Development of a single mode electromagnetic resonant cavity for rewarming of cryopreserved biomaterials

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Abstract

An electromagnetic (EM) heating system is developed to achieve the rapid and uniform warming of cryopreserved biomaterials. Using the heating system, a rectangular resonant cavity is excited in TE101 mode at frequencies near 434 MHz. In experiments, a spherical phantom of biomaterial with a diameter of 36 mm is placed at the center of the cavity. The phantom is first cooled down to about −80 °C within the cavity and then thawed by EM absorption. Results show that EM warming can produce much higher warming rate than conventional water-bath warming method. The spatial temperature distribution in the phantom during EM warming is also more uniform than that during the water-bath warming.

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From previous research in cryopreservation, it has been known that both cooling and warming process are critical for cryo-survival of living cells and tissues [4]. For a given cooling process, a rapid and uniform warming process is desired. This is because a rapid warming rate can prevent harmful ice re-crystallization, and a uniform temperature distribution (i.e., uniform warming) in the frozen sample during the warming process can eliminate the thermal-stress-induced fracture and damage [2]. However, it is impossible to achieve the rapid and uniform warming of a relatively larger biomaterial by using a conventional warming procedure (with heat conduction or convention in nature), e.g. warming of a frozen sample in air or in a stirred water-bath. This is largely due to the low thermal conductivity and high heat capacity of the biomaterials. Electromagnetic (EM) warming, which means heat is generated volumetrically everywhere inside the sample, seems to be a feasible approach to achieve both rapid and uniform warming of cryopreserved cells and tissues.

The study of electromagnetic thawing of cryopreserved tissues and organs began as early as 1970s. Ketterer et al. [3] reported the microwave warming of cryopreserved canine kidney. Success in thawing
canine kidneys using a microwave oven (at a frequency of 2450 MHz) was also performed by Guttman et al. [1]. Pegg et al. [6] attempted to repeat Guttman's experiments but failed. The major problem with microwave warming at the 2450 MHz is that the microwave penetration depth is limited and uniform heating may not be achieved. Lower frequencies are favorable for greater penetration depth. Ruggera and Fahy [9] reported using a resonant helical coil applicator operating at 20–30 MHz to heat frozen cryoprotectant solution rapidly and uniformly. Rachman et al. [7] introduced a UHF warming system, which was used to investigate the warming rate and uniformity of a rabbit kidney phantom. A cylindrical resonant cavity which can be excited in the TE111 and TM010 modes at about 434 MHz was designed. Robinson et al. [8] further developed this EM heating system with a cylindrical resonance cavity. Three fundamental modes of the cylindrical cavity are excited to provide electric field at the center. The frequencies used are 432, 434 and 438 MHz, respectively. Using this system, high warming rate was achieved and the spatial temperature differences in the frozen sample were less than 20 °C.

The objective of the present study is to design and develop an EM rewarming system which is capable of thawing of cryopreserved biomaterials rapidly and uniformly. One of the distinguished features of the present EM warming system is that the source frequency can be adjusted dynamically during the warming process to track the resonant frequency of the cavity.

It should be noticed that our system is almost the same type of EM heater as the one developed by Rachman and Robinson [7,8], except that we used a single mode resonant cavity, i.e., one RF signal source is used, while in their work, three signal sources were used. The main advantage of single mode resonant cavity is that the exact location of peak value of the electric field is known. If the size of the sample is much smaller than the wave length of the EM field, the field distortion caused by the sample is not significant. When the sample is placed at the center of the cavity, it will experience maximum heating effect. In addition, in this study we designed and developed a cavity with rectangular shape, which is easier to manufacture in comparison with the cylindrical cavity used in previously-reported research work. In experiments, frozen CPA solutions were heated and thawed by using both the developed rectangular cavity and conventional heating methods. Results are reported and compared.

**EM rewarming system**

The setup of the whole EM rewarming system is shown in Fig. 1. The whole system includes three major subsystems: microwave subsystem, cavity and cooling subsystem, thermal subsystem.

**Microwave subsystem**

This subsystem provides required microwave power, which is finally delivered to the resonant cavity to thaw/heat the cryopreserved biomaterial. The RF signal source is a frequency synthesizer, which is based on a 400–450 MHz voltage controlled oscillator (VCO). A relative narrow frequency step size, 15.625 kHz has been used in order to increase the accuracy of the frequency tracking. The outputs of the digital frequency synthesizer are fed directly to an 800 W solid state broadband power amplifier (Model SMCC800, IFI). A 20 dB dual directional power meter is used for power level and calibration. A 20 dB dual directional coupler is used for monitoring the reflected power.

**Thermal subsystem**

Power meter is used for monitoring the reflected power. Temperature sensors are used for measuring the temperature of the sample. Dummy load is used for testing the power meter. The schematic diagram of the whole EM rewarming system is shown in Fig. 1.

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**Fig. 1. Schematic diagram of the EM rewarming system.**
coupler (Model 3020A, Narda) is connected at the end of the feedlines to the cavity. The transmitted and reflected powers are measured by a power meter (Model E4419B, Agilent) through two power sensors. A circulator and dry dummy load (Model MFJ-264, MFJ Enterprises Inc.) are connected between the output of the power meter and the dual directional coupler to protect the system.

**Cavity and cooling subsystem**

Copper is used in construction of the resonant cavity because it has relative high electrical conductivity. Electrically conductive adhesive (CHO-BOND360) is used to seal the joints of the cavity walls to reduce microwave leakage. A probe antenna is placed at the cavity wall to excite the electric field. The cavity is designed to work at TE101 mode at the frequency of about 434 MHz. The dimension of the cavity is $0.40 \times 0.35 \times 0.68$ m. The Q-factor of the empty cavity is measured as 136410.

The system is designed to allow the sample to be cooled within the cavity. As shown in Fig. 2, a hollow Styrofoam cylinder is placed at the center of the cavity to hold the sample. The sample can be cooled by the cold nitrogen gas passing through the hollow cylinder.

**Thermal subsystem**

This subsystem consists of fluoroptic thermometer and temperature sensors which are used to measure the temperature rise of the sample during rewarming process. In this system Luxtron 790 fluoroptic thermometer (Luxtron Corporation), which is immune to electromagnetic interference (EMI) and high voltages, is employed. Two fluoroptic sensors are placed inside the sample at different locations to measure the temperature changes during thawing process. One is at the center of the sample, and the other one is at 1/2 radius from the center.

**PC control**

The frequency synthesizer, power meter, and fluoroptic thermometer are connected to a personal computer through different ports. A computer program written in Visual C++6.0 is used to control the whole system. The major functions of the program include: (1) monitoring the transmitted and reflected power; (2) set the frequency of the digital frequency synthesizer; (3) measuring the temperature of the sample.

The frequency controlling algorithm can be described as follows: first, set the starting frequency and the frequency step size of the digital frequency synthesizer, then read the transmitted and reflected power from the power meter, increase and decrease the frequency of the synthesizer by one step size, read the transmitted and reflected power again, the new frequency of the synthesizer is set while the value of transmitted power/ reflected power is maximum. The whole process is repeated until the

![Fig. 2. Schematic diagram of the cavity and cooling subsystem.](image-url)
heating process terminates. In our experiment, depending on the sample, the frequency shift during warming is within 200 kHz.

**Experimental results**

*Experimental results for testing the EM rewarming system*

Experiments are carried out on a test solution to validate the designed frequency tracking mechanism. The solution contains 0.05 M NaCl, 2 M DMSO and 0.167 M trehalose. The sample, together with the temperature sensors are then placed inside the resonant cavity. Cold liquid nitrogen gas is introduced to cool the sample down to about −80 °C, and then shut down and wait about 10 min to allow the sample to reach an equilibrium temperature. Then the power amplifier is turned on, the output power is set to 400 W. The controlling computer program is started to begin EM warming process.

To demonstrate and evaluate the effect of resonant frequency tracking designed in this system, in the first experiment, the frequency tracking function is turned off. The EM source frequency is set to the resonant frequency of the cavity when the test sample is introduced at −80 °C, and the value is kept constant during the whole warming process.

Fig. 3(a) shows the variations of temperature at two locations inside the sample during EM thawing without resonant frequency tracking. Then EM thawing experiment is carried out on the same sample with the frequency tracking function turned on, the output power of the amplifier is also set to 400 W. Experimental results are shown in Fig. 3(b). It can be seen that without resonant frequency tracking, the overall warming process is very slow. Even though there is not too much difference before 50 s after starting, but after that, without frequency tracking, temperature rises at a very slow rate. At 300 s, temperatures of the two sensors only reach about −15 °C. In contrast, for the warming process
with frequency tracking, it takes less 100 s for the temperatures of two sensors to reach $-15^\circ\text{C}$.

The resonant frequency of the cavity changes with the increasing of the sample temperature. Without frequency tracking, the system works at off resonant status. During the experiment, we notice that with the rising of temperature, the reflected EM power from the cavity increases accordingly. At $-15^\circ\text{C}$, the reflection EM power read from the amplifier reaches over 200 W. To avoid damage to equipment, the EM thawing process is terminated at this temperature. But with resonant frequency tracking mechanism turned on, because the source frequency can be adjusted dynamically to track the changing resonant frequency, the reflection EM power from the cavity can be kept very low during the whole warming process. Typical value of reflected power read from the power amplifier is less than 100 W and very steady.

This experiment shows that the resonant frequency tracking mechanism designed works efficiently. With resonant frequency tracking, not only the efficiency of EM warming is increased, but also the reflection power from the cavity is reduced.

To compare EM and conduction heating, we thaw the same sample in a water-bath at 20°C. Fig. 3(c) shows the experimental results. We can see that warming through conduction/convection is generally much slower, with the outside of the sphere taking over 25 min to reach 0°C. With EM heating, the outside of the sample reaches 0°C in about 4 min. For water-bath thawing, the warming rates vary greatly at different positions, leading to temperature difference up to 20°C between the two locations.

**EM thawing of dimethyl sulphoxide (DMSO) solutions**

EM rewarming experiments are carried out on DMSO solutions with different concentrations of 15%, 30% and 50% by volume. The output power of the power amplifier is set to about 400 W. Experimental results are shown from Figs. 3(d)–(f).

As can be seen in Fig. 3(d), for 15% DMSO solution, temperature differences between the two sensors are small in entire thawing process, but it takes a long time, about 300 s, for the cryopreserved solution to reach its melting point. The reason is that for 15% DMSO solution, at temperature below its freezing point, a large amount of ice crystals are formed, which makes the absorption of EM power difficult.

For 30% DMSO solution, as shown in Fig. 3(e), warming rate is faster than 15% DMSO solution, it takes about 200 s to thaw the sample, but temperature differences between the two sensors are much larger. The maximum temperature difference is about 20°C. When the sample begins melting, at about 125 s, the temperature difference is reduced to about 5°C.

Fig. 3(f) shows the variations of temperature at two locations inside the 50% DMSO solution. It can be seen that the warming rate is much faster than both 15% and 30% DMSO solutions. It takes only about 180 s to complete the thawing process. Meanwhile, temperature differences between two locations are very small during the whole thawing process.

These experimental results indicate that higher concentration of DMSO solution is favorable for EM thawing, for it can achieve both faster warming rate and more uniform temperature profile in the sample. This agrees with the results of Wusteman M.C. et al. [10] The uniformity and warming rate of samples are related to their dielectric properties. Michelson and Evans [5] have studied how the uniformity of heating depends on the dielectric properties of the solutions. A further study on the effects of dielectric properties of cryoprotectants on the EM heating is ongoing.

**Conclusions**

An EM warming system was developed for warming/thawing of cryopreserved biomaterials. Experimental results show that much more rapid and uniform warming can be achieved using the developed single mode, rectangular, resonance cavity in comparison with a conventional water-bath warming method.

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**References**