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A needle-form 3-omega sensor for thermal characterization of cryopreserved biological tissues

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Abstract. Thermal properties of cryopreserved tissues are critically important to the biopreservation community, which continues to seek more effective ways to store biological samples for improved outcomes in organ transplants as well as to facilitate the preservation of a record of biodiversity. Here, we present a reusable thermal needle-type 3-omega method designed for in situ characterization of such tissues, as well as other soft materials. The 3-omega method is a classic thermal materials characterization technique, which has been integrated into a modified microfabricated neural probe. This enables the measurement to be robust to environmental and experimental factors in cryopreservation. We demonstrate the viability of such a sensor to measure thermal conductivity for amorphous and crystalline solid samples of biological tissues, as demonstrated on 3mm thick chicken liver. These measurements can also be used for differentiation of solid samples, which is of particular interest for studies involving the kinetic limits of amorphous solidification (vitrification). In this, we demonstrate the value of a packaged thermal sensor to advancing the thermal understanding of cryopreserved biological systems and other solid-liquid phase change systems.

1. Introduction

Late-stage organ disease is one of the most common causes of death in the world [1]. Less than 50% of transplant waitlist patients will receive an organ in a given year, even in highly developed countries in North America and Western Europe [2], and rates are much worse in other parts of the world. The major technical barrier to improving rates and success rates of organ transplants is in increasing the amount of time for which we can store a viable organ [3].

To slow metabolic activity in order to prevent tissue death, organs should be kept as cold as possible, but their solidification into the crystalline structure associate with typical ice Ih causes ischemic cell death. Therefore, it is critical to prevent such ice formation from occurring in preserved tissues. There are several methods by which this can be achieved, one of which is preservation by vitrification, in which the tissue is rapidly cooled while suppressing ice formation, reaching a metastable amorphous/glassy phase [4].

Vitrification allows for nearly limitless storage times, and has been successfully demonstrated on mammalian organs as large as a rat liver [5]. However, there are still many medical and materials advances to be made in the process of scaling up to human organs. In order to do so, it is critical to be able to reliably sample thermal data for biological tissues under cryogenic conditions. To this end, we present a needle-form 3-omega (3ω) sensor for thermal conductivity.

2. Methods

The 3ω method is a mature thermal characterization technique in microfabricated materials and thin films [6]. It relies on a fabricated 4-point probe line, into which a constant AC current is applied. This causes time-periodic heating, the thermal response to which can is captured in the third harmonic (3ω) of the voltage signal measured across the central line.

The 3ω method has been demonstrated before on soft materials, including biological tissues [7], and other electrothermal methods have been used for room-temperature biothermal characterization [8]. Here, we demonstrate the 3ω method on cryopreserved tissues using a reusable microfabricated needle-form sensor, which is placed in the center of tissues.

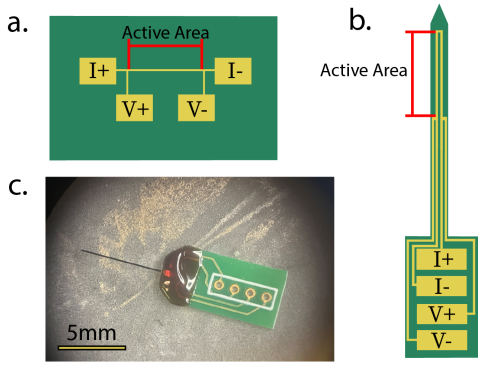

Figure 1. Schematic comparison of a. typical microfabricated planar 3ω sensor to b. needle-form sensor as presented in this work. c. Photograph of sensor.

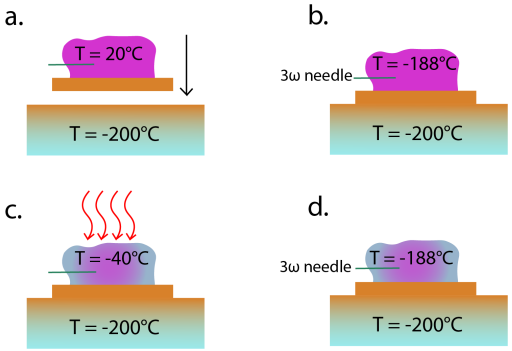

Figure 2. Protocol for vitrification and crystallization of single sample. a. Plunge cool by bringing into contact with cooled copper heat sink. b. Measure vitreous sample. c. Heat to incite crystallization. d. Measure crystalline sample.

Using the 3ω method with a needle-form sensor provides several advantages over other comparable approaches for use in cryobiology. Compared to a planar 3ω sensor, we achieve increased signal due to minimal parasitic effects from the fabrication substrate. Containing the line inside the tissue also avoids condensation near the sensor, which can can have deleterious thermal, electrical, and mechanical effects. Compared to other electrothermal techniques, such as transient hot wire, the 3ω method with frequency sweeping is notably robust to thermal contact resistances, which is particularly beneficial in highly variable systems.

The 3ω needles are a custom microfabricated design from NeuroNexus, Inc and can be seen in Figure 1c. Each needle is 6 mm in length and is comprisded of silicon with an internal gold line. The fabricated line operates on the principles of a 4-point probe, with a current run across the line via 2 connections, and the voltage measured across the line from the others, as depicted schematically in Figure 1a and 1b. The needle is 100 um wide and 15 um thick.

Data was collected using a Stanford Research Systems (SRS) SR830 lock-in amplifier and a custom circuit, which converts the built-in voltage source into a current source (currents used in this experiment were 6.82 mA) at the specified frequency. This circuit also uses an analog operation to cancel the first harmonic of the signal by running a similar phase-shifted current through an IET PRS-330 programmable resistor matched to the resistance of the measurement line. This leads to a better signal-to-background ratio in the third harmonic.

Sample temperatures were measured using an SRS SR630 and an Omega T-type thermocouple. These were validated using resistance thermometry on the line of the sensor, which was characterized before measurements. Data was taken on a standard frequency range of 1000 Hz to 2 Hz. At each frequency, a time-averaged voltage was recorded after settling.

Vitreous samples were created by adding cryoprotectants to samples before cooling, as is typical for such preservation protocols. Before cooling, 3mm chicken liver slices were soaked in 8.5M ethylene glycol (EG) for 36 hours. The sample was fixed to a thin copper plate, which was then cooled by contact with a copper heat sink quenched in liquid nitrogen.

To ensure that vitreous and crystalline measurements were taken on geometrically and thermally similar samples, a heat gun was used to raise the temperature of the vitreous sample to -50°C, where it was held for roughly 3 minutes, which included visual indication of crystallization. After the phase transition, the stable crystalline phase was brought back to the measurement temperature. The cooling and warming process is depicted schematically in Figure 2.

All measurements were taken at a temperature of -188.8±.5°C, indicating that any difference in thermal conductivity would not be due to changes associated with temperature.

3. Results

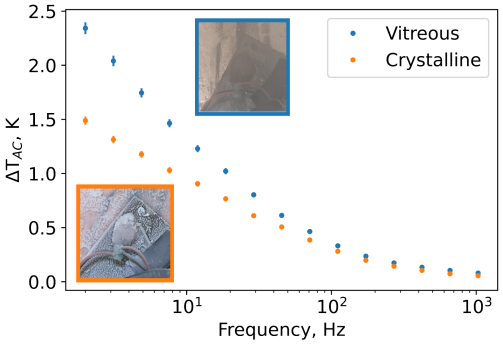

Figure 3. 3ω data for vitreous (blue) and crystalline (orange) chicken liver + 8.5M EG samples at -188.8°C. The diverging slopes indicate a thermal conductivity change.

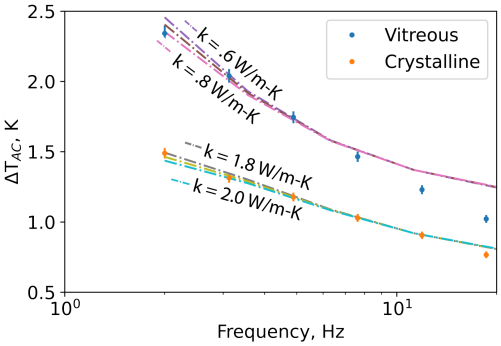

Figure 4. Fitting curves in the low-frequency regime. Points are from Figure 3 and dashed lines indicate simulated results of 3-D Comsol model. Curves are shown for liver thermal conductivity of k = .6, .7, and .8 $\frac{\text{W}}{\text{m-K}}$ (upper model curves) and k = 1.8, 1.9, and 2.0 $\frac{\text{W}}{\text{m-K}}$ (lower model curves).

Figures 3 and 4 show the AC temperature rise for the crystalline and amorphous sample, which is calculated from the measured 3ω voltage using the equation below. The 3ω voltage is a product of the 2ω heat transfer response to a time-periodic input with the 1ω electrical input. We can convert to an AC temperature rise, as shown in the plots above, by using the temperature coefficient of resistance of the sensor line and dividing out the voltage of the current.

$$\Delta T_{AC} = \frac{2V_{3\omega}}{\alpha_{TCR} I R_0}$$

Where $V_{3\omega}$ is the measured voltage of the third harmonic signal, α_{TCR} is the measured temperature coefficient of resistance of the sensor, I is the current provided by the current source, and R_0 is the average resistance of the active sensor line.

In Figure 4, fitting lines are included, in which experimental data is matched to a numerical simulation at the lowest frequencies of the curve. Frequencies below 20 Hz are shown, where the curve is most sensitive to the the surrounding tissue, as the penetration depth scales inversely with the square root of the oscillation frequency, and is insensitive to effects near the probe.

The simulation data was manipulated using an arbitrary constant offset. In 3ω analyses, thermal conductivity most strongly correlates with $\partial T/\partial f$, where T is the AC temperature rise

and f is frequency. Fitting to this value with an arbitrary offset parameter, as shown in Figure 4, is typical in 3ω studies. Offsets ranged from .62 K to 1.07 K.

From this slope fitting, it can be seen that the amorphous sample can be fit to approximately k = .8 $\frac{\text{W}}{\text{m-K}}$ and the crystalline sample can be similarly approximated as k = 1.9 $\frac{\text{W}}{\text{m-K}}$. While the form of the curves do not match well beyond very low frequencies, the fitted thermal conductivities agree well with previous experimental data for such systems. Previously measured biological systems have thermal conductivities between 1.5 and 2.5 $\frac{\text{W}}{\text{m-K}}$ in the crystalline states, while vitrified tissue samples have shown thermal conductivities between .8 and 1 $\frac{\text{W}}{\text{m-K}}$ [9].

4. Discussion

While these data agree well with accepted values, biological tissues are highly non-homogeneous and further evaluation of this methodology could be helped by investigating pure substances, especially water and cryoprotectants, which are well-studied at cryogenic temperatures [10].

It can be seen that in the numerical simulation, linear behavior began to break down much earlier with an increase in oscillation frequency. This, along with the slope changes being relatively small for an incremental increase in thermal conductivity, indicate that better characterization is needed for a more robust measurement. Future work will aim to refine the numerical model to better capture the real-world effects of the system.

Accurate characterization of geometric factors and contact resistances would also provide enough information to do parallel characterization of heat capacity in such systems, as then the offset parameter which was arbitrarily set in this analysis could be mapped to toher properties, which include heat capacity. This may also improve the accuracy of the thermal conductivity fitting. With sufficient precision, this method may also be able to determine the extent of ice formation in partially frozen tissues, a critical need in some preservation protocols [11].

The sensor and method demonstrated here already constitute a significant contribution to cryopreservation research. In addition to providing an *in situ* method for determining thermal conductivity on a wide variety of tissues during preservation, it can also be used to diagnose the solid phase of tissues without damaging cells.

Acknowledgments

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