Study of low-temperature exposure on biotissue using an elongated cryoaplicator

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Abstract. The article presents the results of a study of low-temperature exposure on animal biological tissue using the novel prototype of a liquid nitrogen cryoaplicator. The data obtained are compared with the cryoaplicator characteristics cooled by nitrogen dioxide that are currently used for the atrial fibrillation treatment. Data analysis confirmed the liquid nitrogen cryoaplicator's effectiveness and made it possible to highlight their advantages.

1. Introduction
Cryomedicine currently includes three areas: cryopreservation, cryotherapy, and cryosurgery. Cryosurgery is used in pediatric surgery, neurosurgery, oncology, cardiac surgery, urology, etc. [1 - 5]. From one hand, literature review has shown the cryoablation widespread use for the atrial fibrillation treatment [6, 7]. On the other hand, methods for achieving target temperatures are often not effective enough and require further investigation [8].

Currently, the operating modes of the cryosurgical devices are selected based on the manufacturers' own experience. The emphasis is on safety, with no potential for efficiency increasing. Together with physicians, was made a conclusion about possible improvement in cryoablation efficiency. The idea was to decrease the cryoexposure duration, and therefore, the surgical intervention total time. It is possible due to lower temperature of liquid nitrogen cryoaplicator.

At the first stage, the characteristics of the developed elongated cryoaplicator prototype were studied in detail [9, 10]. The minimum achievable temperature on the applicator surface without heat load was minus 190 °C for about 1 minute from the gas supply start. On biotissue phantom was shown that it is possible to create extended freezing regions using an applicator, which is relevant for the atrial fibrillation treatment. The purpose of this study was to determine the characteristics of the low-temperature exposure using the developed elongated cryoaplicator prototype for the atrial fibrillation treatment using the animal biotissue sample. Also, in order to clearly show the effectiveness of the developed elongated cryoaplicator, its characteristics were compared with the currently used
cryoapplicator. The results of the experiments really confirmed the assumption of an enhancement in efficiency.

2. Materials and methods
The study of the low-temperature exposure dynamics of an elongated cryoapplicator on animal biotissue was carried out in vitro on pig hearts. There were 4 samples. Literature data indicate the absence of significant differences between the response to myocardium cryogenic exposure of pigs and humans. [11]. No animals were killed specifically for this study. After 10-15 min, all hearts were perfused with a heparinized 0.9% saline to clear them from blood and after placed in a container (4 °C) for transportation. All further processing was performed on a sterilized table not later than 40-45 min after slaughter.

During the experiment, the heart was placed in a transparent square plastic container (4) (side 250 mm, depth 200 mm) filled with saline. The container was connected to an electric thermostat (6) by two fittings (for the inlet and outlet flows). The thermostat was maintained the saline temperature at the level of 37 °C, the approximate heart temperature accordingly. The saline of the container (4) was pumping (8) through the coil of the thermostat (6). The heart was located on an additional stand, which ensured the absence of contact between the saline and the cryoapplicator (part of the heart protrudes above the saline surface). The cryoapplicator location relative to the biotissue is shown in (5), where (5.1) is the unfrozen biotissue, (5.2) is the frozen biotissue. Data from thermocouples located in area (5.2) were collected with PC (7). The detailed temperature sensors location relative to biotissue and cryoapplicator are shown in fig. 2, fig. 3.

![Figure 1](image1.png)

**Figure.1.** The scheme of the experimental installation (1 – compressor, 2 – receiver, 3 – Dewar vessel, 4 – experimental capacity, 5 – cryoexposure process, 5.1 – unfrozen biotissue, 5.2 - frozen biotissue, 6 – electric thermostat, 7 – PC, 8 – pump).

The circulation of liquid nitrogen through the cryoapplicator was carried out under overpressure created in the Dewar vessel (3). The overpressure was created using compressor (1) and receiver (2) (to compensate pressure fluctuations after the compressor). Dewar vessel (3) has a safety-valve.

![Figure 2](image2.png)

**Figure.2.** Layout of temperature sensors. Cross section. (1 – biotissue, 2 - elongated cryoapplicator, 3 – needle thermocouples (T1, T2, T3)).
To measure the temperature, 2 Owen TRM202 measuring modules with 3 needle thermocouples were used. It should be noted that needle thermocouples are used as temperature sensors in cryosurgical operations for the treatment of prostate cancer, therefore, their use is permissible both from the point of view of measuring low temperatures and the compatibility of the needle material with biological tissue.

Needle thermocouples are inertial (indicator of thermal inertia is 2 second) due to the fact that the sensitive element is located in the metal tube of the needle. To improve the measurement accuracy and reduce the inertia, a part of the tip of the needle thermocouple was cut off (a part of the tip was left so that the needle could better penetrate the biological tissue). In the course of an experiment with a model medium, the effectiveness of this method of decreasing the inertia of a needle thermocouple was confirmed. Indicator of thermal inertia became 1 second.

The experiment consists of the following stages. A visual inspection of the biotissue provided for the experiments. The target area of cryoexposure is determined. The heart is positioned on an additional stand in the container (4). Thermostat (6) with saline, preheated to the 37 °C temperature, is connected to a container (4) using fittings. Within 60 minutes, the biotissue is heated to the 37 °C target temperature. Temperature control is performed using needle thermocouples. Needle thermocouples are located along the heart wall thickness using a plastic grid. PC (7) collects data from the temperature and pressure sensors. Pressure is created in the Dewar vessel (3), the cryoapplicator enters the operating mode. The cryoexposure starts. The freezing zone growth and the temperature decrease are monitored. The temperature distribution is recorded for further processing.

3. Results and discussion
Experiments with two types of cryoapplicators were carried out. The temperature on the cryoapplicator surface cooled with nitrogen dioxide was about minus 70 ºС – cryoapplicator "No. 1". The temperature along cryoapplicator surface cooled by liquid nitrogen was about minus 190 °C – cryoapplicator "No. 2".

During the literature analysis, it was revealed that the target temperature (necrosis temperature) minus 40 °C is sufficient to create a transmural area [12, 13].

![Figure 3](imageurl) **Figure 3.** Layout of temperature sensors. Front section. (1 – biotissue, 2 - elongated cryoapplicator, 3 – needle thermocouples (T₁, T₂, T₃)).

![Figure 4](imageurl) **Figure 4.** Temperature distribution over the biotissue thickness when using the nitrogen dioxide cryoapplicator.
Figure 5. Temperature distribution over the biotissue thickness when using the liquid nitrogen elongated cryoapplicator.

Figure 6. Cryoexposure with the liquid nitrogen elongated cryoapplicator.

Figure 7. Cryoexposure with the liquid nitrogen curved elongated cryoapplicator.

Figure 8. Frozen zone after cryoexposure.

Figure 9. Frozen zone after cryoexposure with the curved elongated cryoapplicator.

Figure 10. Transmurality of the cryoexposure region. View 1.

Figure 11. Transmurality of the cryoexposure region. View 2.
Figures 4 and 5 show temperature graphs at the biotissue control points when using cryoapplicators No. 1 and No. 2. At the initial temperature of cryoapplicator No. 1 about minus 70 °C, the necrosis temperature at the lowest point of biotissue was reached only at the 5th minute of the experiment (269th second). That is a common disadvantage of such cryoapplicators [11], leading to an increase in the total operation time. The second case (fig. 5), the required temperature at the same point was reached already in the first minute (20 second). Such a significant difference in the time of the cryonecrosis is associated with a significant difference in the initial temperature of the two types of cryoapplicators.

Figures 6-11 show photos at different stages of cryoexposure on biotissue. Figure 6 includes the straight cryoapplicator. In the figure 7, curved cryoapplicator is shown during cryoexposure. The ability to curve the cryoapplicator is important for operations to treat atrial fibrillation (as the target, transmural lines on the heart muscle are non-linear). It should be noted that the developed elongated cryoapplicator prototype can be used several times with proper sterilization. Figures 8 and 9 show the areas of cryoexposure with a straight and curved cryoapplicator. Figures 10 and 11 show that transmurality is achieved with using of liquid nitrogen cryoapplicator (the cryoexposure area is frozen the entire thickness). This means that the goal of cryoexposure has been achieved.

4. Conclusion
As a result of the study, the characteristics of the low-temperature exposure using the developed elongated cryoapplicator prototype for the atrial fibrillation treatment were determined using the animal biotissue sample. The temperature distribution in the biotissue target area was obtained. The time of reaching cryonecrosis across the biotissue thickness using this type of cryoapplicator was determined. Received data were compared with similar for an alternative cryoapplicator cooled with nitrogen dioxide. It was found that the use of liquid nitrogen as a cooling substance in the cryoapplicator in the atrial fibrillation treatment is more effective.

The key results are follows:
1. When using liquid nitrogen as a cryoagent in a cryosurgical apparatus, the condition of transmurality (freezing of the myocardial wall up to 10 mm thick), is performed in less than 1 minute.
2. The advantage of using liquid nitrogen as a cryoagent in a cryosurgical apparatus was revealed in comparison with circulating nitrogen dioxide. The cryoexposure time to the cardiac tissue decreases, and, consequently, the total surgical exposure time.
3. It is important for understand physician. If cryoapplicator reaches a steady state of operation increasing the time of cryoexposure on biotissue, regardless of the cryoagent, will not lead to an increase either the length or the volume of the frozen area.

What is more, it should be noted, that on the one hand it is important to achieve transmurality of the frozen extended area. On the other hand to prevent the excessive freezing of deep-lying tissues freezing modes must be observed. The required freezing modes for the developed cryoapplicator will be presented after more experiments and the collection of a sterling database.

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