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**THE DYNAMICS OF HEAT SHOCK RESPONSE INDUCED BY
ULTRASOUND THERAPEUTIC TREATMENT**

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Abstract: We consider hyperthermia, procedure of raising the temperature above 37°C, as a treatment modality. A Finite Element Method model for obtaining an appropriate heating scheme on the tissue level is considered. Next, the resulting temperature time-course profile is plugged into a recently published mathematical model of heat shock response in eukaryotic cells. The dynamics and biological consequences of the model's behavior are discussed.

1. Introduction

The heat shock response (HSR) is a highly evolutionarily conserved defense mechanism allowing the cell to promptly react to elevated temperature and other forms of environmental, chemical or physical stress. Exposure to shock conditions leads to misfolding of proteins, which in turn accumulate and form aggregates with disastrous effect for the cell. However, damage to cells can initiate one of two opposite responses: either apoptosis, the process of programmed cell death which prevents inflammation in multicellular organisms, or heat shock response which enables recovery and survival of the cell. Thus, these two pathways and the interplay between them have the decisive influence on the biological consequences of the stress. At least to two main reasons why the heat shock response has been subject to intense research recently (see e.g. [10]) should be mentioned. First, as a well-conserved mechanism, it is considered a promising candidate for deciphering the engineering principles being fundamental for any regulatory network. Second, regardless of their regulatory functions in HSR, heat shock proteins have fundamental importance to many key biological processes. Therefore, profound understanding of the HSR mechanism is hoped to have far-reaching consequences for the cell biology and to contribute to the development of new treatment methods for a number of diseases, e.g. neurodegenerative and cardiovascular disorders, cancer, ageing, see [1], [5], [6], [7].

The key part of the heat shock response is an abrupt upregulation of the heat shock proteins (HSPs) which prevent the accumulation and aggregation of misfolded proteins. Two groups of heat shock proteins can be distinguished. Some heat shock proteins are constitutively and ubiquitously expressed in all eukaryotic cells. These proteins are called heat-shock cognates and are involved in house-keeping roles, e.g. assist nascent proteins in the establishment of proper conformation, transport (shuttle) other proteins between different compartments inside the cell and participate in signal transduction. The second group contains those which expression is induced by stress. They act as chaperones, i.e. help proteins to maintain their structural integrity or assist the damaged proteins in re-establishment of the functional structure. Moreover, some of them can act as negative regulators of the apoptotic cascade ([2]) or aid the apoptotic machinery through their chaperone functions, see [11] for the review of this issue. These two functions fulfilled by the heat shock proteins, i.e. protein chaperoning and modulation of survival and death-signaling pathways, make them an attractive therapeutic target, for example in the case of neurodegenerative diseases ([4]) or cancer. Furthermore, the heat-induced expression of HSP genes is itself a mechanism of particular interest as it enables the design of heat-responsive gene therapy vectors.

In this study we consider hyperthermia, procedure of raising the temperature above 37°C, as a treatment modality both on the tissue and cellular level. Theoretically, a properly tuned tempo-spatial temperature distribution in tissue would lead to desired heat shock response in the cells and, in consequence, enhanced expression of heat shock proteins which are important from the therapeutic point of view. One of the most relevant problems which arise in this context is related to the question whether an effective and controlled application of hyperthermia in the considered type of tissue is practically feasible. At the same time it is also important to assure that the temperature itself is kept within the therapeutic range, i.e. up to 43°C. Imposing these conditions influences the choice of the heating scheme of the tissue employed for therapeutic reasons. In this presentation, we utilize a Finite Element Method (FEM) model for obtaining an appropriate heating scheme. Next, the resulting temperature time-course profile is plugged into the basic HSR mathematical model presented in [10]. The dynamics and biological consequences of the model's behavior are discussed.

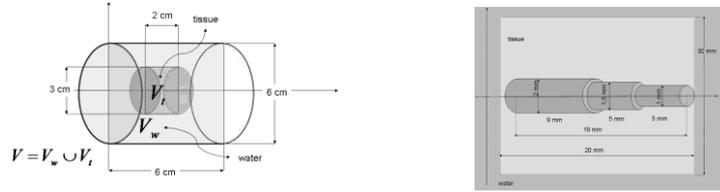
2. Numerical model of the tissue

The very simple Finite Element Method model described below is used to compute tempo-spatial temperature fields generated in soft tissues by ultrasound treatment. Ultrasound irradiation does not stimulate ion activity within the cells, which is an undesired side effect of other irradiation techniques, and is non-invasive, i.e. does not require surgical intervention. The model's construction has been based on an *in vitro* experiment performed in order to investigate the possibilities of

inducing temperature fields in soft tissues by the use of focused ultrasound. The experiment was designed to take into account the physiological conditions of the surrounding environment (37°C), however, since performed *in vitro*, no perfusion was present. Hence, only the heating process with respect to the material properties was considered. For a detailed discussion of the experimental setup we refer to [3].

The bioheat transfer equation in an inhomogeneous thermally anisotropic medium, occupying domain V in the 3D real space, may be written as:

$$\rho(\mathbf{x})C(\mathbf{x})\frac{\partial T(\mathbf{x},t)}{\partial t} = \nabla \cdot K(\mathbf{x}) \cdot \nabla T(\mathbf{x},t) + Q_{ext}(\mathbf{x},t), \quad \text{for } \mathbf{x} \in V, \quad V = V_t \cup V_w.$$

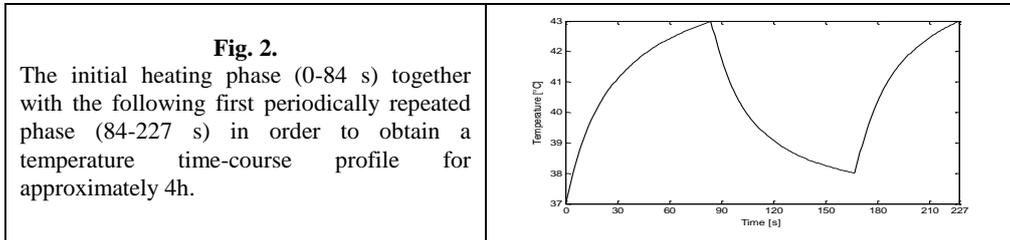


a) Two domains occupied by water and tissue considered in numerical computations b) The heat sources form assumed in numerical calculations

Fig. 1.

The properties of the two homogeneous and isotropic materials are the following. For water: $\rho_w=1000 \text{ kg/m}^3$, $C_w=4200 \text{ J/(kg K)}$, $K_w=0.6 \text{ W/(m K)}$, and for the soft tissue: $\rho_t=1060 \text{ kg/m}^3$, $C_t=3800 \text{ J/(kg K)}$, $K_t=0.5 \text{ W/(m K)}$. The temperature on the boundary of the domain V is assumed to be constant, namely $T(\mathbf{x},t) = 37^\circ \text{C}$, $\mathbf{x} \in \partial V$. The external heat Q_{ext} is modeled by uniformly distributed heat sources of the global power 0,16 in the domain depicted in Fig.1b). The heat sources are assumed to be produced by the focused acoustic beam inside the tissue and their geometric location is chosen so as to minimize, as far as possible while keeping the geometry simple, the difference between numerical results and the experimental data presented in [3].

Finite Element Method simulations were performed and the temperature time-course profiles in the neighborhood of the ultrasound transducer physical focus point were considered. Based on these results, a heating scheme satisfying previously mentioned requirements was obtained. First, the heat sources were turned on at the initial temperature of 37°C. The heating was turned off when the temperature reached 43°C. The cooling process was interrupted by turning on the heating again when the temperature decreased down to 38°C. The last two phases, i.e. cooling and heating, where repeated periodically in order to obtain a temperature time-course profile for approximately 4h. The initial heating phase together with the following first periodic phase are depicted in Fig. 2.



3. Dynamics of the heat shock response

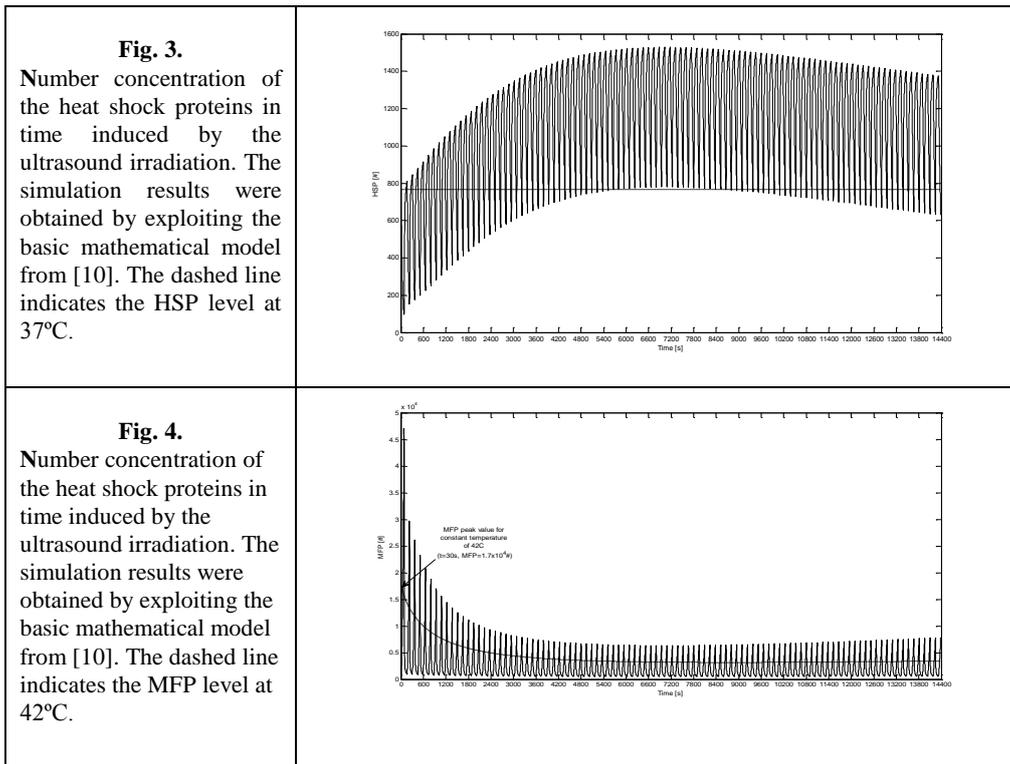
In order to investigate how the obtained temperature regime influences the heat shock response on the cellular level, the basic mathematical model of the heat shock response in eukaryotic cells, recently presented in [10], was exploited. The model is based on a system of ordinary differential equations obtained from an associated biochemical model by assuming the law of *mass-action* for all considered reactions. The model consists of three main modules: the dynamic transactivation of the hsp-encoding genes, their backregulation and the chaperone activity of the heat shock proteins. The heat-induced protein denaturation is modeled by adapting the temperature-dependent formula from [9] for fractional protein denaturation. The biochemical model takes into account only well-documented reactions and does not include any “artificial” elements such as experimentally unsupported components or reactions. For a detailed description of the model with the full list of reactions we refer the reader to [10].

Instead of setting the temperature to a constant value as in [10], we composed the obtained time-dependent temperature profile covering approximately 4 hours with the protein denaturation formula. In this way the basic model from [10] was adapted for simulation of the cellular behavior in response to ultrasound induced heating. The simulation results of the number concentrations of the heat shock proteins (HSP) and misfolded proteins (MFP) in time are depicted in Fig. 3 and 4, respectively.

The dynamics of HSP (Fig. 3) shows that in comparison with the physiological conditions (37°C, dashed line) the applied heating scheme is capable of inducing significant increase in the level of free HSP molecules in the cell. Although the time variation of the HSP level is rather big, still it is considerably higher than in normal conditions in the most of the considered time range. However, for therapeutic applications it is important not only to increase the number of free HSP molecules, but also to keep the level of misfolded proteins low. Otherwise the treatment would lead to the cell’s death rather than its healing. The obtained results (Fig. 4) show that under the proposed heating regime the MFP level evenly oscillates around the reference level of constant 42°C heating (dashed line), i.e. except for the first minutes, the reference line points lie approximately in the middle of the oscillation ranges. The response at constant 42°C is chosen as the reference one on account of the fact that cells are capable of surviving in such environment. In general the oscillations of the MFP level

seems to stabilize in a rather reasonable range for therapeutic purposes, but surely lower peak values of the oscillations would be desired. However, alarming is the protein misfolding during the first few minutes of the heat shock. In order to improve on that, the “self-learning” property of the HSR system could be utilized, i.e. numerical simulations indicate that the response to a second consecutive heat shock is significantly lower. The presented heating scheme could be preceded by some proper irradiation which would prepare the cells for further treatment and, in result, eliminate the undesirable initial MFP level peaks. It should be finely tuned in order to minimize its negative, from the therapeutic point of view, influence on the previously induced free HSP level increase.

However, the obtained results reveal one particular characteristic of the basic mathematical HSR model presented in [10]. Namely, the model immediately reacts to rather fast changes in temperature, which is a direct consequence of lack of implementation of any delay mechanism. This dynamic behavior suggests that the model might not be robust. Robustness is a rather common feature for all biological systems and the behavior displayed by the mathematical model might be unrealistic with respect to the energy resources required for such dynamics. This problem asks for a more thorough investigation including biological verification.



4. Conclusions

In this study hyperthermia was considered as a treatment method. A FEM tissue heating model was described and used together with a new mathematical HSR model recently presented in [10] to make a first attempt to obtain a realistic heating regime which would lead to a proper heat shock response at cellular level. The obtained results of the model dynamics show that the proposed heating scheme leads to a response which, although not to the full extent, still has the desired properties.

This work is a preliminary step on the way to develop non-invasive heating methods for therapeutic clinical applications. Further research, both theoretical and experimental, is needed for this goal.

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