Diffusion of Cryoprotectant Through the Membrane of Reproductive Cells During Equilibration

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Abstract. In order to develop a new technology for low-temperature preservation of fish reproductive cells, and sturgeon fish in particular, mathematical modeling of acoustic impact on biological objects has been performed. A mathematical model of cryoprotectant diffusion through the reproductive cell membrane is constructed. It is assumed that a special piezoactuator creates an acoustic field in the cryoprotectant. By virtue of this, the corresponding velocity field of the environment is assumed to be set. The resulting boundary-value problem is solved numerically using the finite element method.

Keywords: Diffusion · Cryoprotectant · Piezoactuator · Reproductive cell · Sturgeon fish

1 Introduction

Currently, an important task is to develop new technologies for low-temperature preservation of fish reproductive cells. Such technology will undoubtedly be effective not only for artificial reproduction, but will also allow the creation of cryobanks in order to preserve the genetic diversity of valuable fish species.

A review of the literature devoted to the problems of semen cryopreservation shows that the most important research topics are the development of preservation protocols, cryoprotectant compositions, dilution coefficients, equilibration time, etc. At the same time, specific breeds of fish or marine animals are mostly considered. Thus, work [1] considers the current situation and future prospects for standardization of oyster sperm cryopreservation application. Work [2] investigates the impact of cryoprotectants during cryopreservation on sperm of stonefish Kareius bicoloratus, including the effect of dilution factor, equilibration time, etc. Paper [3] describes positive results of experiments on vitrification of sperm of two fish species: freshwater Eurasian perch Perca fluviatilis and sea European eel Anguilla anguilla. The development of vitrification protocols is given, the temperature regime, composition of vitrifying solutions, dilution coefficients and related issues are discussed. In [4], the development of methods for sperm cryopreservation in pechreys Odontesthes bonariensis is presented. The issues of composition and concentration of cryoprotective media are discussed, and the possibility of cryopreservation of pechreys sperm using simple protocols is demonstrated.

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However, there are a number of difficulties on the way to solving this problem. The main problem of cryopreservation is the need to protect the cells from damage during sperm freezing. The main way to protect reproductive cells from damage is to place the sperm in a cryoprotectant, designed to protect the cell during the equilibration stage.

One way to increase the intensity of cryoprotectant penetration into the cell at the equilibration stage is to increase the permeability of the cell membrane. Such an effect can be achieved, for example, by electrostimulation, that is, by applying an acoustic field of a certain power and frequency to the cell [5]. *In-situ* experiments show that the survival rate of spermatozoa using electrostimulation after defrosting increases compared to sperm frozen according to the traditional method by 20% (Fig. 1) [6]. Semen of such high quality can further be used for long-term storage and subsequent artificial insemination of sturgeon caviar.

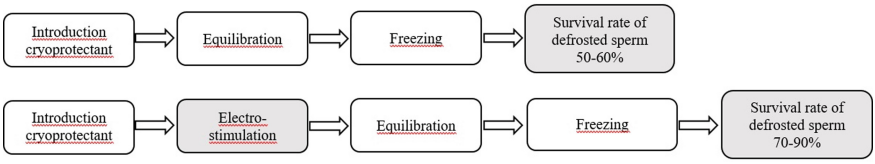

Fig. 1 Application of electrostimulation in sperm cryopreservation

A direct continuation of this work is a series of works by the authors [7–9], in which it is proposed to use a piezoactuator to create an acoustic field. The work also considers the question of determining the optimal effective characteristics of the piezoactuator under its acoustic influence.

A circular plate with a diameter of 0.02 m was chosen as the piezoactuator [7–9]. The plate is glued on the outer side in the center to the bottom of a standard glass laboratory low graduated beaker B-1-50 XC. The beaker has the following dimensions: volume 50 ml, diameter 38 $\pm$ 1.0 mm, height 70 $\pm$ 2 mm (Russian GOST 25336-82).

The suspension (mixture of reproductive cells with cryoprotectant) fills the beaker to about one-third of its height and has a temperature of about four Celsius degrees. The experiment is performed at room temperature.

The operating element of the piezoactuator is a 0.003 m thick piezo-ceramic element PZT-4 with the pre-polarization vector, directed along the thickness. The face surfaces of the piezo-actuator are electrodeposited by the silver burning method and electrical wires are connected to them. The thickness of the applied electrodes can be neglected due to their smallness. The outer surface of the electrodes is covered with epoxy resin 0.001 m thick, which acts as a protective layer. Variable potential difference according to the harmonic law with the amplitude V_0 is supplied to the electrodes through electric wires.

The bottom of the beaker and the piezo element form a semi-passive bimorph, so the application of a variable potential difference to the piezo-electrodes leads to transverse vibrations of the bottom of the vessel. This causes a steady vibration of the piezo-actuator and, consequently, the beaker. The acoustic field created in the suspension, in turn, leads

to an acoustic effect on fish reproductive cells. In this case, the operating frequency is the first frequency of bending vibrations of the bottom of the vessel.

A mathematical model of the impact of the piezoactuator on fish reproductive cells during cryopreservation was constructed within the framework of continuum mechanics. For this purpose, the equations of deformable solid mechanics in the axisymmetric formulation (the theory of elasticity and electroelasticity) and the equations of motion of liquid and gaseous media (in acoustic approximation) [9] were used. Such a model represents an initial boundary-edge problem and, in the general case, its solution can be constructed only numerically with the use of appropriate finite-element analysis software packages.

In the result of the numerical analysis, it is shown that the acoustic field in the volume of the suspension is effectively excited in the first bending mode. However, this field is not homogeneous, as evidenced by the velocity distribution. Therefore, in the process of acoustic influence it makes sense to use stirring of the solution. At the same time, the presence of areas with intense positive and negative vertical velocity components should probably automatically lead to the mixing process.

The next logical step in the continuation of the study is to consider the diffusion of the cryoprotectant through the cell membrane. This process is generally described by the following system of differential equations, based on Fick's laws [10]:

$$\rho\left[\frac{\partial \boldsymbol{u}}{\partial t} + (u\nabla u)\right] = -\nabla p + \eta\nabla^2\boldsymbol{u} + \left(\frac{\eta}{3} + \xi\right)\nabla div\boldsymbol{u} + \rho\boldsymbol{g} \tag{1}$$

$$\frac{\partial n}{\partial t} + div(n\boldsymbol{v}) = 0 \tag{2}$$

$$\frac{\partial c_i}{\partial t} + \boldsymbol{v}\nabla c_i = -div\boldsymbol{j}_i \tag{3}$$

$$\begin{aligned} \boldsymbol{j}_1 &= -\left(D_{11}^*\nabla c_1 + D_{12}^*\nabla c_2\right) \\ \boldsymbol{j}_2 &= -\left(D_{21}^*\nabla c_1 + D_{22}^*\nabla c_2\right) \end{aligned} \tag{4}$$

The following notations are used here: $\boldsymbol{u}$ is the vector of mass average velocity; $\boldsymbol{v}$ is the vector of number average velocity; ρ is the density; p is the pressure; η and ξ are shear and volume viscosity coefficients; $\boldsymbol{g}$ is the vector of free fall acceleration; n is the numerical density; t is the time; T is the temperature; c_i is the concentration of i–th component; $\boldsymbol{j}_i$ is the diffusion flux density vector of the i–th component; D_{ij}^* are practical diffusion coefficients, determined through mutual diffusion coefficients.

The above system of Eqs. (1)–(4) must be supplemented by the equation of state of the medium: $\rho = \rho(c_1, c_2, p)$, $T = const$.

2 Research Method

We will assume that the velocity vector $\boldsymbol{u}$ from Eqs. (1), (2) is defined in works [7–9]. Therefore, further we focus our attention on the solution of Eq. (3).

In addition, another important simplification should be made. The transfer of substances inside the cell can be carried out in two different ways: free diffusion or active

transfer with energy expenditure [11]. Two main stages in the evolution [12] of models of intracellular transport of substances are distinguished:

(1) *Compartmental models*, which describe the transfer of substances (receptors and their complexes) along isolated areas of the cell. That is, from the outer membrane to the cytoplasm, from the cytoplasm to the lysosomes, etc.
(2) *Spatial models*, which take into account the spatial localization of reactions, calculate the change of values (amount of substance or density) at a specific point in space. We can distinguish between models that take into account stochastic effects (attachment/detachment of motor proteins from a microtubule, transported substance from motors) and models that use averaged values of the transfer rate.

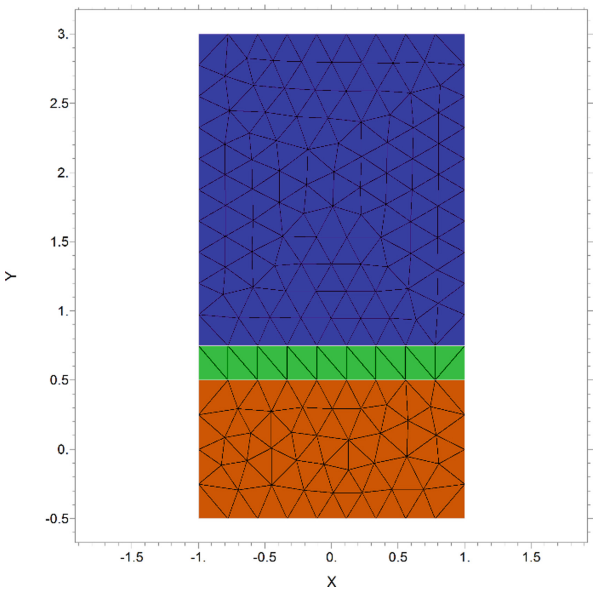

Fig. 2 Generated finite-element mesh

Models of intracellular transport also differ in the way they describe the microtubule network and its dynamics [13–15]. Within the framework of the mechanical model considered in this paper, we assume that the corresponding diffusion coefficients (4) are predetermined from biological experiments.

The numerical experiment was performed using the FlexPDE program of scenario models for solving differential equations using the finite element method. Figure 2 shows the generated finite-element mesh. This figure shows the cryoprotectant in red, the cell membrane in green, and the intracellular biological fluid in blue.

Figure 3 shows the resulting distribution of c_1. Here, the value of c_1 denotes the concentration of biological intracellular fluid. It can be seen that it is inside the cell and does not go out through the membrane.

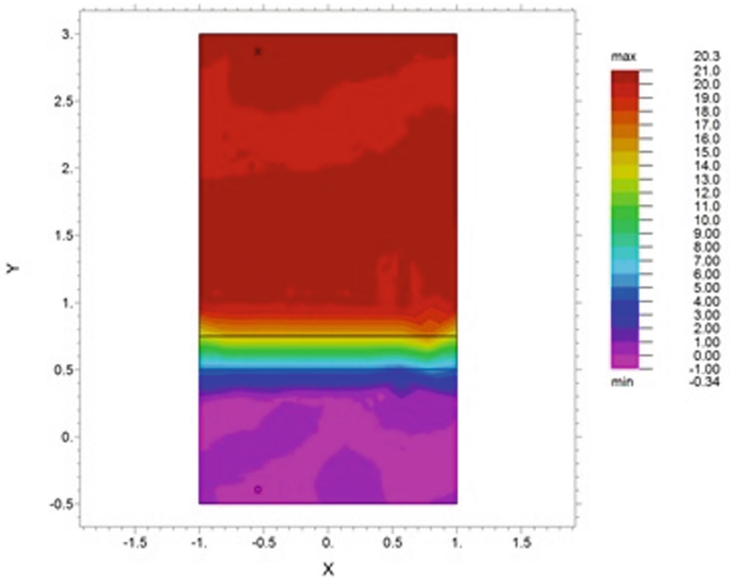

Fig. 3 Distribution c_1

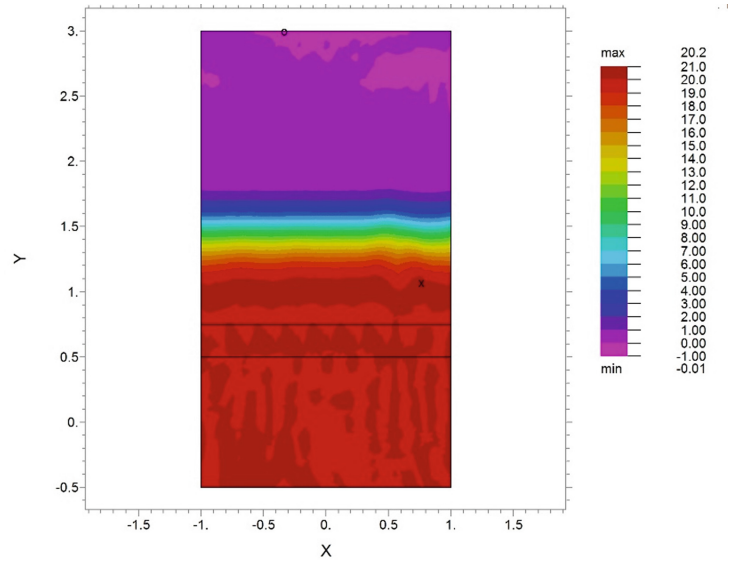

Fig. 4 Distribution c_2

Figure 4 shows the distribution of c_2. Here the value of c_2 denotes the concentration of the cryoprotectant, which was outside the cell before the experiment. It can be seen that the cryoprotectant now penetrates inside the cell in the result of diffusion. Numerical

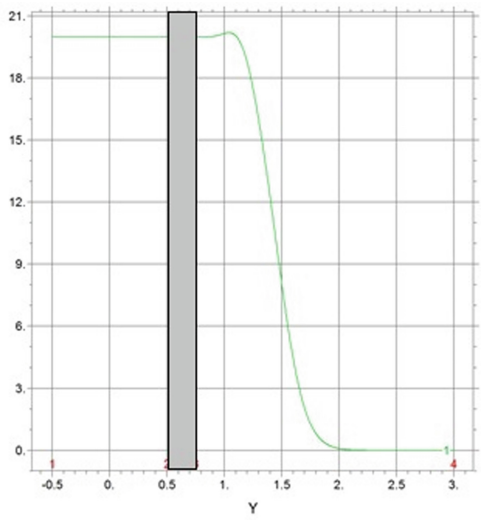

Fig. 5 Numerical distribution of c_2

distribution of c_2 concentration as a function of distance from the membrane is shown in Fig. 5. The vertical gray rectangle corresponds to the cell membrane.

The following numerical values were used in the numerical calculations:

(i) frequency of oscillations, $\omega = 1000\text{c}^{-1}$;
(ii) projection of cryoprotector velocity on the horizontal axis (parallel to the cell membrane), $V_x = 0$;
(iii) projection of cryoprotector velocity on the vertical axis (perpendicular to the cell membrane), $V_y = 2\sin(\omega t)$;
(iv) diffusion coefficients: $D^*_{11} = 0.001$; $D^*_{12} = 1.0$; $D^*_{21} = 0.001$; $D^*_{22} = 0.01\left(1.1 + \text{sign}\frac{\partial c_2}{\partial y}\right)$;
(v) process time, $t = 80c$.

Numerical calculations showed the dependence of cryoprotectant diffusion inside the cell on the velocity amplitude and exposure time, but a weak dependence on the frequency of oscillations.

3 Conclusion

A mathematical model of cryoprotectant diffusion through the reproductive cell membrane is constructed. It is assumed that a special piezoactuator creates an acoustic field in the cryoprotectant. By virtue of this, the corresponding velocity field of the environment is assumed to be set. The resulting boundary-value problem is solved numerically using the finite element method in the FlexPDE software package.

In the result of numerical experiments, it was found that the diffusion of the cryoprotectant inside the cell depends on the amplitude of velocity and time of exposure, but

weakly depends on the frequency of oscillations. Thus, for effective protection of reproductive cells with cryoprotectant, the suspension should be exposed to piezoactuator near the resonance frequency.

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