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The Effects of Radiofrequency or Cryothermal Ablation on Biomechanical Properties of Isolated Human or Swine Cardiac Tissues

STEPHEN G. QUALLICH[1], KEVIN E. KRIEGE[2], AND PAUL A. IAIZZO[3], (Member, IEEE)
[1]Medtronic, Inc., Minneapolis, MN 55112, USA
[2]Department of Integrative Biology and Physiology, University of Minnesota, Minneapolis, MN 55455, USA
[3]Department of Surgery, University of Minnesota, Minneapolis, MN 55455, USA
CORRESPONDING AUTHOR: P. A. IAIZZO (iaizz001@umn.edu)

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ABSTRACT Changes in cardiac tissue properties following the application of various ablation modalities may lead to the development of an array of associated complications. The application of either radio frequency (RF) or cryothermal ablations will alter the biomechanical properties of various cardiac tissues in a differential manner; in some cases, this may be attributable to increased incidences of cardiac tamponade, pulmonary vein stenosis, and/or atrial-esophageal fistula. Thus, a greater understanding of the underlying changes in tissue properties induced by ablative therapies will ultimately promote safer and more efficacious procedures. The effects of applied RF or cryothermal energies on the biomechanical properties of the pulmonary vein, left atrial, or right atrial samples ($n = 369$) were examined from fresh excised porcine ($n = 35$) and donated human tissue ($n = 11$). RF ablations were found to reduce the tensile strength of the porcine cardiac specimens ($p < 0.05$), and a similar trend was noted for human samples. Cryoablations did not have a significant impact on the tissue properties compared with the untreated tissue specimens. Locational and species differences were also observed in this experimental paradigm ($p < 0.001$). Incorporating these findings into cardiac device design and computational modeling should aid to reduce the risks of complications associated with tissue property changes resulting from cardiac ablative procedures.

INDEX TERMS Atrial fibrillation, biomechanical properties, cryoablation, radiofrequency ablation.

I. INTRODUCTION

Atrial fibrillation (AF) continues to affect millions of individuals in the United States alone, and the incidence of this disease is expected to grow rapidly, 2.5 fold by 2050 [1]. In general, individuals over the age of 80 elicit incidences of AF above 10% [1], [2]. Unfortunately, complications associated with catheter ablation procedures for AF occur at approximate rates of 4-6% [3], [4]. Some of these complications are the secondary result of the application of either radiofrequency (RF) or cryothermal ablative energies, such as cardiac tamponade, pulmonary vein (PV) stenosis, and/or atrial-esophageal fistula. While it is generally considered that the heating or cooling of tissues will alter their biomechanical properties, the exact therapeutic thresholds have not been identified in controlled experiments. Additionally during such RF clinical procedures, surpassing the minimum target temperature of 50°C for myocardial scar formation is quite plausible, especially when aiming to create transmural lesions [5]. It is considered that RF energies build up within the associated tissues during RF ablations [6]. Exceeding ablation temperatures of 60-65°C has been reported to result in the denaturation of collagen, temperatures higher than 80°C cause elastin denaturation, and both scenarios contribute to a loss of total tissue compliance [7]. In contrast, it is considered that ice formation during cryoablations induces altered alignment of structural proteins without compromising their integrity, although there have been noted modifications to their elastic moduli [8]. In other words, the application of ablation energies changes biomechanical as well as tissue properties that may play a role in the manifestation of the aforementioned complications. Thus, a greater understanding of how and why these transformations occur could ultimately reduce their elicitation relative to these cardiac treatments.

Detailed biomechanical characterizations of various myocardial structures within the heart as a whole are currently underway. For instance, the biomechanical properties of the fossa ovalis have been investigated in an effort to reduce iatrogenic atrial septal defect formations [9]. Additionally, our laboratory has studied the relative contact forces required to cause perforations and the associated relationships with various ablation modalities [10]. Furthermore, interest in engineered heart tissues, as well as rapid utilization of transcatheter valves, has fueled the study of both valves and chordae tendineae properties to better mimic their native behavior [11], [12]. Therefore, a detailed understanding of biomechanical as well as biothermal tissue properties associated with ablative therapies remains an intense area of interest. Further, as cardiovascular technologies continue to advance, the role of computational modeling in both device development and personalized medicine will likely become readily available and increasingly important [13].

II. METHODS

Human heart specimens (n = 11) were acquired from nonviable organ transplant donors through LifeSource (St. Paul, MN, USA) as well as the University of Minnesota Bequest Program (Table 1). Yorkshire cross swine (n = 35) cardiac tissue was also obtained from the University of Minnesota Meat Sciences Lab as well as our laboratory. Fresh atrial and ostial PVs (n = 369) were carefully dissected into dog-bone-shaped tissue bundles and 2-0 silk sutures (Surgical Specialties Corp., Reading, PA, USA) were tied to both ends (Fig. 1). This dog-bone orientation allowed for repeatable failures of these tissues near their midpoint; evulsions occurred at the tissue regions with the smallest cross-sectional area. Pectinate muscles from the right and left atrium were used; pulmonary vein samples were dissected so that the axial direction of the vein aligned with the long axis of the dog-bone shape. Dimensions of all tissue samples were measured with calipers to allow for subsequent data normalization. All swine samples were tested within 24 hours post explantation; some human specimens were tested beyond this timeframe, but within 48 hours. Samples were stored in saline at 4°C prior to uniaxial testing. The prepared samples were randomized to the following study groups: 1) no treatment, 2) RF ablation for 1 minute at 30 W, with a 65°C temperature limit, or 3) focal cryoablation for 2 minutes. Specimens were then pulled uniaxially until we observed failure at a rate

TABLE 1. Human heart demographics.

Heart #	Age (Yr)	Weight (kg)	Gender	Cause of Death	Cardiac History
1	45	96	M	CVA	Hypertension, alcoholism
2	34	86	M	CVA	None
3	62	73	F	CVA	Hypothyroidism, hyperlipidemia
4	52	74	F	CVA	None
5	81	75	F	Natural	AF, mitral regurgitation
6	67	82	M	Bladder cancer	None
7	69	77	M	COPD	None
8	68	137	M	CVA	Hypertension, hyperlipidemia, CABG
9	58	93	F	Head trauma	Hypertension
10	52	94	M	Drug overdose	None
11	57	106	M	CVA	None

AF=atrial fibrillation; CABG-coronary artery bypass graft; COPD=chronic obstructive pulmonary disease; CVA=cerebrovascular accident

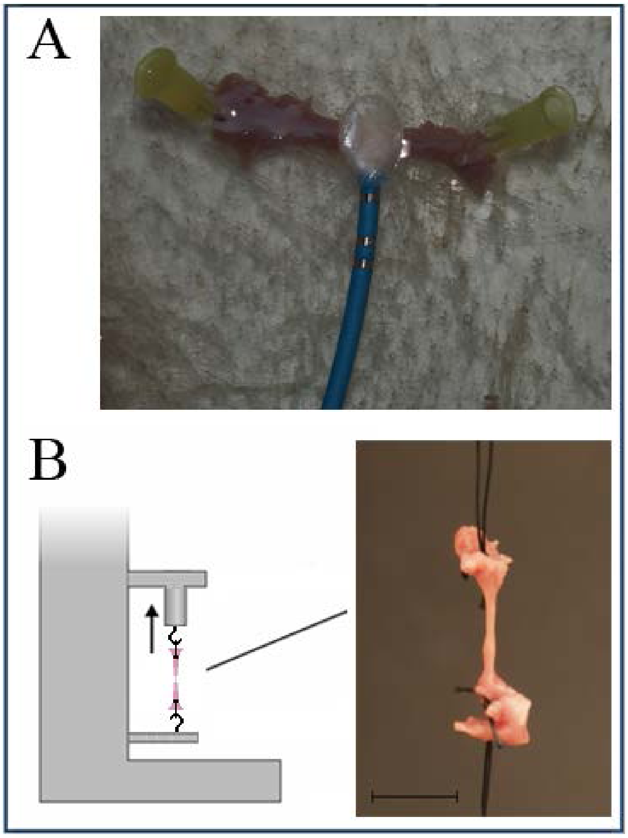

FIGURE 1. A prepared dog-bone shaped specimen which was submerged in saline during dissection. Following dissection an ablation treatment (in this case cyrothermal) was performed (A). Sutures were affixed to each end of the sample. The specimen was then mounted in the mechanical force tester looping the sutures onto hooks. The undeformed strain was measured after the sample was mounted but prior to the uniaxial pull. The scale bar depicts 1 cm (B).

of 100 mm/min with a mechanical force tester (Chatillon, Largo, FL, USA). A typical stress response plot for each treatment modality is displayed in Figure 2. Lagrangian strain was used in this experimental paradigm, and the initial tissue length was measured after the application of ablation modalities.

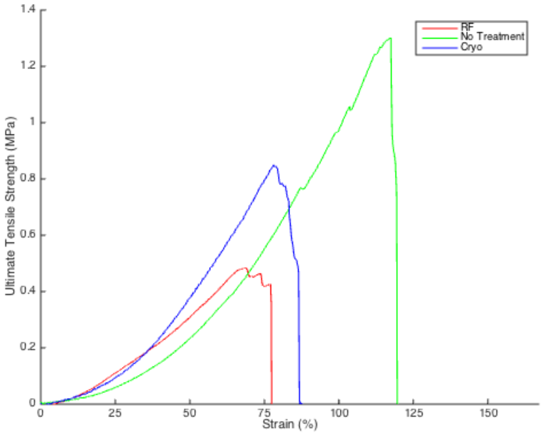

FIGURE 2. During uniaxial pulls the stress-strain response of each treatment (RF = red, no treatment = green, and cryothermal = blue) was measured.'

A. DATA ANALYSES

Ultimate tensile strength, strain at failure, and Young's modulus parameters were calculated and analyzed with Matlab (MathWorks, Natick, MA, USA) and Minitab (State College, PA, USA). All determined values are presented as mean ± standard deviation. Analysis of variance (ANOVA) for groups of 3 or more and t-test with Bonferroni correction for individual comparisons were used to examine normally distributed data. P-values ≤ 0.05 were considered as significant.

III. RESULTS

The relative tensile strength, strain at failure, and derived Young's modulus parameters for tissues were found to be significantly different, i.e., between the right/left atrial and PV specimens as shown in Table 2 ($p < 0.05$ for RA and LA compared to PV). Furthermore, there were significant differences between species, with human hearts having greater ultimate tensile strength and Young's modulus parameters, but lower strain at failure, as provided in Table 3 ($p < 0.001$).

TABLE 2. Comparisons of porcine biomechanical properties sorted by tissue type.

	Location			
	RA	LA	PV	P-value
Ultimate tensile strength (MPa)	0.69±0.35	1.04±0.76	1.64±0.95	<0.001
Strain at failure (%)	87±33	117±39	153±68	<0.001
Young's modulus (kPa)	15±8	19±10	31±23	<0.001
Number	33	23	39	

LA=left atrium; PV=pulmonary valve; RA=right atrium

TABLE 3. Comparisons of pulmonary vein biomechanical properties sorted by species: human versus swine.

	Species		
	Human	Swine	P-Value
Ultimate tensile strength (MPa)	5.90±5.22	1.64±0.95	<0.001
Strain at failure (%)	58±28	153±68	<0.001
Young's modulus (kPa)	224±219	31±23	<0.001
Number	29	39	

TABLE 4. Comparisons of the biomechanical properties of porcine pulmonary veins, right atrium, and left atrium in each treatment group: controls (NT), radiofrequency (RF) therapy or cryothermal therapy (Cryo).

	Ablation Modality			
Pulmonary Vein	NT	RF	Cryo	P-value
Ultimate tensile strength (MPa)	1.64±0.95	1.09±0.59	1.41±0.49	0.012
Strain at failure (%)	153±68	128±62	147±72	ns
Young's modulus (kPa)	31±23	21±13	27±18	ns
Number	39	27	32	
Left Atrium				
Ultimate tensile strength (MPa)	1.04±0.76	0.64±0.30	0.83±0.49	0.044
Strain at failure (%)	117±39	90±33	85±21	0.001
Young's modulus (kPa)	19±10	15±10	21±12	ns
Number	23	25	29	
Right Atrium				
Ultimate tensile strength (MPa)	0.69±0.35	0.54±0.40	0.63±0.30	ns
Strain at failure (%)	87±33	69±36	84±32	ns
Young's modulus (kPa)	15±8	14±8	15±9	ns
Number	33	37	40	

The applied ablation modality and therapeutic application site within these hearts were both shown to be factors influencing the resultant biomechanical properties. Following RF applications, the relative tensile strength for both the porcine pulmonary veins and left atrial tissue specimens was different, as shown in Table 4 ($p < 0.05$ for PV and LA NT compared to RF). Note that post-treatment responses of the porcine right atrial and human PV samples followed the same trends, but these effects did not achieve significance (Table 5). Further, the application of RF energies reduced the tensile strength of all investigated tissues; typically this resulted in reduced evulsion forces by approximately one-third following RF ablations. In contrast, cryoablative therapies elicited no statistical impact on the ultimate tensile strength, regardless of the tissue tested.

IV. DISCUSSION

The biomechanical properties of cardiac tissues observed here were similar to those reported in the literature. For instance, studies examining the biomechanical properties

TABLE 5. Comparisons of human pulmonary vein biomechanical properties associated with treatment group: controls (NT), radiofrequency (RF) therapy or cryothermal therapy (Cryo).

	Ablation Modality			P-value
	NT	RF	Cryo	
Ultimate tensile strength (MPa)	5.90±5.22	3.85±2.62	6.69±5.91	ns
Strain at failure (%)	58±28	55±21	64±25	ns
Young's modulus (kPa)	224±219	144±108	219±225	ns
Number	29	30	25	

of porcine peripheral and coronary arteries revealed ultimate tensile strength and Young's modulus parameters in agreement with this study [14], [15]. It should be noted that there were expected differences between arteries and the venous/cardiac tissues used in this experiment. Also, Venkatasubramanian et al. reported a shift in Young's modulus of the physiological region of the frozen samples so that they acted stiffer [14]. Similar behavior was observed in this study during cryoablation with a shift of Young's modulus (data not shown). Furthermore, it was reported that RF energy application caused a loss of pulmonary vein compliance and contraction of the tissue, which was exacerbated with temperature increases beyond 60-65°C [7]. Note that a temperature limit of 65°C was utilized in our experiments, and although there was an increase in compliance, inversely related to Young's moduli, no significant changes were observed. It is important to acknowledge that in the current study samples were examined following the ablation, so this shrinkage would not be observed as it was out of our window of interest. Additionally, similar effects of ablation were exhibited for the esophagus, with RF significantly reducing the ultimate tensile strength and cryoablation having no noticeable impact [16]. In general, the effects of RF and cryothermal ablation observed in this study are in agreement with those manifested in a variety of other tissue types including veins and esophagus.

The biophysical disruption of the structural integrity of tissues in relation to heating has been characterized numerous times [7], [17]–[20]. As temperatures exceed 65°C collagen denaturation occurs, and at temperatures above 80°C elastin denaturation is incited [7]. In this experimental paradigm a temperature limit of 65°C was used, which allowed for collagen breakdown in these tissues following the application of RF therapeutic ablation. Therefore, reduced structural integrity was expected and was found to be in agreement with our presented results. In contrast, cryoablative therapy is considered to have minimal effects on the integrity of structural proteins, so reductions in ultimate tensile strength would not be anticipated and were not observed in this study. Yet, the relative induced changes that RF and cryothermal ablative energies had on the structural proteins were associated with the tissue and species specific biomechanical responses.

Although we identified significant difference between the swine and human biomechanical properties, there are a number of other factors one needs to consider. The tissue samples studied here were from 6-9 month old swine, while samples from human donors ranged between the ages of 34-81 years. Aging processes will alter biomechanical properties; e.g., studies examining the chordae tendineae reported that with age the collagen orientation becomes more disorganized and alterations in the collagen wave structure occur [21]. More specifically, the large ranges in the age and associated disease state of the donor human specimens studied here may have contributed to the observed variabilities and differences when compared to the swine samples. Note that some of the donors had cardiac conditions, which may have altered their anatomy and biomechanical properties of the tissue. Also, given the relatively small sample size for human tissue, additional testing would aid in verifying these findings. Nevertheless, swine hearts were used as a translational model to approximate tissue biomechanics following ablation.

The biomechanical properties assessed in our investigations of both human and swine tissues should be of interest to scientists, engineers, and electrophysiologists utilizing these therapies to treat patients. We consider here that this is one of the first studies to examine the effects of ablative energies on the biomechanical properties of various cardiac tissues. Greater awareness of the resultant differences that RF and cryothermal ablation applications have on tissues may influence future iterations of such delivery devices and/or be used to fine tune clinical procedures, so to minimize circumstances leading to the development of associated complications. For instance, the knowledge that RF weakens the ultimate tensile strength of various tissues could be advantageous in particular applications. For example, the Baylis RF transseptal needle (Baylis Medical, Montreal, CA) takes advantage of these induced effects and uses RF energy to more readily cross the septum, i.e., as an alternative to solely applying mechanical force. Additionally, the results presented here may have numerous implications for device design and implementation in numerous clinical scenarios that help to improve the safety and efficacy of all types of ablation procedures.

This study had some inherent limitations which may include: 1) tissue temperatures during applied therapies were not monitored; 2) only uniaxial force responses were studied; and 3) the relative viability of the human tissues was not always optimal. Monitoring of the contact tissue temperatures would have allowed for a more accurate examination of the influence temperature has on biomechanical properties. However, we employed clinical systems for therapeutic delivery, and this type of monitoring would in turn be invasive and may have compromised the measured tissue properties of interest. Furthermore, samples were typically near or less than 1 mm^2 in cross-sectional area, so catheter tip temperatures were expected to be quite close to maximum tissue temperature. Noted above, biomechanical properties were only examined uniaxially and these tissues likely have directional differences, since they are not homogeneous in nature. Furthermore, some of the human tissue used was not tested within 24 hours post explantation. Although, we did not observe significant differences between the relative properties of our obtained human samples, this may have been masked by the small sample size. Therefore, further experiments need to be conducted to investigate these potential study limitations.

V. CONCLUSIONS

The effects of RF and cryothermal ablation on the biomechanical properties of cardiac tissues were investigated using a translational approach. Applied RF energies induced significant denaturation of structural proteins, ultimately leading to decreases in tensile strength, but without associated changes in strain at failure and/or derived Young's modulus. Cryoablations did not elicit significant effects on any tissue properties compared to untreated specimens. There are tissue specific and species variations that need to be accounted for in applications related to the biomechanical properties of cardiac tissues. Thus, special consideration should be taken when using animal models as substitutes for human tissues in device design efforts. This research highlights the need for further studies to investigate the tissue property transitions that occur during and following the application of ablation modalities, especially with respect to clinical complications.

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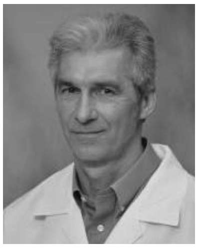
STEPHEN G. QUALLICH received the Ph.D. degree from the Visible Heart Laboratory, University of Minnesota. He is currently a Senior Reliability Engineer with Medtronic PLC. His primary research interests include cardiac ablation tissue property changes, fossa ovalis tearing, RF ablation steam pops, and infrared imaging of cryoablation.

KEVIN E. KRIEGE is currently pursuing the bachelor's (Hons.) degree in physiology with the University of Minnesota. He has volunteered in the Visible Heart Laboratory for more than two years, and aspires to attend medical school.



PAUL A. IAIZZO received the Ph.D. degree. He is currently a Professor with the Departments of Surgery, Integrative Biology and Physiology, and the Carlson School of Management at the University of Minnesota, Minneapolis. His main research focus is translational systems physiology, and his research group has developed a unique isolated working large mammalian heart model. Other research interests include cardiac pacing, skeletal muscle pathophysiology, thermoregulation, black bear hibernation, wound healing, and spine biomechanics.