

Study of local low-temperature effect on biotissues

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Abstract. Local low-temperature exposure on biological tissues consists in the removal of heat and, accordingly, a decrease of its temperature. Such impact may be divided into groups: (1) «destruction», for example, cryosurgery, (2) «preservation», for example, cryoconservation, (3) «therapy», for example, joint cryophysiotherapy. Obtaining different effects depends on the depth and rate of the biological tissue target region cooling. As part of the research, a concept for improving the accuracy of low-temperature exposure is proposed. The conducted studies make it possible to take a step towards the possibility of carrying out precision of local low-temperature impact on biological tissues in different directions.

1 Introduction

The use of cold in medicine has been known since ancient times. The writings of Hippocrates describe the use of cold procedures to stop bleeding and reduce swelling [1]. There are several rounds of rapid development of the method, from the end of the 19th century to the present. They are associated with the possibility of developing artificial methods for obtaining cold, high-tech equipment for ensuring and controlling the process. The local low-temperature effect depends on the final temperature of the target area and the parameters for achieving it. Allocate destructive, therapeutic and preserving effects, which are considered further.

1.1 Destructive effect

The destructive effect of local low-temperature exposure is widely used in various medicine fields. Operations are performed in dermatology, urology, gynecology, pediatric surgery and oncology [2]. This is collectively called cryosurgery. The effect is based on the freezing of biological tissue, the formation of ice crystals both in the intracellular and extracellular spaces, cell rupture and other specific damaging processes. From the heat transfer point of view, the process may be divided into cooling to a cryoscopic temperature, an extended phase transition from a liquid to a solid state, and a further decrease in temperature. The impact is carried out using cryosurgical equipment. It can be divided into groups according to the physical processes of obtaining low temperatures: due to the phase transition heat of the working substance, the working body expansion in throttle devices, the Peltier thermoelectric effect.

In equipment using the phase transition heat, the minimum temperature of the cryoinstrument working

part can be maintained close to the cryoagent boiling point. The most common in practice are devices that use liquid nitrogen. It has a low boiling point of minus 195.75 °C, is non-toxic, and does not ignite.

For devices operating on the Joule-Thomson effect, the characteristic temperatures depend on the working substance, the cryoprobe design and the cycle parameters. As a rule, such systems operate at very high pressures. For example, the inlet pressure of gaseous argon into the cryosurgical apparatus is maintained at 240 atmospheres. When using this substance on the cryoinstrument working surface, temperatures of minus 80 °C are achieved in contact with the target object.

Apparatuses based on the thermoelectric Peltier effect are rare in cryosurgery practice. The minimum temperature can be at the level of minus 43 °C. Such systems are mainly used to remove the lens of the eye.

The basic parameters of cryosurgical equipment are geometry, material, temperature distribution on the cryoinstrument working surface, which determine the cooling power (cooling capacity). A regulation range of the given parameters is provided, which forms one or another exposure mode depending on the target object characteristics. Different types of tissues react individually to exposure. The freezing zone is not a guarantee of destruction. The temperature of irreversible damage to cells and tissues is called the temperature of «necrosis» and for different tissues can vary from minus 20 to minus 40 °C [3, 4]. When planning, it is important for physicians to understand what temperatures in the target object volume can be obtained with a particular operating mode of cryosurgical equipment. From the heat transfer point of view, this is a solution to the Stefan problem. A problem that arises in the study of physical processes associated with the substances phase transformation, in which the questions of finding the temperature distribution and the law of phase boundary motion are raised. To calculate

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the temperature fields in the target object, a geometric model of a cryoinstrument and a biological object are formed, initial and boundary conditions are set. In most works, on the cryoinstrument working surface, a first kind boundary condition is specified in the form of a temperature distribution on the surface, which, along with the instrument geometry, is the determining criterion in calculating the effect on biological tissue.

1.2 Therapeutic effect

The cryotherapy is associated with the impact on the neuromuscular apparatus, the mechanisms of thermoregulation including thermal receptors, etc. [5]. According to modern understanding, prolonged cooling causes their inhibition and partial paralysis. A person first feels cold, then a burning sensation and tingling, and then pain, which is replaced by anesthesia and analgesia. The initial reaction of small and medium-sized vessels to cooling is expressed by narrowing of small capillaries and arteries of the skin, slowing down the blood flow. The second protective reaction is associated with the expansion of the lumen of blood vessels and an increase in the blood flow intensity. Local cryotherapy is accompanied by an analgesic effect due to the pain receptors blocking in the skin. In addition, it leads to the suppression of inflammatory processes [6]. From the heat transfer point of view, the process can be divided into cooling to a target temperature (above 0 °C) without a phase transition and maintaining it for a certain time. The impact is carried out using local cryotherapy devices. They have different physical principles for obtaining low temperatures. Among them are air cooling with the use of vapor compression refrigeration machines, blowing with liquid nitrogen vapor and others. As part of the work, the contact method is considered using a specific cryobag and varying working substances.

1.3 Preserving effect

The preserving effect is mainly used for cryopreservation of biological materials. E.g., it is based on an ultra-fast decrease in the object temperature with its transition to a glassy state without the crystals formation. For these purposes, together with low-temperature exposure, cryoprotective solutions are used. From the heat transfer point of view, the process is similar to cryosurgical, but the rate of temperature change differs. In this direction, the immersion of a biological object in a liquid nitrogen volume is mainly used, followed by storage in liquid nitrogen vapor in special cryogenic storage facilities.

1.4 Dosing accuracy problem

Today, local low-temperature exposure is mainly carried out on the general recommendations of equipment manufacturers. This is a mass approach for the simplest cases implementation and potentially leads to failure to achieve the planned effect, up to tragic consequences.

Any method of applying a physical factor requires accurate dosing. It should be based on an understanding of the processes occurring in tissues and organs. The temperature and the parameters of its achievement are convenient and understandable indicators that characterize certain processes in the target area. Based on the above, the concept of increasing the accuracy of dosing of local low-temperature exposure is proposed further. In the future, this will make it possible to move from mass recommendations to personalized ones and expand the method application.

2 Unified approach concept

Conceptually, the idea of increasing the accuracy of the local low-temperature exposure contains the following elements. Firstly, this is the division of the process into three stages: planning, ensuring the impact under control, analysis of the results. Secondly, this is the identification of features and criteria that affect the each stage implementation. Thirdly, it is a search for ways to improve the accuracy of the each stage execution. A promising way lies both in the creation of experimentally verified mathematical models, and in the conduct of specially prepared experiments aimed at identifying the process features that increase the planning accuracy. In this paper, examples of solving problems of increasing the dosing accuracy for destruction, therapy, and preservation are considered.

2.1 Destruction

In the direction of destruction, the authors considered several problems. A particular problem of increasing the accuracy of planning minimally invasive prostate cryoablation has been solved. This type of surgery is performed with several argon cryoprobes near a critical area - the urethral canal, inside which a heating system is placed and saline at 38 °C circulates. There is a problem of correctly positioning cryoprobes to achieve maximum necrosis and prevent damage to the urethral canal and healthy surrounding tissue. As a result, both experimental data and results using thermophysical modeling were obtained [7].

In this study, the task of developing a modeling program for local low-temperature exposure to biological tissue using a nitrogen sapphire applicator is considered. Previously, this material was shown to be effective and promising for cryosurgery [8]. Conventionally, the whole process can be divided into 2 stages. The first stage consists in pouring liquid nitrogen into the cryodestructor. In this case, liquid nitrogen boils and the body and applicator are cooled down. After a stable temperature is established on the applicator surface, a transition to the second stage takes place. It consists in positioning the applicator working part on the biological tissue target area. The process of removing heat from the biological tissue and lowering its temperature begins. Of interest to a physician is understanding the dynamics of changes in the temperature field in the target biological tissue. Therefore, in this work, attention was focused on this

aspect. This is described by the heat equation (1) with a source member (2), which was proposed by Pennes H.H. in 1948 [9].

$$\rho(T)c_p(T)\frac{\partial T}{\partial t} = \frac{\partial}{\partial x}\left(k(T)\frac{\partial T}{\partial x}\right) + \frac{\partial}{\partial y}\left(k(T)\frac{\partial T}{\partial y}\right) + \frac{\partial}{\partial z}\left(k(T)\frac{\partial T}{\partial z}\right) + q(t), \quad (1)$$

$$q(t) = w^*(\rho C)_b(T_b^* - T) + q_{met}^* \quad (2)$$

where: ρ - tissue density, C_p - tissue specific heat capacity, T - temperature, t - time, k - thermal conductivity of the tissue; w^* - blood perfusion flow rate per unit volume of tissue; ρ_b - blood density; C_b - blood specific heat capacity; T_b^* - temperature of the blood within the vessels; q_{met}^* - heat generation from metabolism.

Previously, physical experiments were carried out and the temperature of the applicator minus 190 °C was determined, which can later be used as a boundary condition [8].

Generalized graphically, the model for calculation can be represented in the form of Figure 1. This is the stage of impact on biological tissue. The sapphire applicator comes into contact with the biological tissue surface and the process of temperature change begins. A necrosis zone is formed, limited by the necrosis temperature and a freezing zone between the necrosis and the cryoscopy temperature.

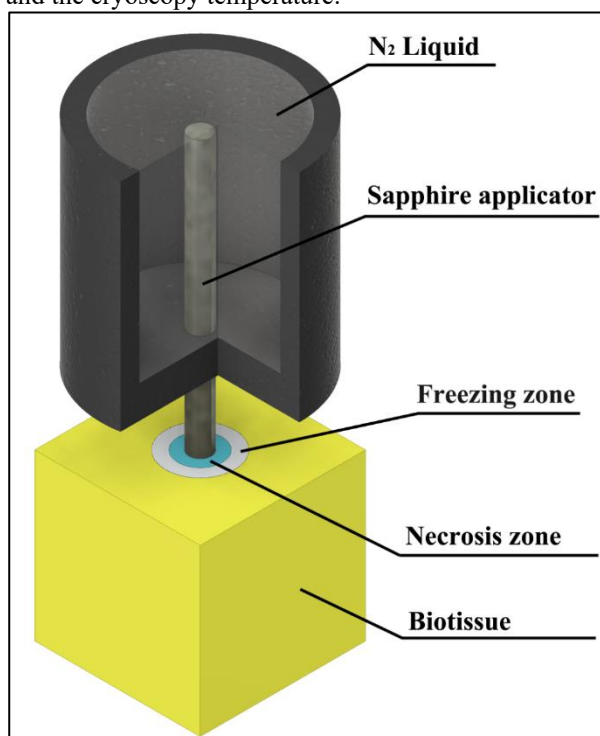


Fig. 1. The model for «Destruction» calculation.

To develop the calculation program, it was decided to use the numerical simulation package ANSYS 2020R1 on workstation with Intel Xeon Gold 6246 3.3GHz CPU with 256 GB RAM. The main calculations were carried out using CFX, since it has specifying thermal properties by piecewise given functions. The main equation that the ANSYS calculates for each mesh

element is heat transfer equation (1). CFX uses the finite volume method.

A geometric model of the applicator and conditional biological tissue has been developed. Calculations were carried out in the same formulation as the physical model experiments described below. The dimensions of the sapphire applicator were as follows. Diameter - 12 mm, length immersed in liquid nitrogen - 90 mm, length outside - 43 mm. The initial temperature was set as in physical experiments and was 24 °C. The process of liquid nitrogen boiling inside the cryodestructor was not modeled. An assumption is made and a first kind boundary condition is set on the applicator surface inside the destructor tank in the form of minus 190 °C [8]. The calculation time was 1200 sec.

The thermophysical properties of tissues and model mediums were used on the basis of experimentally obtained ones [10]. The phase transition was taken into account by setting the thermophysical properties as functions of temperature. There were jumps in the heat capacity during the phase transition, as well as changes in thermal conductivity and density.

The results of modeling the freezing zone for various four points in time are presented (Fig. 2.)

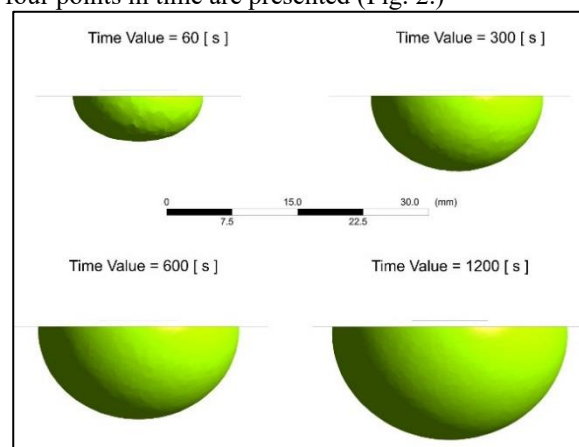


Fig. 2. The results of modeling the freezing zone in a model environment (cryoscopic isotherm limitation).

To verify the program, an experimental installation was developed (Fig. 3) and model experiments were carried out.

Before the experiment, the preparatory stage was carried out (Fig. 3a). Liquid nitrogen was poured from a thermos (position «3») into a small-sized laboratory cryosurgical apparatus with a sapphire applicator (position «1»), which was attached to a clamp (position «2»). After stabilization of the temperature on the sapphire applicator surface, the cryosurgical apparatus was positioned for contact with the model medium surface and the stage of direct low-temperature exposure began (Fig. 3b). The temperature was measured in a model medium «Mediagel» ultrasonic gel (manufactured by Geltek-Medica, Moscow, Russia) at 5 and 10 mm depths. A specially designed and printed on a 3D printer (position «5») stand and pt100 resistance temperature sensors (position «4») were used. The readings from the sensors were transmitted to the secondary transducer - analog input module OWEN MV110 (position «6») and then to the personal

computer (position «7») for recording and processing the results. Additionally, during the experiment, video recording of the freezing zone growth took place.

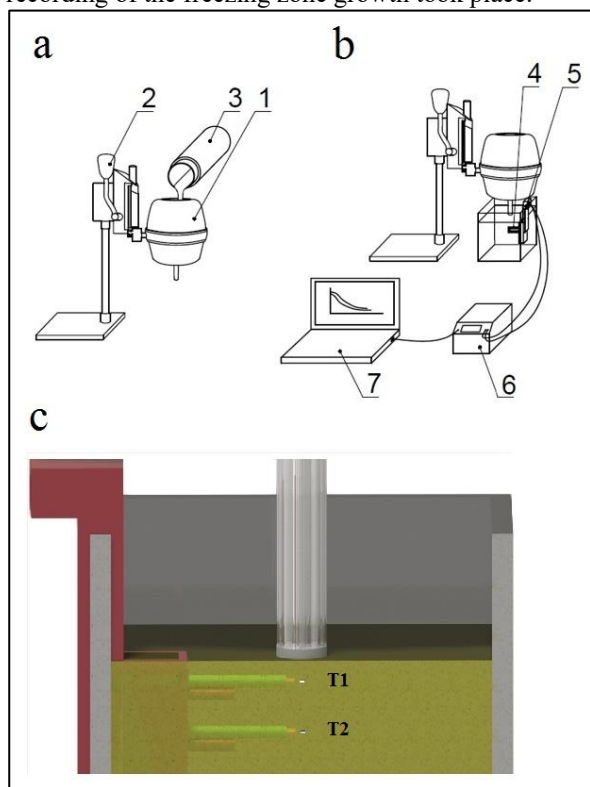


Fig. 3. Experimental installation for «Destruction»
a - general view; b - the working area is enlarged (contact of the applicator working surface and the model medium).

Three repeated experiments were carried out to confirm the results reproducibility. Next was a comparison with the simulation program results.

The comparison was based on the freezing zone size and the temperature values at the control points at a depth of 5 and 10 mm from the impact surface. The difference in temperature in absolute terms between experimental and numerical did not exceed 3 °C (Fig. 4.). Comparison by freezing zones is shown (Fig. 5).

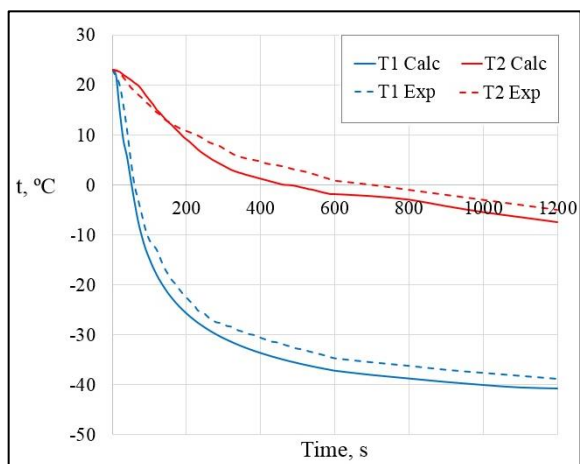


Fig. 4. Comparison of temperature values at the control points T1 and T2.

The numerical result is shown as a green surface for better visibility. The discrepancy between the linear dimensions of the freezing zones does not exceed 2 mm.

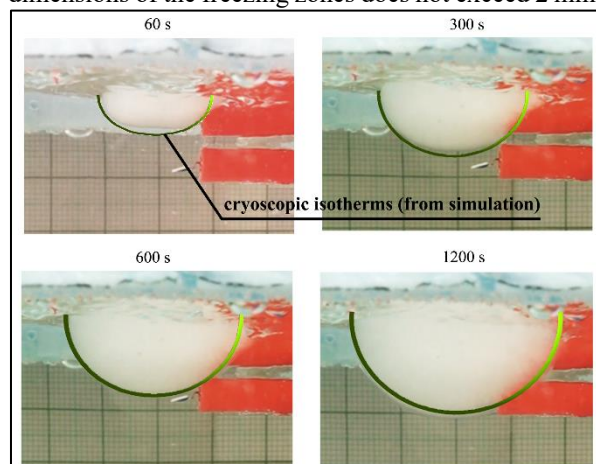


Fig. 5. Comparison of the ice balls volumes obtained experimentally and numerically.

Thus, the modeling program was verified and the application possibility was proved. In the future, it is planned to consider the joint use of forecasting using a modeling program and the optical control possibility of the freezing zone using an artificial sapphire applicator.

2.2 Therapy

In this direction, an approach was used with specially prepared physical experiments. The focus was on the study of various methods and modes of local cryotherapy. The sources analysis showed the target surface temperature of the impact area is 10 ± 2 °C and the critical temperature for safety is 0 °C. In the identified range, the basic modes were selected on a model medium for blowing with air and liquid nitrogen vapor [11]. In the current study, an experimental installation has been developed and modes for the contact method of cooling have been considered. The experimental installation consists of 10 elements. (Fig. 6). A cryobag «MUELLER» (position «1») was used as a local cryotherapy tool. This cryobag is supported by a tripod with a clamp (position «2») to ensure stability during the experiment. To simulate the biological tissue, a model medium «Mediagel» ultrasonic gel (manufactured by Geltek-Medica, Moscow, Russia) is employed, which is contained within a glass container measuring 90x90x100 mm (position «3»).

The system for creating and maintaining the initial temperature 37 °C of the model medium consists of several components. A liquid thermostat (position «4») (Termex VT10, Russia) is employed to control and regulate the temperature. A pump (position «5») (KNF, PML13229-NF 60) is utilized to circulate warm water within an external glass container (position «6»).

The measurement system has several elements. Pt100 resistance thermometers (position «7») are used to measure the model medium temperature accurately. A mounting system for temperature sensors

(position «8») is employed to secure and position the thermometers properly. A secondary transducer - analog input module OWEN MV110 (position «9») is used to facilitate the analog input from the temperature sensors. Additionally, a personal computer (position «10») is incorporated into the setup for recording and processing the results.

As the first working substance, a water and ice mixture was used in a ratio of two to one by weight, the second working substance was a NaCl solution at a concentration of 23.1% by weight. The solution, when stored in a freezer at minus 18 °C, remains in a liquid state, which guarantees a tight fit of the bag with the working substance to the exposure surface.

Three repeated experiments of each mode were carried out to confirm the results reproducibility. The difference in temperature in absolute terms did not exceed 0.5 °C. The possible temperature range of influence is revealed (Fig. 7).

When cooled with NaCl solution, the surface temperature of the model medium decreased to 10 °C for 6 minutes of exposure and after 17 minutes reached its minimum value of 6.2 °C. In the case of using a water and ice mixture the minimum temperature of 12.5 °C was reached in 58 minutes. The use of applications with a NaCl solution made it possible to achieve the accepted target temperature (10 ± 2 °C) in contrast to the method with a water and ice mixture. Cooling by both methods did not lead to cooling below 0 °C. That is, the use of these methods should not lead to tissue damage. Comparison of the use of NaCl solution and a mixture of water with ice was carried out under the condition of their equal volume in the cryobag, the possibility of a snug fit to the exposure surface and the manufacturer's recommendations for the ratio of ice and water.

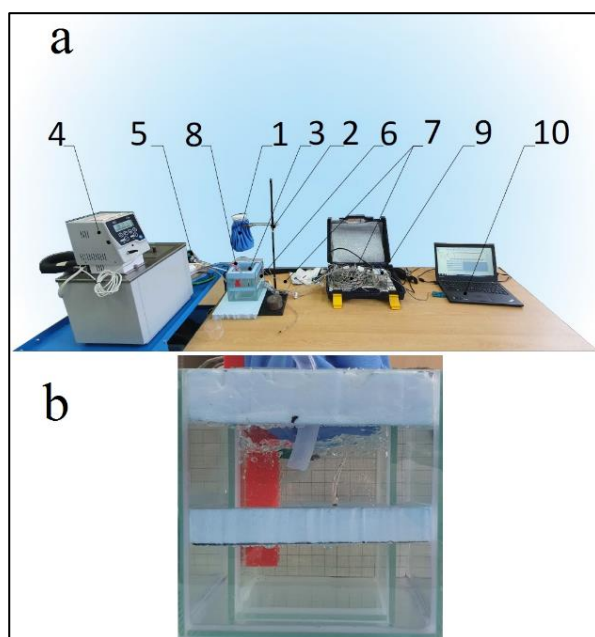


Fig. 6. Experimental installation for «Therapy»
 a - general view; b - the working area is enlarged (contact of the cryobag working surface and the model medium).

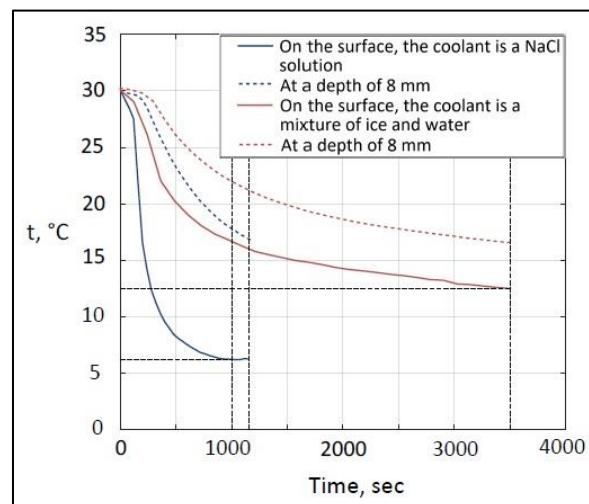


Fig. 7. Possible temperature range of exposure.

As a result, the most appropriate mode of local cryotherapy by the contact method was determined. This is a cooling mode using NaCl solution as a working substance with an initial temperature of minus 18.4 °C with a possible exposure time of 6 to 19 minutes. In the future, it will be used as a base when planning tests on volunteers.

2.3 Preservation

In the direction of preservation, an urgent problem for regenerative medicine is being solved - biotissue decellularization. This is the preservation of the biological tissue framework when the layer of donor cells is removed. Usually, chemical agents are used for these purposes, which entails a number of difficulties in terms of toxicity and a lot of time spent on the washing process. The potential use of cold seemed promising to the authors. The paper considers the cyclic freezing and thawing processes of animal pericardial samples by varying the number of cycles and the intensity of both freezing and thawing. Freezing of samples in special containers was carried out by immersion in liquid nitrogen at a -196 °C, defrosting by placing them in a phosphate-salt solution at a 37 °C temperature in a liquid thermostat (Termex VT10, Russia). A series of experiments were carried out and preliminary positive results were obtained. For the appearance of significantly decellularized areas of the pericardium, the number of cycles reached 30, each lasting up to 5 minutes. This was confirmed by histological studies of pericardial tissues. This shows the possibility and prospects of using the cold physical factor for the decellularization purposes. In the future, additional experiments and studies involving samples of other types are planned.

3 Conclusion

In this paper, a unified approach concept is described - the idea of increasing the accuracy of the local low-temperature exposure on biological tissues. The results of solving problems in different directions are shown. For destruction selected correctly

positioning cryoprobes to achieve maximum necrosis and prevent damage to the urethral canal and healthy surrounding tissue. A program for modeling local low-temperature exposure to a nitrogen cryoapplicator made of artificial sapphire was also developed and experimentally verified. An applicator made of this material makes it possible to apply optical methods to control the growth of the freezing zone. Simultaneous application of the program for planning and further optical control will potentially improve the accuracy of the impact.

As part of the therapy, a specially prepared experimental stand was developed. The results on the most suitable mode of the contact cooling method are obtained. In the future, experiments on volunteers are planned.

As part of conservation, the problem of biological tissues decellularization is being solved without the use of toxic chemicals. The first encouraging results on the regimes of cyclic freezing and thawing have been obtained.

The conducted studies make it possible to take a step towards the possibility of carrying out precision of local low-temperature impact on biological tissues in different directions.

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References

1. N.N. Korpan, J. Am. Coll. Surg., **204(2)**, 314-324 (2007)
2. A.V. Shakurov, A.V. Pushkarev, V.A. Pushkarev, D.I. Tsiganov, Sovrem Technol Med, **9(2)**, 178–189 (2017)
3. K.F. Chu, D.E. Dupuy, Nat. Rev. Cancer, **14(3)**, 199-208 (2014)
4. M. Shurrab, H. Wang, N. Kubo, T. Fukunaga, K. Kurata, H. Takamatsu, Int J Refrig, **67**, 308-318 (2016)
5. R. Bouzigon, F. Grappe, G. Ravier, B. Dugue, J. Therm. Biol., **61**, 67-81 (2016)
6. A.V. Shakurov, Yu.S. Lukina, A.S. Skriabin, L.L. Bionyshev-Abramov, N.B. Serejnikova, D.V. Smolencev, J. Therm. Biol., **113**, 103501 (2023)
7. A.V. Pushkarev, S.S. Ryabikin, N.Yu Saakyan, D.I. Tsiganov, I.A. Burkov, A.O. Vasilev, Cryobiology, **109**, 10-19 (2022)
8. A.V. Pushkarev, S.S. Ryabikin, D.I. Tsiganov, A.K. Zotov, V.N. Kurlov, I.N. Dolganova, J of Biomedical Photonics & Eng, **8(4)**, 040501 (2022)
9. H.H. Pennes, J Appl Physiol, **1(2)**, 93 – 122 (1948)
10. I.V. Agafonkina, Y.M. Berezovsky, I.A. Korolev, A.G. Belozerov, A.V. Pushkarev, D.I. Tsiganov, A.V. Shakurov, A.A. Zherdev, J. Eng. Phys. Thermophys., **94(3)**, 790-803 (2021)
11. N.Yu. Saakyan, A.V. Pushkarev, *Target Temperature Range of Local Cryotherapy*, in Proceedings of International Theoretical and Practical Conference, Low Temperatures in Science and Industry, 12–14 October 2022, Moscow, Russia (2022)