabla \Psi) + w_{bl} c_{bl} (T_a - T) + q_v \quad (15)$$

in the isotropic case (where Ψ is a scalar) and in the form

$$\rho \frac{\partial H}{\partial \tau} = \nabla \cdot (\mathrm{div} \boldsymbol{\Psi}) + w_{bl} c_{bl} \left(T - T_a \right) + q_v , \tag{16}$$

where

$$\nabla \cdot \mathrm{div} \boldsymbol{\Psi} = \Psi_{ij,ji}$$

in the general case (where $\boldsymbol{\Psi}$ is a second-rank tensor).

This formulation is a good starting point for the development of numerical schemes, cf. [12].

The formulation, using the enthalpy defined by equation (13), is also the basis for the so-called Alternating Phase Truncation Method devised by ROGERS et al. [33]. An application of this method to the cryosurgery problem is presented in [17].

When effects of the blood perfusion are neglected the problem is modelled by the usual Fourier–Kirchhoff equation. Its analytical solutions for various geometrical and boundary conditions can be found in [5], [9].

3. Cellular damage mechanisms

In both the field of cryosurgery and cryopreservation, the problem of tissue damage caused by freezing and exposure to low temperature is of paramount importance. In the cryosurgery, the damage is the desired effect of the treatment (destruction of the undesirable tissues, for instance during treatment of cancer) and the prediction of affected area is vitally important for the development of successful surgical procedures. In cryopreservation, the damage to the biological tissue is an altogether undesired effect and one tries to minimize it.

One can distinguish two groups of basic mechanisms of cellular damage occurring at low temperatures: those occurring in phase transition temperature range and those taking place after solidification, cf. [23], [25], [27], [29], [30], [34]. More complex mechanisms such as the post-thaw vascular injury characterized by stasis, thrombosis and increased vascular permeability (macromolecular leakage) can be viewed merely as the consequences of the basic mechanisms described below. They, however, contribute to the entire injury response and prolong the period needed for healing [13].

3.1. Damage mechanisms occurring during freezing

The first group includes destructive processes taking place in phase transition temperature range and related to the dynamics of the freezing/thawing process. Usually accepted view relates cellular damage to the rate of freezing when the temperature drops below upper freezing limit, see [4], [7], [9], [22], [34], [37], [41] and the references therein. The ice nucleation is the most intensive in the larger extracellular spaces of single cell systems or the larger vascular/extracellular space of

the whole tissues, so that ice forms first in the extracellular fluid, while cells remain unfrozen. As ice forms extracellularly the concentration of the solutes in extracellular space increases and concentration difference between cell interior and exterior develops. To equilibrate the chemical potential between the supercooled interior of the cells and the partially frozen exterior, the water leaves cells through the partially permeable cell membranes and freezes outside. This process increases the intracellular osmolality and causes chemical damage to the cells due to dehydration. The experimental data (see, e.g., [22]) prove that the amount of intracellular ice being formed in cells ranges from almost none (for cooling rates < 5 K/min), to over 88 per cent of the original cellular water (for cooling at the rate > 50 K/min). This range of values is typical of the regions from the middle to the periphery of the cryosurgical iceball. Therefore, for accurate modelling and simulation of the damage processes occurring during freezing, the problem of water transport between the intracellular and extracellular/vascular space of the tissue is of vital importance. In order to predict the dehydration experienced by embedded tissue cells due to the extracellular ice formation, microcompartment models were developed, see [22] and the references cited therein. The change in the single cell volume caused by water loss can be modelled by the Krogh cylinder tissue model [7], [8], [22]:

$$\frac{\partial V}{\partial T} = -\frac{L_p A_c RT}{G v_w}\left(\ln\frac{V - V_b}{V - V_b + \varphi_s n_s v_w} - \frac{\Delta H_f}{R}\left(\frac{1}{T_R} - \frac{1}{T}\right)\right), \tag{17}$$

where: V is the cell volume, L_p is the permeability of the plasma membrane to water, A_c is the original effective membrane surface area for the water transport, R is the universal gas constant (8.314 J/mole K), G is the cooling rate, v_w is the partial molar volume of water, V_b is the osmotically inactive cell volume, n_s is the total number of moles of solutes in the cell, $\varphi_s = 2$ is the dissociation constant for NaCl in water and ΔH_f is the latent heat of fusion for water.

The permeability of cell membrane is temperature-dependent and can be modelled via the exponential law, cf. [7], [8], [42]:

$$L_p = L_{pg} \exp\left(-\frac{E_{Lp}}{R}\left(\frac{1}{T} - \frac{1}{T_R}\right)\right), \tag{18}$$

where: E_{Lp} is the activation energy, T_R denotes the reference temperature and L_{pg} is the permeability of cell membrane at that temperature. The coefficients E_{Lp} and L_{pg} are identified experimentally by means of the cryomicroscopy or Differential Scanning Calorimetry (DSC) measurements. For the detailed description of the DSC experiments the reader is referred to [7].

Krogh model was originally developed for single cell freezing but – after some modifications – it can be applied to tissue freezing (see [7] and [22] dealing with freezing of rat liver tissue). It can also be adapted to account for the presence of added *cryoprotectant* (cryoprotective agent – CPA) such as dimethylsulfoxide (DMSO), see

[37]. Such cryoprotectants can substantially decrease tissue damage due to the dehydration. MAZUR [19] reports that they appear to protect rather against the dehydration than against the intracellular ice formation. On the other hand, TONER et al. [42] argue that the CPA's modify the cell membrane slowing down the process of surface-catalysed intracellular ice formation. The hypotheses concerning the mechanisms of action of such protective additives are discussed in detail in [19].

With increased cooling rate more ice starts to form intracellularly and the damage due to the dehydration is reduced as the water transport is reported to cease at ca. –10 °C [22], [37]. This "shut-off" temperature varies slightly with cooling rate (it is lower for higher cooling rates) and depends on the presence of CPA. As experimental data indicate increasing DMSO concentration causes significant drop in the "shut-off" temperature (–15 °C for the cooling rate of 5 K/sec. and –25 °C for the cooling rate of 50 K/sec. at DMSO concentration of 2M), cf. [37]. This effect is offset by the fact that the initial dehydration rate is much lower in the presence of CPA and the final cell volume is greater.

When cooling rate is high enough the intracellular ice formation starts to have mechanical effect on the tissue – the second process resulting in damage during freezing. The volume of intracellular ice that the cell can withstand without damage depends on the cell type. Cracks are often reported during rapid freezing (such as using liquid nitrogen) unless the size of the sample is too small (individual cells). It is postulated that the direct cause of the damage is the excessive stress resulting from volumetric expansion of water contained in the tissue, cf. [27], [36]. Similar phenomena occur in inorganic saturated porous media [10].

TONER et al. who has studied the kinetics of intracellular ice formation (IIF) suggests that IIF is triggered by two kind of effects: the surface effects of the cellular membrane and the volume effects [42]. The influence of the extracellular ice formation on the membrane provides the centres of nucleation of intracellular ice triggering surface catalysed nucleation (SCN). The nucleators already present in some kinds of cells provide means of volume-catalysed nucleation (VCN) [42]. The mechanisms of SCN are active in the presence of the extracellular ice and become less pronounced with dropping temperature. The VCN becomes more important at very low temperatures (of the order of –40 °C).

There are multiple effects of the extracellular ice on the cell membrane resulting in SCN, i.e. chemical (desorption of the membrane components), electrical (membrane breakdown), mechanical (adhesion, squeezing) and thermal (change of viscoelastic properties, lipid transitions) [42].

TONER et al. suggest that the action of the CPA has a substantial effect on the membrane processes only rendering them ineffective. In such a case, the IIF relies more on the volume effects which are less effective in high temperature range. For more detailed description the reader is referred to [42].

RABIN and STEIF [25] have shown that plastic deformation induced by thermal dilatation during freezing always starts at the cryoprobe surface; it may also form at the

freezing front position in the case of high cooling rates. However, in their model, freezing occurs at a single temperature. Moreover, the volumetric transition caused by water-to-ice transition is omitted though these phenomena could have a dominant effect.

When whole tissues are frozen it is argued that vascular expansion, due to the cell dehydration, is another destructive mechanism, cf. [22].

The competition of the dehydration and intracellular ice formation in a tissue during freezing determines the final volume of the cell and its survival chances. The least damage is reported for some intermediate cooling rates when the changes in cellular volume are minimal. Exact value of this "intermediate rate" depends on the type of the cell, its volume to surface area ratio and permeability to water. It should be lower for larger, spherical cells and for those less permeable, cf. [19]. In figure 2, the survival ratio is given for various types of cells as a function of the cooling rate, see [19]. The maximum survival peak is clearly visible in all presented cases.

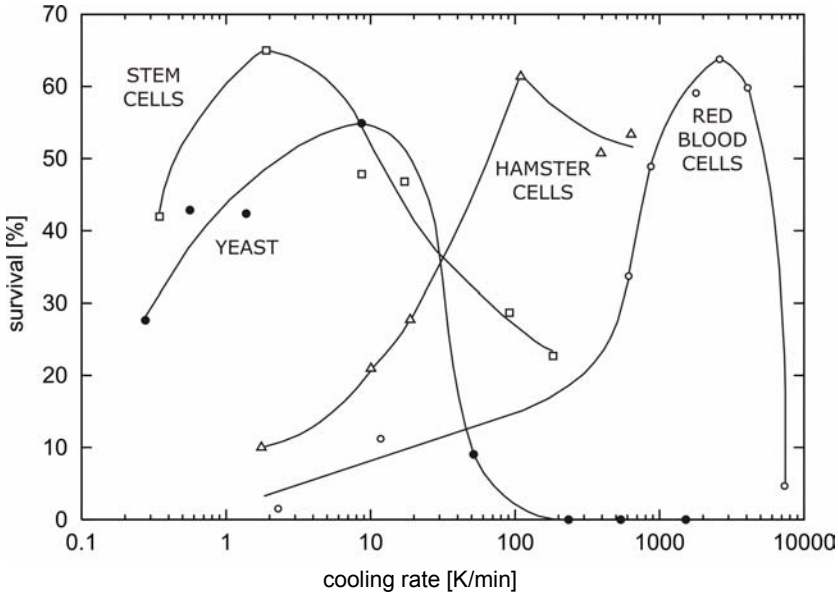

Fig. 2. Relationship between cell survival ratio and cooling rate for different kinds of cells, alter [9]

In real situation, the cryosurgery usually involves a metal-tipped cryoprobe introduced into tissue. As the freezing front moves away from the probe the cooling rate changes from very fast (in the immediate vicinity of the probe) to very slow (at the periphery of the iceball). Taking into account all the above considerations of damage mechanisms, it should be concluded that the damage distribution after cryosurgical treatment can be quite complex.

Apart from the two main mechanisms of damage (cell dehydration and intracellular ice formation) other effects are also observed [4]. These are the denaturation of

membrane lipid–protein complexes, damage to small blood vessels, microcirculatory arrest and ischaemia [4]. These effects contribute to the overall damage so that the portion of the tumor cells excised immediately after cryosurgery can remain viable and be grown *in vitro*, while the complete cellular death occurs if the tumor is left in the host [4]. BROWN et al. [4] measured blood flow during and after cryosurgery treatment of the rat cremaster muscle. They reported that in 15 minutes following the treatment the blood flow ceased in all vessels with some oscillatory movement in large arterioles and venules. Half an hour after the treatment the flow was restored in large venules. Fifteen minutes later the flow was observed in large arterioles and venules but there was no flow or only small flow in smaller vessels. Another fifteen minutes later the flow in all the vessels ceased and did not return for at least one hour [4]. Similar studies on liver tumor indicate that the final period of complete vascular stasis may last eight hours [3]. Little is known about the mechanisms that control the blood flow during cryotherapy.

3.2. Damage mechanisms in solid state

The second group comprises destructive mechanisms occurring after the phase transition is complete, i.e. in the solid state. Damage is caused by mechanical stress at this stage. It was shown that the stress resulting from a constrained contraction of the frozen tissues (called *thermal stress*) can easily reach the yield strength of the frozen tissue resulting in plastic deformation or fracture, cf. [27], [30] and the references therein. The contraction is driven by temperature gradients developed during cooling.

The free water in cells forms ice crystals during freezing so frozen tissue is often expected to have the characteristics similar to pure ice during thermal contraction. The uniaxial compression experiments performed by RABIN et al. [29] indicate that although the elastic modulus of frozen rabbit liver, kidney and brain is within the range of values reported for sea ice, the maximum strength appears to be one order of magnitude higher than the yield strength of the ice. These large discrepancies may be explained by the contribution of biological fibers to the frozen solid, making it a composite material [29]. It should also be noted that the maximum strength of the frozen tissue is usually different from the yield strength in uniaxial compression so the above comparison can be misleading.

SHI et al. [35] modelled the gradual freezing of the tissue (freezing over the temperature range and the thermal expansion during water–ice phase transition). Their research covered also experiments with potato specimens. The authors concluded that most of the ice formation and fracture occurs in the temperature range from 0 to –20 °C, while the most severe fractures occur at high (200 K/sec) rates of cooling. Faster cooling at lower temperature seemed to have little influence on tissue damage. The step-freezing cryoprotection protocols with an initial low cooling rate are therefore reported to be superior to constant-freezing-rate protocols.

Thermally-induced damage analysis performed in [23] revealed that the highly inhomogeneous structure of the frozen tissue encourages crack formation during loading. It also plays a quite different role. The cracks that would propagate undisturbed in the homogeneous material are frequently halted or diverted. The highly heterogeneous structure of frozen tissue allows substantial degree of cracking before final failure takes place.

When cryoprotectants (e.g. dimethylsulphoxide or glycerol) are used they tend to vitrify the intracellular fluid. We recall that vitrification is an amorphous solidification of liquid caused by enormous increase of viscosity, cf. [19], [30]. Below the temperature of glass transition the solution becomes more brittle and the coefficient of its thermal expansion drops significantly (see figure 4). In attempts to cryopreserve bulky biological systems (e.g. tissues and organs) coexistence of amorphous and crystalline phases often exists, cf. [30].

The experimental and theoretical analysis of freezing/thawing of water solutions (phase change over a temperature range) reveals that most severe fractures occur at the early stages of thawing, not freezing, cf. [27], [28]. This asymmetry is due to the fact that the deviatoric stress on the freezing front is zero, while no such a condition is required for the thawing front [26], [27].

The property of the tissue describing its mechanical behaviour with changing temperature is the thermal expansion coefficient which is defined in the linear thermoelasticity in the following manner [11], [21].

The constitutive Duhamel–Neumann law is introduced. It relates the stress tensor $\boldsymbol{\sigma} = (\sigma_{ij})$ to the small strain tensor $\mathbf{e} = (e_{ij})$, $i, j = 1, 2, 3$, and temperature:

$$\sigma_{ij} = C_{ijkl} e_{kl} - \beta_{ij}\left(T - T_0\right). \tag{19}$$

Here T_0 is the reference temperature at which no thermal stress exists, $\mathbf{C}$ is the tensor of elastic moduli and $\boldsymbol{\beta}$ is the tensor of the thermal moduli. In the general case of anisotropic hyperelastic solid, the following symmetries hold true:

$$C_{ijkl} = C_{klij} = C_{jikl}, \quad \beta_{ij} = \beta_{ji}.$$

In the isotropic case, the elastic and thermal moduli can be described in terms of the Young modulus E, Poisson ratio ν and thermal expansion coefficient α (thermal coefficient of linear expansion):

$$C_{ijkl} = \frac{\nu E}{(1-2\nu)(1+\nu)}\delta_{ij}\delta_{kl} + \frac{E}{2(1+\nu)}(\delta_{ik}\delta_{jl} + \delta_{il}\delta_{jk}),$$

$$\beta_{ij} = \frac{\alpha E}{1-2\nu}\delta_{ij}.$$

It should be stressed that equation (19) is valid only in the small-strain regime (linear thermoelasticity).

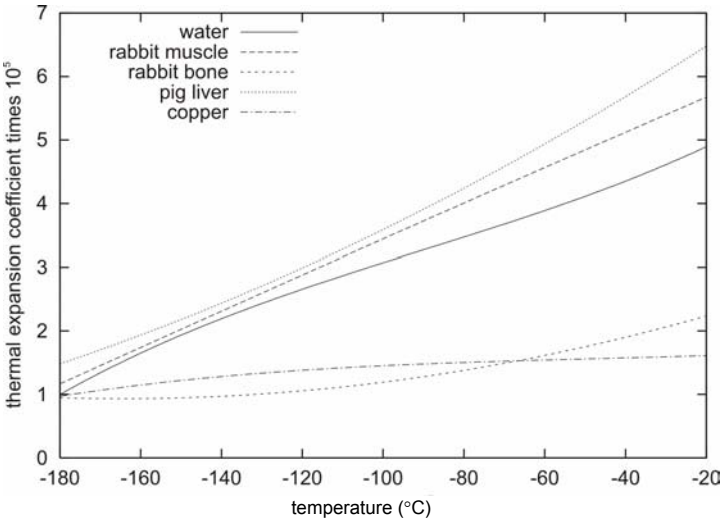

Fig. 3. Thermal expansion coefficients $\left[\frac{1}{K}\right]$ of various tissues compared to water and copper in cryogenic temperature range, after [30]

In figure 3, polynomial approximations of the thermal expansion coefficients of various tissues are compared to these of water and of copper (after [30]). The tissues with high water content (liver and muscle tissues) exhibit characteristics similar to that of pure water, while thermal expansion coefficient of hard tissue (bone) is significantly lower.

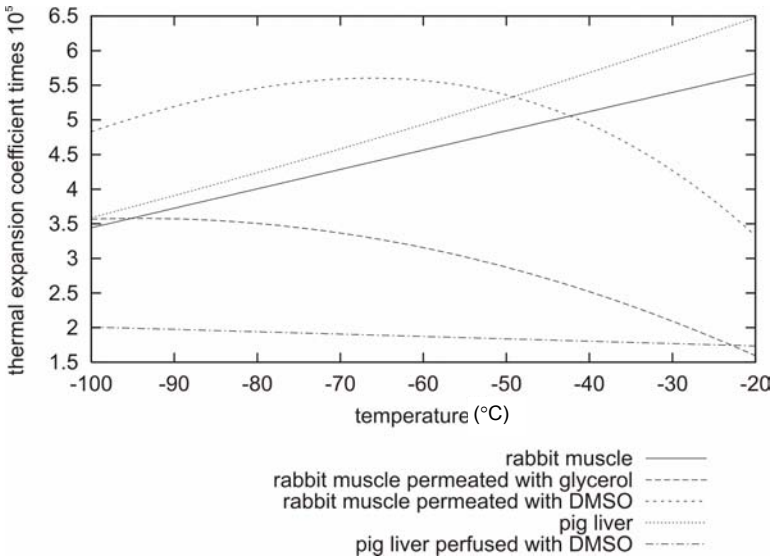

Fig. 4. Effect of cryoprotectant (dimethyl sulphoxide – DMSO or glycerol) on thermal expansion coefficients $\left[\frac{1}{K}\right]$ of soft tissues, after [30]

In figure 4, the influence of cryoprotectant on the thermal expansion coefficient of two kinds of tissues is shown [30]. The quantitative effect is significantly different for muscle and liver tissue, suggesting that some soft tissues are more susceptible to cryopreservation with a given cryoprotectant than others.

4. Cryotherapy

Since the cryotherapeutic treatments properly designed do not involve freezing and tissue damage due to the mechanisms described, these treatments are now briefly described, separately from the problems of cryosurgery and cryopreservation. We now focus on the applications of the cryotherapy to the treatment of joint diseases and osteoporosis.

Cryotherapy devised by T. Yamaguchi was first performed in Japan [15], [45], [46]. It can be applied to the whole body or to selected groups of joints. The duration of the treatment is 1–3 minutes, depending on the individual predispositions. The mixture of air and nitrogen is blown over the skin of the patient. The temperature of the stream is below –100 °C. In the case of the whole-body application, the patient is put inside the cryochamber where similar temperature is maintained.

The purpose of the treatment is to induce the physiological reactions to the low temperatures, namely:

- Cold air blown over the surface of the skin causes constriction of the small blood vessels lasting several seconds. Then, the vessels enlarge in diameter, the arterio-venous fistulas close resulting in more intensive circulation [15], [16].
- The mechanisms of reception and transmission of nervous signal are weakened and partially disabled at low temperature [15], [46]. This results in pain relief.
- Increased blood flow caused by the low temperature reduces swelling, edema and inflammation [16], [46].
- Low temperature is also responsible for relaxation of pathologically stressed muscles and increase in their maximum force [15], [16].

The cryotherapeuic treatment is applicable in the case of the arthritis: rheumatoid arthritis, ankylosing arthritis, ankylosing spondylitis, bacterial arthritis, reactive arthritis and also in the case of degenerative arthritis [46].

The cryotherapy finds also its applications in the sports medicine, rehabilitation and in the treatment of musculoskeletal injuries. The beneficial effects are similar to those expected in orthopaedics: pain relief, reduction of edema formation etc, cf. [14]. The cold is applied by means of contact of ice packs with skin. The temperature is thus higher than in the case of liquid nitrogen vapours, but the duration of the treatment can be of the order of half an hour.

JUTTE et al. [14] measured intramuscular (two centimeters deep under the subcutaneous fat layer) and skin surface temperature during the cryotherapy performed in

a small region in the middle of the right anterior human thigh. Their results are presented in figure 5.

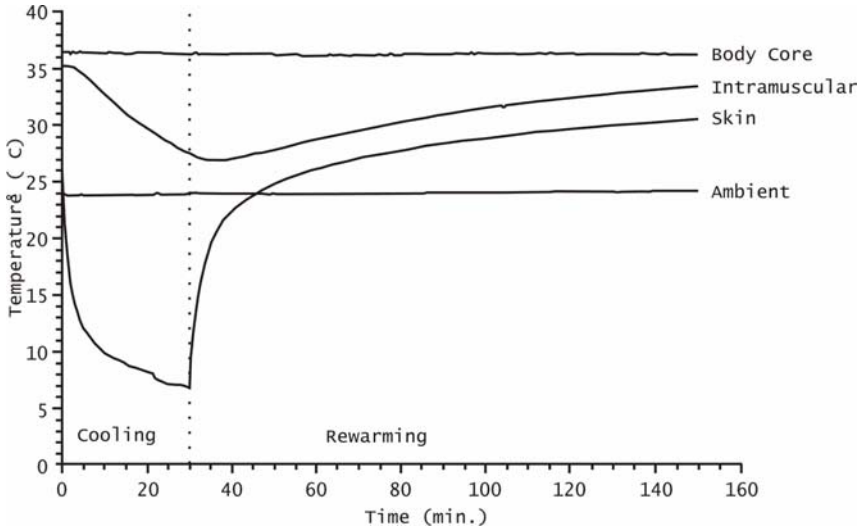

Fig. 5. Temperature history during cryotherapeutic treatment (ice packs), after [14]

Theoretical prediction of their results should incorporate modelling of the heat transfer in the freezing soft tissue. Various bio-heat equations can be found in the first part of the present paper [39], see also equation (11).

Soft and hard tissues are complex deformable porous media. It seems that the general thermomechanical approach developed by FRÉMOND [10] to study free surface problems can also be exploited in low temperature biomechanics.

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References

[1] BALDERSON D.D., CURREY J.D., PEGG D.E., *Both freeze-drying and desiccation of cortical bone allografts preserve mechanical properties satisfactorily*, Acta Bioengng. Biomech., 2002, 4, (suppl. 1), 427–428.

[2] BARRON R.F., *Cryogenic heat transfer*, Taylor&Francis, Bristol PA, 1999.

[3] BROWN N.J., BAYJOO P., REED M.W.R., *Effect of cryosurgery on liver blood flow*, Br. J. Cancer, 1993, 68, 10–12.

[4] BROWN N.J., POLLOCK K.J., BAYJOO P., REED M.W.R., *The effect of cryotherapy on the cremaster muscle microcirculation in vivo*, Br. J. Cancer, 1994, 69, 706–710.

[5] CARSLAW H.S., JAEGER J.C., *Conduction of heat in solids*, Oxford University Press, 1954.
[6] COOPER T.E., PETROVIC W.K., *An experimental investigation of the temperature field produced by a cryosurgical cannula*, J. Heat Transfer, 1974, 96, 415–420.
[7] DEVIREDDY R.V., BISCHOF J.C., *Measurement of water transport during freezing in mammalian liver tissue*. Part II. *The use of differential scanning calorimetry*, J. Biomech. Eng., 1998, 120, 559–569.
[8] DEVIREDDY R.V., SMITH D.J., BISCHOF J.C., *Effect of microscale mass transport and phase change on numerical prediction of freezing in biological tissues*, J. Heat Transfer, 2002, 124, 365–374.
[9] DILLER K.R., *Modelling of bioheat transfer processes at high and low temperature*, Advances in Heat Transfer, 1992, 22, 177–357.
[10] FRÉMOND M., *Non-smooth thermomechanics*, Springer, Berlin, 2002.
[11] FUNG Y.C., *Foundation of solid mechanics*, Prentice Hall, 1965.
[12] HOFFMANN N.E., BISCHOF J.C., *Cryosurgery of normal and tumor tissue in the dorsal skin flap chamber*. Part I. *Thermal response*, J. Biomech. Eng., 2001, 123(4), 301–309.
[13] HOFFMANN N.E., BISCHOF J.C., *Cryosurgery of normal and tumor tissue in the dorsal skin flap chamber*. Part II. *Injury response*, J. Biomech. Eng., 2001, 123(4), 310–316.
[14] JUTTE L.S., MERKRICK M.A., INGERSOLL C.D., EDWARDS J.E., *The relationship between intramuscular temperature, skin temperature, and adipose thickness during cryotherapy and rewarming*, Arch. Phys. Med. Rehabil., 2001, 82, 845–850.
[15] KSIĘŻOPOLSKA-PIETRZAK K., *Cryotherapy use in osteoporosis treatment* (in Polish), Pol. Mer. Lek., 1998, 5(28), 930–933.
[16] KSIĘŻOPOLSKA-PIETRZAK K., GAJEWSKI M., POKRZYWNICKA-GAJEK I., JÓŹWICKA M., MAŁDYK P., MAŚLIŃSKI S., LESIAK A., *The effect of cryotherapy on oxygenic stress in rheumatoid arthritis* (in Polish), Reumatologia, 1999, 37(4), 353–359.
[17] MAJCHRZAK E., *Numerical modelling of bio-heat transfer using the boundary element method*, J. Theor. Appl. Mech., 1998, 36(2), 437–455.
[18] MARTIN R.B., SHARKEY N.A., *Mechanical effects of postmortem change, preservation and allograft bone treatments*, [in:] *Bone mechanics handbook*, S.C. Cowin, Ed. CRC Press, Boca Raton, 2001, pp. (20–1)–(20–24).
[19] MAZUR P., *Cryobiology – the freezing of biological systems*, Science, 1970, 168, 939–949.
[20] MOCHNACKI B., DZIEWOŃSKI M., *Numerical analysis of cyclic freezing/thawing process of biological tissue*, Acta Bioengng. Biomech., 2001, 3, 361–368.
[21] OSTROWSKA-MACIEJEWSKA J., *Mechanics of deformable bodies* (in Polish), Wydawnictwa Naukowe PWN, Warszawa, 1994.
[22] PAZHAYANNUR P.V., BISCHOF J.C., *Measurement and simulation of water transport during freezing in mammalian liver tissue*, J. Biomech. Eng., 1997, 119(4), 269–277.
[23] RABIN Y., OLSON P., TAYLOR M.J., STEIF P.S., JULIAN T.B., WOLMARK N., *Gross damage accumulation in frozen rabbit liver due to mechanical stresses at cryogenic temperatures*, Cryobiology, 1997, 34, 394–405.
[24] RABIN Y., SHITZER A., *Numerical solution of the multidimensional freezing problem during cryosurgery*, J. Biomech. Eng., 1998, 120(1), 32–37.
[25] RABIN Y., STEIF P.S., *Analysis of thermal stresses around a cryosurgical probe*, Cryobiology, 1996, 33, 276–290.
[26] RABIN Y., STEIF P.S., *Thermal stresses in a freezing sphere and its application to cryobiology*, J. Appl. Mech., 1998, 65, 328–333.
[27] RABIN Y., STEIF P.S., *Thermal stress modeling of the freezing of biological tissue*, Advances in Heat and Mass Transfer in Biotechnology, 1999, 183–187.
[28] RABIN Y., STEIF P.S., *Thermal stress modeling in cryosurgery*, Int. J. Solids Structures, 2000, 37, 2363–2375.

[29] RABIN Y., STEIF P.S., TAYLOR M.J., JULIAN T.B., WOLMARK N., *An experimental study of the mechanical reponse of frozen biological tissues at cryogenic temperatures*, Cryobiology, 1996, 33, 472–482.

[30] RABIN Y., TAYLOR M.J., WOLMARK N., *Thermal expansion measurements of frozen biological tissues at cryogenic temperatures*, J. Biomech. Eng., 1998, 120(2), 259–266.

[31] RINCÒN L., SCHATZMANN L., BRUNNER P., STÄUBLI H.U., FERGUSON S.J., OXLAND T.R., NOLTE L.P., *Design and evaluation of a cryogenic soft tissue fixation device – load tolerances and thermal aspects*, J. Biomech., 2001, 34, 393–397.

[32] RODRIGUES J.F., *The variational inequality aproach to the one-phase Stefan problem*, preprint, Centro de matemática e aplicaçoes fundamentais das universidades de Lisboa.

[33] ROGERS J., BERGER A., CIMENT M., *Alternating phase truncation method for a Stefan problem*, SIAM Journal of Numerical Analysis, 1979, 16, 562–587.

[34] RUBINSKY B., SHITZER A., *Analysis of Stefan-like problem in a biological tissue around a cryosurgical probe*, J. Heat Transfer, 1976, 98, 514–519.

[35] SHI X., DATTA A.K., MUKHERJEE S., *Thermal fracture in a biomaterial during rapid freezing*, J. Therm. Stress, 1999, 22, 275–292.

[36] SHI X., DATTA A.K., MUKHERJEE Y., *Thermal stresses from large volumetric expansion during freezing of biomaterials*, J. Biomech. Eng., 1998, 120(6), 720–726.

[37] SMITH D.J., SCHULTE M., BISCHOF J.C., *The effect of dimethylsoxide on the water transport response of rat hepatocytes during freezing*, J. Biomech. Eng., 1998, 120(5), 549–558.

[38] STAŃCZYK M., TELEGA J.J., *Modelling of heat transfer in biomechanics – a review*. Part II. *Orthopaedics*, Acta Bioengng. Biomech., 2002, 4(2), 3–31.

[39] STAŃCZYK M., TELEGA J.J., *Modelling of heat transfer in biomechanics – a review*. Part I. *Soft tissues*, Acta Bioengng. Biomech., 2002, 4, (1), 31–61.

[40] STEFAN J., Ann. Phys. Chem., 1891, 42, 269–286.

[41] TATSUTANI K., RUBINSKY B., *A method to study intracellular ice nucleation*, J. Biomech. Eng., 1998, 120(1), 27–31.

[42] TONER M., CRAVALHO E.G., KAREL M., *Thermodynamics and kinetics of intracellular ice formation during freezing of biological cells*, J. Appl. Phys., 1990, 67, 1582–1593.

[43] VOLLER V.R., SWAMINATHAN C.R., *Treatment of discontinuous thermal conductivity in control-volume solutions of phase change problems*, Numerical Heat Transfer, 1993, 24, 161–180.

[44] WINZELL B., *Finite element Galerkin methods for multi-phase Stefan problems*, Appl. Math. Modelling, 1983, 7, 329–344.

[45] YAMAUCHI T., *The cryogenic therapy and the 24 hours rehabilitation*, [in:] IX European Congress of Reumatology, Wiesbaden, 1979, p. 1025.

[46] ZDROJEWICZ Z., ŁASISZ B., STRYCHALSKI J., DUL W., *Cryotherapy of rheumatoid arthritis* (in Polish), Wiad. Lek., 1988, 41, 930–933.