Effect of Formalin Fixation on Thermal Conductivity of the Biological Tissue

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Effect of formalin fixation on thermal conductivity of the biological tissues is presented. A self-heated thermistor probe was used to measure the tissue thermal conductivity. The thermal conductivity of porcine aorta, fat, heart, and liver was measured before the formalin fixation and then 1 day, 4 days, and 11 days after formalin fixation. The results indicate that the formalin fixation does not cause a significant change in the tissue thermal conductivity of the tissues studied. In the clinical setting, tissues removed surgically are often fixed in formalin for subsequent pathological analysis. These results suggest that, in terms of thermal properties, it is equally appropriate to perform in vitro studies in either fresh tissue or formalin-fixed tissue. [DOI: 10.1115/1.3147745]

Keywords: tissue thermal conductivity, self-heated thermistor, bioheat transfer, formalin fixation, tissue fixation, formaldehyde

1 Introduction

The ultimate goal of this paper is to measure the effect of formalin fixation on the thermal conductivity of biological tissues. The tissue thermal conductivity was measured using the self-heated thermistor technique. This paper presents thermal data comparing the thermal conductivity of tissue samples before formalin fixation and 1 day, 4 days, and 11 days after formalin fixation.

The surgeon after removing tissue has three immediate options: freeze the tissue, keep it cold, or stabilize the tissue in a fixative. Each generates a different type of specimen for analysis [1]. Cells contain lysosomes that contain digestive enzymes. On cell death, these enzymes are released breaking down the cells, a process called autolysis. One way to halt autolysis is to fix the tissues. Presence of bacteria may break down the tissues by a process called putrefaction. Also the risk of infection of investigators working with human tissues, from a variety of modern pathogens, including the human immunodeficiency virus or the hepatitis B virus, has increased recently. New safety procedures are needed to reduce that risk. Fixatives arrest the autolysis and putrefaction and minimize the risk from contagious diseases [2,3]. Therefore, formalin fixation is widely used by both clinicians and researchers to study the tissue for prolonged durations after the tissue extraction.

Because biological processes and temperature are interdependent, heat transfer plays a major role in many diagnostic and therapeutic devices. Accurate knowledge of thermal properties is important in the design and testing of these devices. If formalin fixation were shown to not have large changes in thermal conductivity, then an abundant source of human tissues routinely collected in the pathology lab could be safely and appropriately used for in vitro bioheat transfer research. Therefore, knowledge of variations in the tissue properties due to the formalin fixation is vital for these researchers.

2 Background

The aim of fixation is to preserve the structural and biochemical constituents of cells in as close to in vivo conditions as possible. There are two major consequences of analyzing cells without fixation. First of all, cells are naturally present in a hydrated form, and the removal of intercellular water molecules, which are bound to macromolecules (proteins, phospholipids, and carbohydrates), can result in the collapse of internal structures, leading to the delocalization of biomolecular species [3]. The removal of cells from pH-buffered growth medium and subsequent air-drying can also influence the osmotic pressure within these cells, resulting in cell shrinkage or swelling. This may lead to membrane rupture and leaching of intercellular components. Second, fixation is necessary in cell biology to arrest autolysis, preventing protein denaturing, dephosphorylation of mononucleotides, phospholipids, and proteins [3].

Ferdinand Blum was the first person to use formaldehyde as a tissue fixative [4]. Most laboratories use neutral-buffered formalin (10%), which contains 4% formaldehyde. Formaldehyde is the most widely used universal fixative. It preserves a wide range of tissues and tissue components. The most important molecular change induced by formaldehyde is the formation of cross links between proteins, or between proteins and nucleic acids, involving hydroxymethylene bridges [5–7]. Formaldehyde is not a coagulating fixative, so tissues fixed in formaldehyde do not contain clumps of coagulated materials. Also the cellular detail is not distorted by the formation of coagulum [4]. Peculiar to formaldehyde is its slow formation of covalent bonds in aqueous solution yet rapid diffusion in tissue. Shrinkage of tissues is minimal in formaldehyde fixation. Tissues incompletely fixed in formaldehyde or fixed in formaldehyde at different temperatures may have different spatial characteristics than tissues fixed under dissimilar conditions [4]. The speed of fixation depends on the rate of diffusion of fixative into the tissue and the rate of chemical reactions with various components. In practice, it is assumed that these processes require at least 1 h/mm of tissue thickness [1,8]. Srinivasan et al. [1] have shown that it is extremely hard to extract usable DNA from formalin-fixed tissues for molecular biological studies. A peculiar characteristic of formaldehyde fixation is vesiculation of cell membranes [4,9]. Werner et al. [7] and Mason and O'Leary [10] showed that the cross-links and coordinate bonds may be responsible for the masking of epitopes by altering the three-dimensional structure of proteins, but the fixed proteins retain their secondary structure present before fixation.

Halliday [11] and Jones and co-worker [12,13] previously showed that formalin can preserve lipids by the reaction of hydrated formalin (methylene glycol) with double bonds of unsaturated hydrocarbon chains. However, Halliday found that the lipid content of the tissue may not be correctly estimated beyond 3 months [11–13]. Synchrotron-based Fourier transform infrared (SR-FTIR) microspectroscopy is a powerful bioanalytical tech-

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Table 1 Tissue description

Porcine tissue	Location of measurement	Dimensions, length by width by height (cm)
Aorta	Luminal surface	4.2×2×0.3
Fat	Internal surface of a cross section	4×2×0.5
Left ventricular myocardium	Internal surface of a cross section	4.5×2.2×0.5
Liver	Internal surface of a cross section	4×2.2×0.5

nique for the simultaneous analysis of lipids, proteins, carbohydrates, and a variety of phosphorylated molecules within intact cells. Gazi et al. [3] used the SR-FTIR microspectroscopy, to see the effect of formalin fixation on the cells. They found that the formalin-fixed cells retained the lipid content and also the molecular cell structure.

Thermal properties of biological tissue depend strongly on the relative composition of water, fat, and protein structures, with water content being the dominant factor [14,15]. Because formalin fixation does not cause major structural changes or shifts in water or fat, one would anticipate that the effects on thermal properties caused by fixation would be minimal.

3 Methods

Self-heated thermistors have been widely used for the measurement of tissue thermal properties [14–20]. Normally, thermistors are used to measure temperature, but in the self-heated thermistor technique they are also used to heat the tissue samples, in order to measure the tissue thermal properties. The experiments were performed using glass encapsulated bead thermistor probe, P60DA102K (radius=0.75 mm, 1 kΩ at 25°C) from Thermometrics. The probe used for this study was constructed by inserting this thermistor inside a plastic syringe, such that about 0.5 mm of the thermistor bead protruded from the syringe and rest of the syringe was filled with silicone for thermal and electrical insulation [21]. The pulse-power integrated-decay technique was used to measure the tissue thermal conductivity [21]. In this technique the thermistor was heated for about 3 s (during which about 5.35 mW power was applied across the thermistor) and then allowed to cool. The temperature and power across the thermistor was continuously measured during the 3 s heating phase followed by 3 s cooling phase. The thermal conductivity of tissue was measured, while the tissue was maintained at 38°C. The maximum temperature rise was less than 2°C, ensuring no significant thermal damage of the tissue. An empirical calibration process, performed in glycerol and agar-gelled water, provides accurate thermal conductivity measurements. The average measurement accuracy of this technique is about 1.2% [21].

Most biological tissues are anisotropic. Finite element studies have shown that the depth of penetration of the temperature field into the tissue is about five thermistor radii [21], which is about 4 mm for the probe used in this study. Furthermore, these temperature fields are spherical with respect to the center of the thermistor. This 4 mm depth is large compared with the anisotropy of muscle fibers and arterial walls, meaning much of the anisotropy of the tissues will be averaged into a single lumped average thermal conductivity measurement.

Fresh samples of porcine aorta, fat, heart, and liver tissues were used for the experiments. The fat, heart, and liver samples were cut, creating an internal surface on which the measurements were collected. The details of the tissue samples have been summarized in Table 1. All tissue samples were placed on cotton gauze lightly soaked in saline solution (isotonic sterile aqueous solution containing a borate buffer system and sodium chloride) in order to prevent water loss from the tissue during the experiments. During the measurements, the tissue samples were kept in a water-tight chamber immersed in a temperature-controlled water bath, maintained at 38°C. The thermistor probe was placed on the tissue surface, and the measurements were taken only after the initial baseline temperature measured by the thermistor was stable (less than 0.03°C/s). External weights were used to hold the thermistor probe on the tissue, exerting a light but constant pressure of about 150 g/cm^2. The pressure was applied to ensure that all the tissue samples had a firm and uniform contact with the thermistor surface, without squeezing out any water out from the tissue and that none of the thermistor surface was exposed to air. This pressure did not cause any visible damage to the tissue. There were four groups: aorta, fat, heart, and liver. The thermal conductivity measurements were first recorded at eight or more different places in each group (fresh tissue, before formalin fixation). After the initial measurements, all the groups were immersed in 10% neutral-buffered formalin solution and refrigerated in closed glass bottle. After 1 day, tissues were taken out of the air-tight glass bottles and the thermal conductivities were recorded at eight or more locations (postformalin fixation). The tissues were again immersed in formalin and refrigerated. In a similar fashion thermal conductivity of all groups was measured after 4 days and 11 days (postformalin fixation). The thermal conductivity values of tissue samples as obtained by previous researchers have been summarized in Table 2 [22].

4 Results

The calibrated values of the thermal conductivity for all the tissue samples are summarized in Fig. 1. The measurements were

Table 2 Thermal conductivity of unperfused tissues [22]

Tissue	Thermal conductivity (mW/cm°C)
Aorta (human)	4.76
Fat (porcine, subcutaneous)	1.5–1.7
Fat (human, subcutaneous)	2.3–2.7
Cardiac muscle (porcine)	5.33
Liver (porcine)	5.28

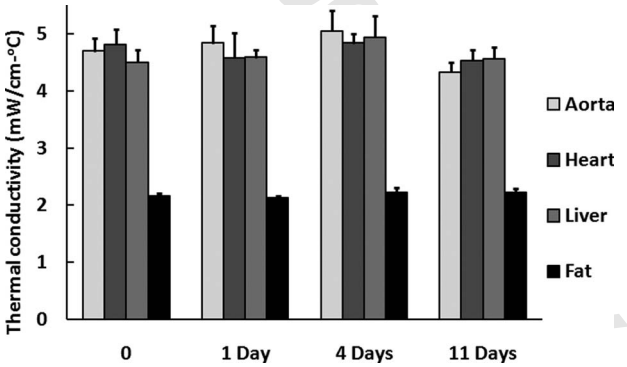

Fig. 1 Thermal conductivity measurements for all the tissue samples

Table 3 Summary of thermal conductivity measurements for all tissue samples (*=mW/cm°C, 0=preformalin fixation) [23]

Tissue type	Time (days)	Mean (*)	No. of samples	Std. deviation (*)	Kurtosis (A)	Std. error of kurtosis (B)	(A/B)	Skewness (C)	Std. error of skewness (D)	(C/D)
Aorta	0	4.70	9	0.23	−0.34	1.40	−0.24	1.06	0.72	1.48
Aorta	1	4.84	9	0.31	−1.64	1.40	−1.17	0.31	0.72	0.44
Aorta	4	5.05	9	0.35	0.14	1.40	0.10	0.96	0.72	1.34
Aorta	11	4.33	9	0.17	−1.57	1.40	−1.12	0.04	0.72	0.05
Fat	0	2.16	10	0.06	0.03	1.33	0.02	−0.96	0.69	−1.39
Fat	1	2.13	10	0.04	−0.25	1.33	−0.19	0.55	0.69	0.80
Fat	4	2.23	10	0.07	−0.37	1.33	−0.27	0.09	0.69	0.12
Fat	11	2.23	10	0.07	−1.19	1.33	−0.89	0.10	0.69	0.14
Heart	0	4.81	8	0.26	1.82	1.48	1.23	−1.23	0.75	−1.64
Heart	1	4.57	8	0.45	0.04	1.48	0.03	−0.79	0.75	−1.05
Heart	4	4.84	8	0.17	1.46	1.48	0.99	−1.14	0.75	−1.51
Heart	11	4.53	8	0.19	1.59	1.48	1.07	−0.97	0.75	−1.29
Liver	0	4.50	9	0.22	1.06	1.40	0.76	0.82	0.72	1.14
Liver	1	4.58	9	0.13	−1.00	1.40	−0.71	−0.61	0.72	−0.85
Liver	4	4.94	9	0.38	2.78	1.40	1.99	1.35	0.72	1.89
Liver	11	4.56	9	0.20	−1.18	1.40	−0.84	−0.53	0.72	−0.73

Table 4 Summary of post hoc test results showing no significant difference between various data sets (p=0.05; T=Tamhane's T2, D=Dunnett's T3)

Preformalin fixation tissue ↓/postfixation day →	Day 1	Day 4	Day 11
Aorta (t=0)	T and D	T and D	T and D
Fat (t=0)	T and D	T and D	T and D
Heart (t=0)	T and D	T and D	T and D
Liver (t=0)	T and D	T and D	T and D

performed on each tissue group at eight or more different locations. Each thermal conductivity value in Fig. 1 is the average of measurements, for that group.

5 Discussion

Statistical analysis was performed using SPSS 16.0 (SPSS, Chicago, IL). The distribution of each data set was tested for normality by evaluating the standard error of skewness and kurtosis. The calibrated values of thermal conductivity along with the statistical analyses for all the tissue samples are summarized in Table 3. As seen from the Table 3, calculated standard values (ratios A/B and C/D) for all tested data sets were between −2 and 2, thereby indicating normally distributed data. Levene's test for equality of variances was implemented to determine if equal variances could be assumed for the analysis of variance (ANOVA) and post hoc tests. If the significance of the Levene's test is greater than 0.1, equal variance could be assumed. The data sets showed that significance was less than 0.01 and hence equal variance was not assumed.

To further determine which data sets were significantly different, two post hoc tests were conducted. Since the data sets were normally distributed and equal variance could not be assumed, Tamhane's T2 and Dunnett's T3 post hoc tests were performed. The summary of both post hoc tests showing no significant difference between various data sets is shown in Table 4. If the significance of the post hoc tests was greater than 0.05 (p=0.05), the null hypothesis concerning the population means was accepted (the mean values of the two data sets being compared were assumed not to be significantly different). As seen from the Table 4, both the post hoc tests showed that the mean values of all the four groups before formalin fixation were not significantly different from the mean values recorded after 1 day, 4 days, and 11 days of formalin fixation.

The major sources of noise in these experiments come from thermistor-tissue contact and thermal fluctuations in the water bath. The same thermistor and the same water bath were used throughout the study, but each of the measurement involved a separate probe-tissue contact and was measured at a separate time. Therefore, it is reasonable to assume the noise is independent both between sets and within sets. The slight variation in the thermal conductivity of various groups post formalin fixation could be due to variations in the tissue water content.

6 Conclusions

Proper thermal contact between thermistor probe and tissue sample and temperature stability are extremely important for this method to work successfully and are the main factors that may have introduced errors in these experiments. The results clearly indicate that the thermal conductivity measurements before formalin fixation and after formalin fixation are not very different for porcine aorta, fat, heart, and liver tissues. Thus, we can conclude that the formalin fixation does not have a large effect on the tissue thermal conductivity of biological tissues. These results suggest that human tissues routinely collected for pathological analysis could be appropriately used for in vitro bioheat transfer research.

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