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Original Article

Thiel-embalming technique: investigation of possible modification in embalming tissue as evaluation model for radiofrequency ablation

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Abstract

Contrary to freezing preservation and formalin embalming, Thiel embalmed cadaver presents soft texture and color very close to that of living organism, and many applications based on Thiel embalmed cadavers have been reported. However, Thiel embalmed cadavers cannot be used as reliable evaluation model for radiofrequency ablation (RFA) due to dramatic changes of electrical conductivity in the embalmed tissue. To address this issue, we investigated various modifications of the original Thiel embalming solution. By altering the chemicals' species and concentration we figured out a formula that can greatly reduce the embalming fluid's electrical conductivity without significantly compromising the 18-day embalmed kidney samples' suppleness and color. We also investigated a two-stage embalming technique by first submerging the kidney sample into original Thiel's tank fluid for 28 days, then the sample was withdrawn from the tank fluid and placed into modified dilution fluids for additional two weeks. Stiffening and discoloration occurred in these diluted samples implying the reversibility of Thiel-embalmed tissues' suppleness and color with the removal of the strong electrolytes. This study presents a modified embalming method which could be used for RFA evaluation and also helps our understanding of the mechanism of embalmment process.

Keywords: Cadaver embalmment, Thiel's embalming fluid, electrical conductivity, radiofrequency ablation

Introduction

Thiel-embalming method[1–2] is a soft-fix embalming method developed by W. Thiel in 1990s, and since its first report in 1992[3], has produced cadavers that are both flexible and able to be long preserved, suitable for many training and research activities[4–6]. Contrary to conventional preservation techniques like freezing preservation and formalin embalming[7], the flexibility of the cadavers is excellent from the start and no additional measures are needed to achieve this[8].

Due to the unique advantage of Thiel embalmed cadavers, they can be employed in multiple research and training procedures such as arteries research[9],

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evaluation of mortuary cooling equipment[10], MRI research, ventilation research, ear motion[11], orthopedic studies[12], research on explanted organs[8,13–14], anesthesia[15–16], oral surgery and[17], neural modulus[18–19], spinal motion[20], and developments of new surgical devices or evaluation of a surgeon's skill[4,21–22].

Despite the outstanding properties of Thiel's embalmed cadavers for various applications in biomedical and biomechanical research, a number of studies have described the unsuitability of the Thiel-embalmed cadavers for studies into failure loads of implants due to significant discrepancies in biomechanical properties between embalmed cadavers and living tissues. It is recommended that such differences need to be taken into consideration when planning studies and interpreting the findings[8,23]. Thiel embalmed tendons for biomechanical investigation was also dissuaded from since the Thiel embalmed tendons did not "faithfully represent the biomechanical characteristics of fresh frozen tendons"[24]. Fessel *et al*, in 2011, by finding the unsuitability of Thiel embalmed for biomechanical studies, suspected that the diminished elastic modulus and failure strength of Thiel preserved tendons may be related to collagen denaturing associated with the high salt (boric acid) concentration in the Thiel embalming solution[24–25].

Hence, Thiel specimens cannot be recommended for biomechanical load to failure test because of a significantly altered failure strain and plastic energy absorption[15,26–27]. The plastic energy absorption of human and bovine specimens was altered significantly by Thiel's method[28]. Moreover, loss of signal and contrast when Thiel-embalmed human cadavers are imaged using clinical magnetic resonance imaging (MRI) sequences, especially those based on spin-echo MRI[29–30]. Thiel-embalmed organs cannot be used as reliable model for radiofrequency ablation evaluation[31] due to the much increased electrical conductivity by the embalming process[32–33]. The aim of this study was to modify the original Thiel embalming technique and produce embalmed organ with electrical conductivity comparable to its normal organ in order for radiofrequency ablation evaluation.

Materials and methods

To understand Thiel's fluid, Benkhadra *et al* conducted a histological comparison between fresh cadavers, formalin preserved cadavers and Thiel-embalmed cadavers[34]. Microscopic images showed that the muscle fibers had a cut-up "minced" appearance, but remained aligned, with conjunctive collagen fibrils also undisturbed, leading them to give the conclusion that the exceptional suppleness of these cadavers was caused by boric acid for its very corrosive effects on proteins[34]. However, the mechanism proposed by Benkhadra *et al* is questionable. If the pH of the Thiel's tank fluid is taken into consideration, one would easily found that the solution is nearly neutral or slightly alkaline (***Table 1***). The corrosive effect of acids is the consequence of high concentration of hydronium ion, but boric acid in this case is almost neutralized by the basic ionic salt such as sodium sulphite, and the concentration of H^+ is not high enough, as is indicated by the pH value, thus not capable to corrode biological tissues as a protonic acid.

While boric acid is able to react with substances with two or more neighboring hydroxyl groups such as saccharides to form stable chelates. The formations of chelate by boric acid and glucose chain in the

Table 1 **Measured pH and electrical conductivity data from each component of the Thiel's embalming fluid**

Chemical*	Concentration in Thiel's tank fluid	Electrical conductivity of individual solution	pH
Boric acid	3.6 g/100 mL H_2O	1.384×10^{-4} Sm^{-1}	4.38
Potassium nitrate	6.0 g/100 mL H_2O	7.25 Sm^{-1}	9.37
Sodium sulphite	8.4 g/100 mL H_2O	6.83 Sm^{-1}	9.24
Ammonium nitrate	12.0 g/mL H_2O	13.33 Sm^{-1}	5.86
Ethylene alcohol	None in tank fluid	7.16×10^{-3} Sm^{-1}	7.18
Propylene glycol	12.0 mL/100 mL H_2O	7.31×10^{-3} Sm^{-1}	7.46
8.9% Formalin	10.0 mL/100 mL H_2O	9.41×10^{-3} Sm^{-1}	6.54
Thiel's tank fluid	–	11.09 Sm^{-1}	7.27

Note: *Chemical used: potassium nitrite (Alfa Aesar, 500 g, 97% cas 7758-09-0, shore road, Heysham, la3 2xy, England), Boric acid (99 + %, Alfa Aesar, cas 10043-35-3, bond street, ward hill, MA 01835), sodium sulphite (anhydrous, 98%. 500 g, cas 7757-83-7, shore road, Heysham, la3 2xy, England), Ammonium nitrate (99%, cas 6484-52-2, VWR chemicals, Prolabor, Leuven Belgium), 1,2-propanediol (99 + %, cas 57-55-6, Merck KGaA, 64271 Darmstadt, Germany), Ethanol (99.8 + %, cas 64-17-5, Sigma-Aldrich, 3050 Spruce Street, St louis, MO 63103), formaldehyde, 37% in aq. Sln stab 10%-15% methanol liquid, cas 50-00-0, Alfa Aesar) glycoprotein might be able to reduce intercellular adhesion by reducing hydrogen bonding and possibly covalent bond as well. However, boric acid alone seems not to be able to make such drastic change of Thiel-embalmed cadavers. Propylene glycol, with its high concentration in the embalming solution is suspected to soften the embalmed tissue by weakening hydrogen bond and damaging cellular membrane structure for its amphiphilic property (soluble both in water and lipid). Also, the high salinity, due to high concentration of inorganic solutes, might contribute to the extraordinary suppleness, since high salinity is usually associated with high ionic strength in the solution which leads to higher solubility to some substances. Our measurement results have demonstrated that electrolytic components, i.e., potassium nitrate, ammonium nitrate and sodium sulphite, are the main contributors to the high electrical conductivity of Thiel's embalming. These three electrolytes are all strong electrolytes and have good solubility in aqueous environment. By solving some substances that are originally insoluble, biological tissue might become softer with increased hydration. Nonetheless, boric acid is not the sole contributor to the suppleness, which is also confirmed by a personal communication with Miss Amanda Hunter, a PhD student at Centre for Anatomy & Human Identification (CAHID) in University of Dundee, who stated that "The mouse embalmed with embalming solution without boric acid seemed to be soft enough as well".

Moreover, the pinkish or reddish color of Thiel-embalmed cadavers can be attributed to the existence of nitrate. Nitrate itself is not directly engaged in the color preserving but its product nitrite is highly suspected to play a vital role. Formaldehyde and sulphite are both agents with considerable reducing capability and can reduce nitrate to yield nitrite. Nitrite and its gaseous products, nitrogen monoxide NO and nitrogen dioxide NO_2 can all coordinate to the ferrous ion Fe^{2+} in myoglobin in myocytes (muscle cells) and hemoglobin in blood red cell. The formation of the coordinate compound of Fe^{2+} with nitrite, NO or NO_2 leads to the pinkish appearance of embalmed cadavers. So is the same in meat industry. To keep meat products, especially red meat product, it is the usual practice to add small amount of sodium nitrite to keep the product in inviting color and from decaying. However, the reaction is not that quick, while the products are supposed to be slowly released into the solution since the solution is not acid enough to realize quick release. Though slow, the reaction can take long enough since the concentration of nitrate, sodium and formaldehyde is high enough.

Nitrite embalmment

To achieve lower concentrations of electrolytes, we substituted nitrite for nitrate. Eight candidate embalming fluids were designed, listed in ***Table 2*** in comparison with Thiel's original tank fluid. Fluid 1 has no electrolytic component in it at all, which aimed to understand the role of the electrolytic components as a whole. The use of sodium sulphite in fluid 2 was aiming to know the role of nitrate/nitrite. Potassium nitrite is the only strong electrolyte in it, which is to understand the role of sulphite. Fluid 4 is a combination of fluids 2 and 4 in order to explore possible synergism of the two. Fluids 5 to 8 have more electrolyte than the first four, trying to meet the balance between the properties of embalmed samples and their conductivity. The electrical conductivity and pH of the eight solutions were measured (***Table 3***). Porcine kidneys were purchased from local butcher shop (Scott Brothers, Dundee, UK). The fresh porcine kidneys were harvested from pigs sacrificed within 48 hours and had been under refrigeration since then. The selected samples had similar size and appearance.

Table 2 **Formulae of candidate embalming solutions**

	Thiel's	Fluid 1	Fluid 2	Fluid 3	Fluid 4	Fluid 5	Fluid 6	Fluid 7	Fluid 8
Hot tap water/mL	500	500	500	500	500	500	500	500	500
$B(OH)_3$/g	18	18	18	18	18	18	18	18	18
NH_4NO_3/g	60	-	-	-	-	6	3	3	3
KNO_3/g	30	-	-	-	-	-	-	-	-
KNO_2/g	-	-	-	3	3	3	1.5	3	1.5
$NaSO_3$/g	42	-	4.2	-	4.2	4.2	8.4	4.2	21
Propylene glycol/mL	60	60	60	60	60	60	75	75	75
Stock Ⅱ/mL	12	12	12	12	12	12	18	18	18
8.9% formalin/mL	51	51	51	51	51	51	51	25.5	25.5

Seven porcine kidneys were embalmed in the fluids for 14 days before we measured their electrical conductivity.

Electrolyte dilution

In this experiment study, porcine kidneys were embalmed with Thiel's tank fluid for 28 days instead of just 18 days used in previous experiments. The purposes were to make sure the sample completely Thiel-embalmed and to also compare duration's influence upon the electrical conductivity. Based on Thiel's original tank fluid, dilution fluids were designed by removing the strong electrolytes while the only weak electrolyte boric acid was kept. Chlorocresol (pre-mixed with propylene glycol, mixture referred to as stock Ⅱ) concentration did not change since it is assumed that this antiseptic agent does not greatly affect the overall quality of the embalmed tissues but purely prevents them from decaying. There were some minor adjustments of the other components. Propylene glycol was increased in dilution fluid 3 to 90 *g* per every 500 mL water but in dilution fluids 1 and 2, the dosage remained unchanged. Since the drop in ionic strength would probably lead to increase of stiffness in the embalmed tissues and formalin can stiffen the tissue, formalin was reduced to its one tenth in dilution fluids 2 and 3. In addition, ethanol is added in all three dilution fluids to strength anti-bacterial activity. Detailed formulae were shown in ***Table 4***.

All three dilution solutions were odourless, achromatic and transparent. Electrical conductivity and pH of the dilution fluid were measured as shown in ***Table 5***.

Three porcine kidneys that had been previously embalmed in Thiel's tank fluid for 28 days were later transferred in to three dilution fluids, respectively. Before dilution process, conductivity measurement was conducted. Then 800 mL of the dilution fluid was used to embalm each kidney for one week, after the first week the dilution fluids was then renewed with the same dilute solution, 800 mL of each. Two weeks of dilution was then followed by conductivity measurement.

Conductivity measurement

The electrical conductivity (DC) meter used was from Mettler TOLDO company and the pH meter was SevenEasy Mettler TOLEDO INV083, with glass Helectrode Mettler TOLEDO InLab @Expert ProPH. For RF ablation, electrical conductivity frequency response is required. To obtain the bio-impedance/conductivity of the samples, a measuring setup was established (***Fig. 1***).

Table 3 **Measurement of modified fluids**

	Electrical conductivity (DC)	pH	Note
Thiel's tank fluid	11.09 Sm^{-1}	7.27	Clear and transparent
Fluid 1	7.91×10^{-3} Sm^{-1}	4.26	Clear and transparent
Fluid 2	4.79×10^{-1} Sm^{-1}	6.81	Clear and transparent
Fluid 3	4.78×10^{-1} Sm^{-1}	5.13	Brownish, transparent
Fluid 4	8.46×10^{-1} Sm^{-1}	6.81	Clear and transparent
Fluid 5	1.248 Sm^{-1}	5.38	Brown but transparent and emitting plenty of achromatic gas, very unstable
Fluid 6	1.095 Sm^{-1}	6.61	Clear and transparent, slightly green
Fluid 7	1.075 Sm^{-1}	6.14	Clear and transparent, slightly yellow
Fluid 8	2.19 Sm^{-1}	7.72	Clear and transparent, almost colorless

Table 4 **Formulae of dilution fluids**

Component	Dilution fluid 1	Dilution fluid 2	Dilution fluid 3
Hot tap water/mL	500	500	500
Boric acid/g	20	20	20
Propylene glycol/mL	60	60	90
Stock Ⅱ/L	18	18	18
Formalin (8.9%)/mL	51	5.1	5.1
Ethanol/mL	30	60	30

Table 5 **Measurement of dilution fluids**

	Dilution fluid 1	Dilution fluid 2	Dilution fluid 3
pH	4.205	4.742	4.195
Electrical conductivity (DC)	6.80×10^{-3} Sm^{-1}	1.672×10^{-3} Sm^{-1}	6.76×10^{-3} Sm^{-1}
Appearance	Achromatic and transparent	Yellowish but transparent	Achromatic and transparent

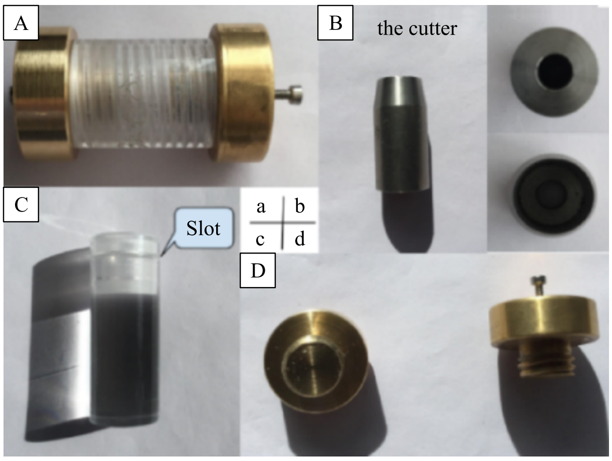

Fig. 1 **Tissue electrical impedance measurement sampling gadgets.** A: the measuring tube in assembled state; B: the steel cutter; C: plastic tube; D: electrodes/sealing lids.

The measuring tube is composed of two metal electrodes and a plastic hollow tube. When in use, the two electrodes are linked to a commercialized impedance analyzer (C60 Impedance-Amplitude-Phase Frequency Response Analyzer, Cypher Instrument Company) with sample of interest sealed in the chamber. The cylindrical chamber has length of 10.54 mm and round cross section whose diameter is 12.0 mm. The steel cutter has inner diameter of 12 mm that can presumably cut tissues into cylindrical pieces with diameter of 12 mm. The plastic mold has the same diameter but has a slot that allows a scalpel to insert in and cut tissue in to desired length. The distance between the slot and the bottom of the hollow space is 11.54 mm. Two electrodes are used to contact the measured tissue and also help to seal the measuring chamber. Besides, a scalpel, from Swann-Moston, Sheffield, England is sterilized with 75% percent of ethylene alcohol (v/v) prepared from pure chemical, purchased from VWR Co. and tap water.

The result given by the built-in software was just conductance G of the tested tissue, while their conductivity κ are given by further calculation with Eq. (1) below,

$$\kappa = G \cdot \frac{l}{A} \quad (1)$$

The length l was 10.54 mm (or 1.054×10^{-2} m) and the cross-sectional area $A = \frac{\pi}{4} \cdot d^2 = 1.13 \times 10^{-4}$ m^2. Conductivity over the range from 10 Hz to 1 MHz was calculated.

Results

Photographs were taken of the embalmed kidneys in comparison with an *ex vivo* fresh porcine kidney (***Fig. 2***). All nine embalmed porcine kidneys, including Thiel's original, were odourless despite there was some unpleasant smell of the volatile and pungent formaldehyde in the embalming fluids. Modified Theil's embalming solution has made specimen with profound color change: Thiel-embalmed porcine kidney looked pale and pinkish. Moreover, after embalmment of 18 days, Thiel's tank fluid used in the embalming process became red, opaque and turbid. However, the modified solutions remained transparent although their color became considerable dark. Among the four embalmed porcine kidneys using the modified fluid, the one embalmed with modified solution 4 seemed to be closer to *ex vivo* one, even closer than the Thiel-embalmed. The reason can be the synergism of sulphite and nitrite in the solution since modified solution 4 was the only one that has both sulphite and nitrite in it, others had only either of the two chemicals.

The embalmed porcine kidneys are assessed by hand touching and feeling. Comparison was drawn in term of suppleness. The *ex vivo*, the freshest of all, was the softest, and was less likely to rupture if under pulling or twisting force, followed by Thiel-embalmed one, which felt like rubber, still soft but not as soft as the *ex vivo* one. However, as for the four modified kidneys, case is different. They tend to be hard and felt like rubber made eraser, much hardened by the embalming process. A further investigation of their elasticity will be carried out in the near future.

Porcine kidneys embalmed by fluids 6, 7 and 8 are generally suppler than the rest. The suppleness difference can be clearly sensed, showing great probability that the newly embalmed samples had significant advantages over other fluids. Among samples 6, 7 and 8, sample 8 stood out, whose suppleness was comparable with Thiel-embalmed samples and even closer to *ex vivo* samples. And as shown in ***Fig. 2B***, unlike modified samples 6 and 7, which looked a little compressed for their smaller size and flattened surface as a consequence of contact with the container's bottom, modified sample 8 had smooth surface and no clear deformation in shape.

Measured results of the electrical conductivity of the samples are shown in ***Fig. 3*** and ***Table 6***. The embalmed samples' conductivities have dramatically decreased to the extent that they are comparable to the fresh (unembalmed) sample.

Mathematical modeling was used to find the relationship between the conductivity of embalming solutions and that of the embalmed porcine kidneys. Blue points in a scatter graph (***Fig. 4***) represents each modified formula and Thiel's formula as well. A linear curve was obtained as drawn in the chart above, which is formulated in Eq. (2), where y represents conductivity of the embalmed porcine kidney and x the conductivity of the embalming fluids.

$$y = 0.195246 \cdot x + 0.115236 \text{ Sm}^{-1} \quad (2)$$

Pearson's correlation coefficient was calculated to be 0.999 (n=7) indicating significant linearity between the two. Therefore, it could be possible to use this equation to predict the electrical conductivity of the embalmed porcine kidneys by the measured or known conductivity of embalming fluid. We could conclude that that the embalming fluid directly changes the electrical conductivity of the embalmed organs.

Electrolyte dilution

The conductivity before and after dilution are compared as shown in ***Fig. 5***.

The three samples' conductivities have been significantly reduced even to the level that they were comparable to *ex vivo* porcine kidney's conductivity. Data have shown the dilution method is successful in removing the ionic components in the Thiel-

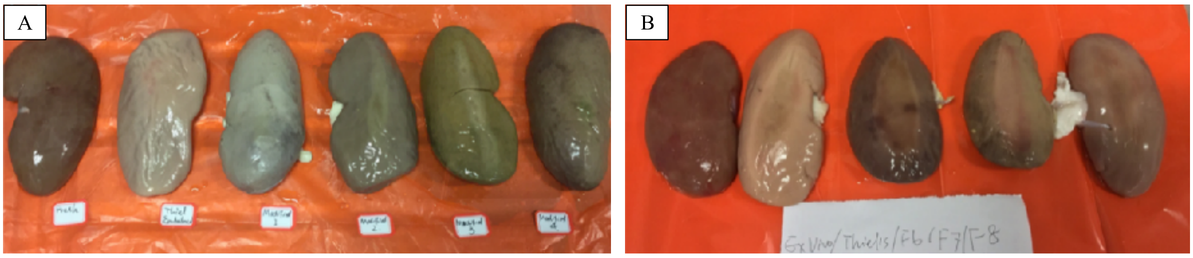

Fig. 2 **Porcine kidneys embalmed by candidate fluids for 14 days in comparison with the fresh and Theil's.** A: From left to right, fresh, Thiel's, samples by fluid 1, fluid 2, fluid 3 and fluid 4; B: From left to right, fresh, Thiel's, samples by fluid 6, fluid 7 and fluid 8.

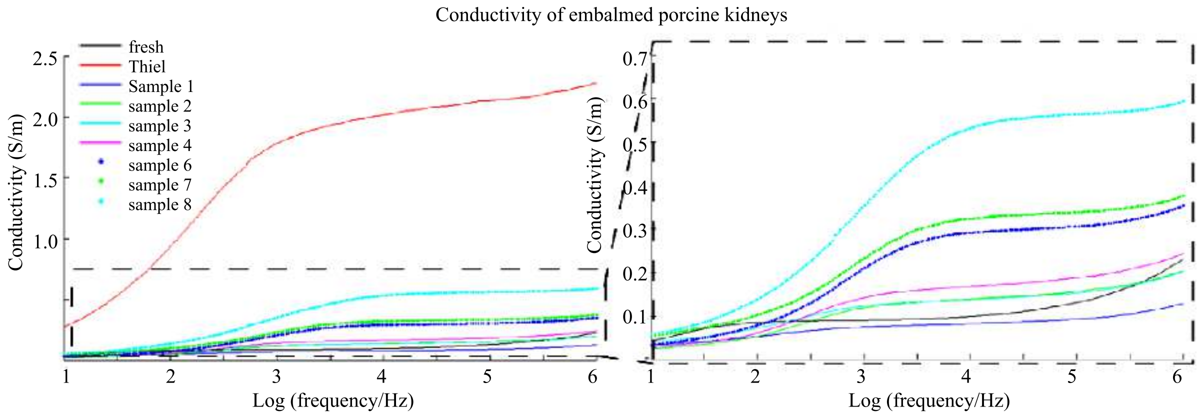

Fig. 3 **Samples' electrical conductivity measure in a range from 10 Hz to 1 MHz.**

Table 6 **Porcine kidneys' electrical conductivity at 500 kHz and 1 MHz**

Frequency	500 kHz	1 MHz
Ex vivo	0.188 Sm^{-1}	0.231 Sm^{-1}
Thiel-embalmed	2.216 Sm^{-1}	2.276 Sm^{-1}
Modification #1	0.112 Sm^{-1}	0.129 Sm^{-1}
Modification #2	0.183 Sm^{-1}	0.203 Sm^{-1}
Modification #3	0.181 Sm^{-1}	0.202 Sm^{-1}
Modification #4	0.244 Sm^{-1}	0.220 Sm^{-1}
Modification #6	0.353 Sm^{-1}	0.330 Sm^{-1}
Modification #7	0.376 Sm^{-1}	0.356 Sm^{-1}
Modification #8	0.593 Sm^{-1}	0.576 Sm^{-1}

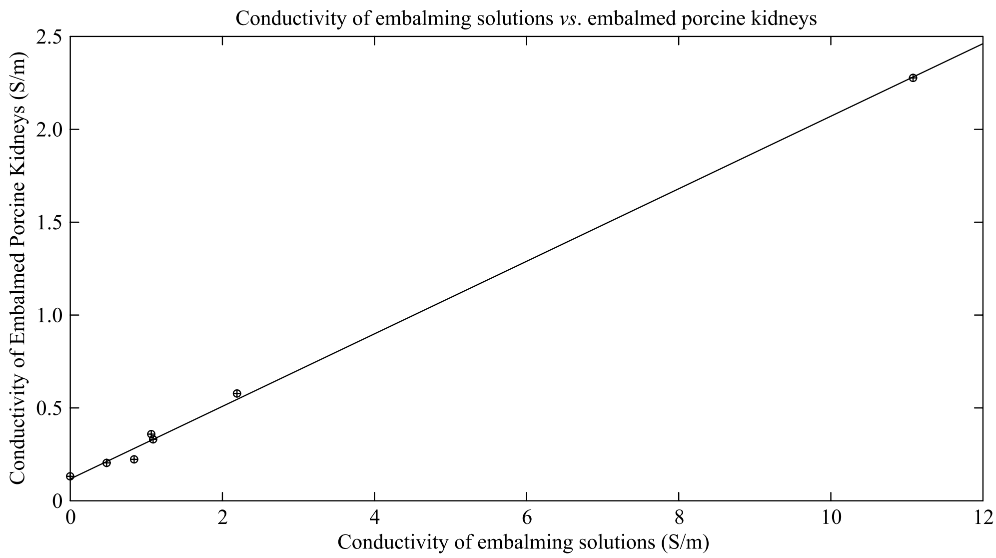

Fig. 4 **The embalmed samples electrical conductivity as a function of embalming solutions.**

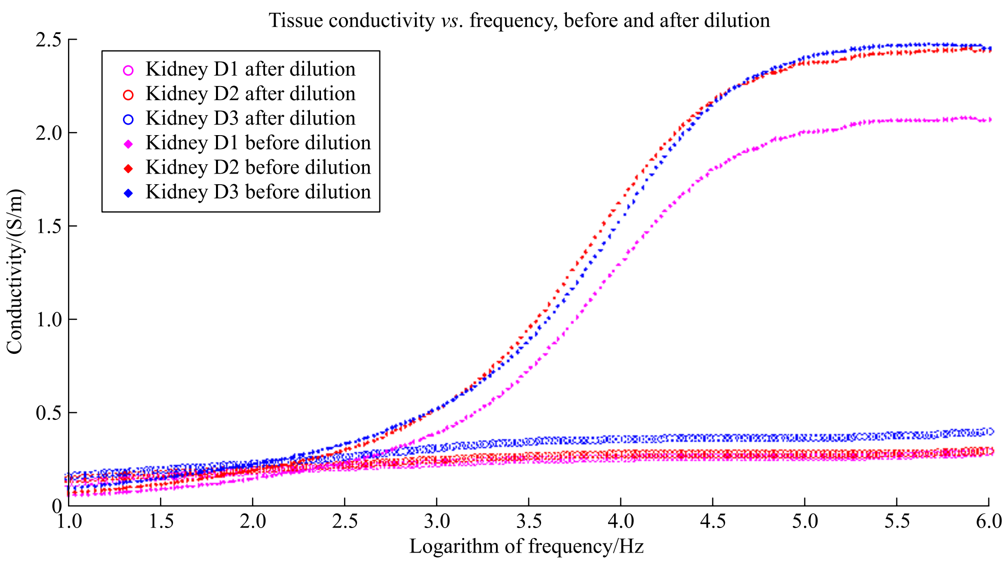

Fig. 5 **Thiel-embalmed porcine kidneys before and after dilution.**

embalmed porcine kidneys.

As shown in ***Fig. 6***, the diluted samples look less red and pink but gray and pale and were stiffer than the Thiel-embalmed: they felt like rubber eraser in contrast to Thielembalmed, which felt like fresh chicken breast. All three samples were somewhat deformed, and their bottom surfaces were flattened due to the gravitational force, which can be noticed by looking at the picture. White curves appeared on their surfaces and they were the boundary of the fattened surface. Also, porcine D1 and D2 seemed to be dehydrated since their compressed size is obvious.

Discussion

Quite a few modifications were made to Thiel's tank fluid, which purely reduced the concentrations of the strong electrolytes, and later reduced the tissue conductivity in the embalmed tissue after 18-day embalmment, but resulted in the stiffness and unfavorable color of the porcine kidneys. It has, however, proven that the reduction in embalming fluid's conductivity can lead to embalmed tissues' reduced conductivity. It also implied a possible relation between the suppleness and concentration of the three electrolytes.

Furthermore, mathematic modeling employing linear regression discovered that embalmed porcine kidney's conductivity (at 1 MHz) increased with the rise of the embalming fluid's conductivity, with perfect linearity (R=0.999, n=7). An equation was draw from the data, which can guide formulating embalming solutions and thus producing embalmed tissues with desired electrical conductivity. Stiffening and discoloration occurred in these diluted samples since significant changes were noticed. Experiments of electrolytic dilution implied the reversibility of Thiel-embalmed tissues suppleness and color which can be damaged if the strong electrolytes in Thiel's recipe are removed.

In conclusion, high electrical conductivity in original Thiel-embalming fluid makes Thiel-embalmed tissue with high electrical conductivity

Fig. 6 **From left to right, Thiel's, samples' dilution by dilution fluids 1, 2 and 3, respectively.**

which was considered to be the main factor for failed radiofrequency ablation. This study encompasses Thiel's embalming method with possible modifications aiming to embalm organ samples for radiofrequency ablation research, development and training, and possibly other applications. Two methods based on Thiel's original embalming technique have been investigated so far in order to reduce the embalmed tissues' electrical conductivity. *Ex vivo* porcine kidneys were used as experimental models due to their appropriate size and wide application in many medical researches.

Much new understanding was accumulated in this study. Results in our experiments showed that acid embalming fluid did not yield soft tissues while neutral or slightly alkaline ones (Thiel's tank fluid and modified fluid 8) did. Modification fluid 8 can significantly reduce embalmed sample's electrical conductivity without compromising suppleness. We also found that strong electrolytes in Thiel's embalming fluid are very important components since removal of them caused sample to become stiff and dehydrated. This study provides a useful modified method for preserving cadaveric tissues for radiofrequency ablation and related research and development, and meanwhile helps further our understanding of the mechanism of this embalming process.

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