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Quantitative Imaging Assessment of an Alternative Approach to Surgical Mitral Valve Leaflet Resection: An Acute Porcine Study

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Abstract—This study reports the initial *in vivo* use of a combined radiofrequency ablation and cryo-anchoring (RFC) catheter as an alternative to surgical mitral valve (MV) leaflet resection. Radiofrequency ablation thermally shrinks enlarged collagenous tissues, providing an alternative to leaflet resection, and cryo-anchoring provides reversible attachment of a catheter to freely mobile MV leaflets. Excised porcine MVs ($n = 9$) were tested in a left heart flow simulator to establish treatment efficacy criteria. Resected leaflet area was quantified by tracking markers on the leaflet surface, and leaflet length reductions were directly measured on echocardiography. Leaflet area decreased by 38 ± 2.7%, and leaflet length decreased by 9.2 ± 1.8% following RFC catheter treatment. The RFC catheter was then tested acutely in healthy pigs ($n = 5$) under epicardial echocardiographic guidance, open-chest without cardiopulmonary bypass, using mid-ventricular free wall access. Leaflet length was quantified using echocardiography. Quantitative assessment of MV leaflet length revealed that leaflet resection was successful in 4 of 5 pigs, with a leaflet length reduction of 13.3 ± 4.6%. Histological, mechanical, and gross pathological findings also confirmed that RFC catheter treatment was efficacious. The RFC catheter significantly reduces MV leaflet size in an acute animal model, providing a possible percutaneous alternative to surgical leaflet resection.

Keywords—Percutaneous mitral valve repair, Mitral regurgitation, Radiofrequency ablation, Cryo-anchoring.

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ABBREVIATIONS

MR	Mitral regurgitation
MV	Mitral valve
RF	Radiofrequency ablation
RFC	Radiofrequency ablation and cryo-anchoring
PM	Papillary muscle
AL	Anterior leaflet
PL	Posterior leaflet

INTRODUCTION

The current standard in long-term treatment of degenerative mitral regurgitation (MR) is open-chest surgical repair or replacement.[7] There are a variety of surgical repair techniques such as leaflet resection, annuloplasty and chordal replacement that can be employed by the cardiac surgeon to address the assortment of lesions that may be present in degenerative MR. Percutaneous alternatives to annuloplasty and chordal replacement are currently in development, but there is no percutaneous technique to approximate surgical leaflet resection,[3,5] which is used in 70–80% of all degenerative mitral valve (MV) repairs.[6,8] Furthermore, it is estimated that up to 50% of high-risk patients with severe symptomatic MR are not referred for surgery due to co-morbidities that may confer higher perioperative risk.[13] Thus, there is a need for a percutaneous alternative to conventional surgical leaflet resection,[3] which can be combined with percutaneous annuloplasty and neochordae placement to

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provide the potential for a complete percutaneous MV repair.

We have previously reported on the development of a combined radiofrequency (RF) ablation and cryo-anchoring (RFC) catheter (Figs. 1a and 1b), which is intended as a percutaneous alternative to the surgical technique of leaflet resection. The RFC catheter uses intraluminal delivery of pressurized nitrous oxide to cool a catheter tip to temperatures between −50 and −20°C to provide stable and reversible catheter adhesion to moving MV leaflets without negatively restricting leaflet motion or altering leaflet structure.[2,4] RF energy delivery to an RF electrode on the catheter is then used to thermally shrink, or resect, enlarged and myxomatous leaflet tissue by heating collagen to temperatures greater than 65°C,[16,17] and RF energy has been shown to reduce leaflet size by up to 45% in a static bench environment.[2]

The objective of the present work is to evaluate the feasibility of the RFC catheter to approximate surgical leaflet resection using an acute, non-survival porcine animal model. Initially, RFC catheter treatment efficacy criteria was identified using *ex vivo* testing of excised porcine MVs placed in a left heart flow simulation device. The primary goal of the *ex vivo* study was to use quantitative leaflet length measurements, obtained on echocardiographic views of the A2–P2 imaging plane, as a predictive imaging correlate for total leaflet area reductions. Leaflet area reductions of MVs in the left heart flow simulator were measured by tracking the positions of four suture markers placed on the MV anterior leaflet (AL) surface with echocardiography in an intercommissural imaging plane to capture all markers on 2-dimensional (2D) views. Corresponding leaflet length reductions were captured in the A2–P2 imaging plane and compared to directional and areal measurements obtained in the intercommissural plane.

Following the establishment of efficacy criteria, five consecutive *in vivo* studies in healthy pigs were performed open-chest to test the feasibility of the RFC catheter to resect leaflet tissue. The data that follow demonstrate that RFC catheter treatment, guided by echocardiography, produces significant resection of MV leaflets without compromising hemodynamic function during treatment. Post-treatment echocardiographic images, quantification of leaflet length, as well as gross morphological, histological, and mechanical analyses provide evidence that RF ablation can produce significant reductions in MV leaflet area in a beating heart environment.

MATERIALS AND METHODS

Ex Vivo Establishment of Efficacy Criteria

Intact, normal porcine MVs were excised from hearts obtained from the local abattoir (Hampton Meats, Hopkinsville, KY) within one hour of slaughter, including the annulus, leaflets, chords, and papillary muscles (PMs). Four tracking markers (3-0 braided polyester suture with 3–4 square knot throws) were sutured to the central region of the AL on the atrial surface with approximately 1 cm spacing to track leaflet size with echocardiography. Additionally, the outer margin of the AL was marked by staining with trypan blue to provide an estimate of leaflet area pre- and post-treatment. The intact valves were then placed in a left heart flow simulator (Fig. 1c and Online Video 1) capable of reproducing physiologic pressure and flow waveforms (average flow rate of 5 L/min, heart rate of 70 beats/min, transmitral pressure of 100–120 mmHg), as described previously.[4]

Mock circulatory flow loops have been used to create models of MR and to study the hemodynamic response of MVs to various surgical treatments.[14] In this study, after a normal, baseline PM configuration was established, both PMs were displaced towards the MV annulus until significant leaflet billowing (greater than 4 mm beyond the annular line) was seen on echocardiography (Fig. 1c). This provided an approximate simulation of bi-leaflet prolapse to more easily visualize leaflet morphologic changes following treatment with the RFC catheter.

To provide a clinically relevant assessment of RFC catheter treatment efficacy, two-dimensional (2D) echocardiography (Acuson Antares™ scanner, Siemens Healthcare, Ultrasound Business Unit) was used to image MVs in the left heart flow simulator. Views of the MV apparatus were captured in two imaging planes: an intercommissural plane to track the four suture markers on the surface of the AL, and an A2–P2 plane to record leaflet morphology, length, and prolapse (Fig. 1d and Online Video 2). Baseline echocardiographic recordings were captured, the PMs were displaced towards the annulus, and the prolapse condition was recorded (Fig. 1e). The AL was targeted for RFC catheter treatment first, and was treated several times with the RFC catheter with a cryo-anchor temperature of approximately −50°C and RF ablation power of 65 W for 90 s each. The first five ablations to the AL were performed within the area constrained by the four tracking markers, and marker positions were recorded during mid-diastole after each ablation in the intercommissural plane (Fig. 1f). To determine reductions in marker area, circumferential length, and radial length, the deformation gradient tensor was calculated

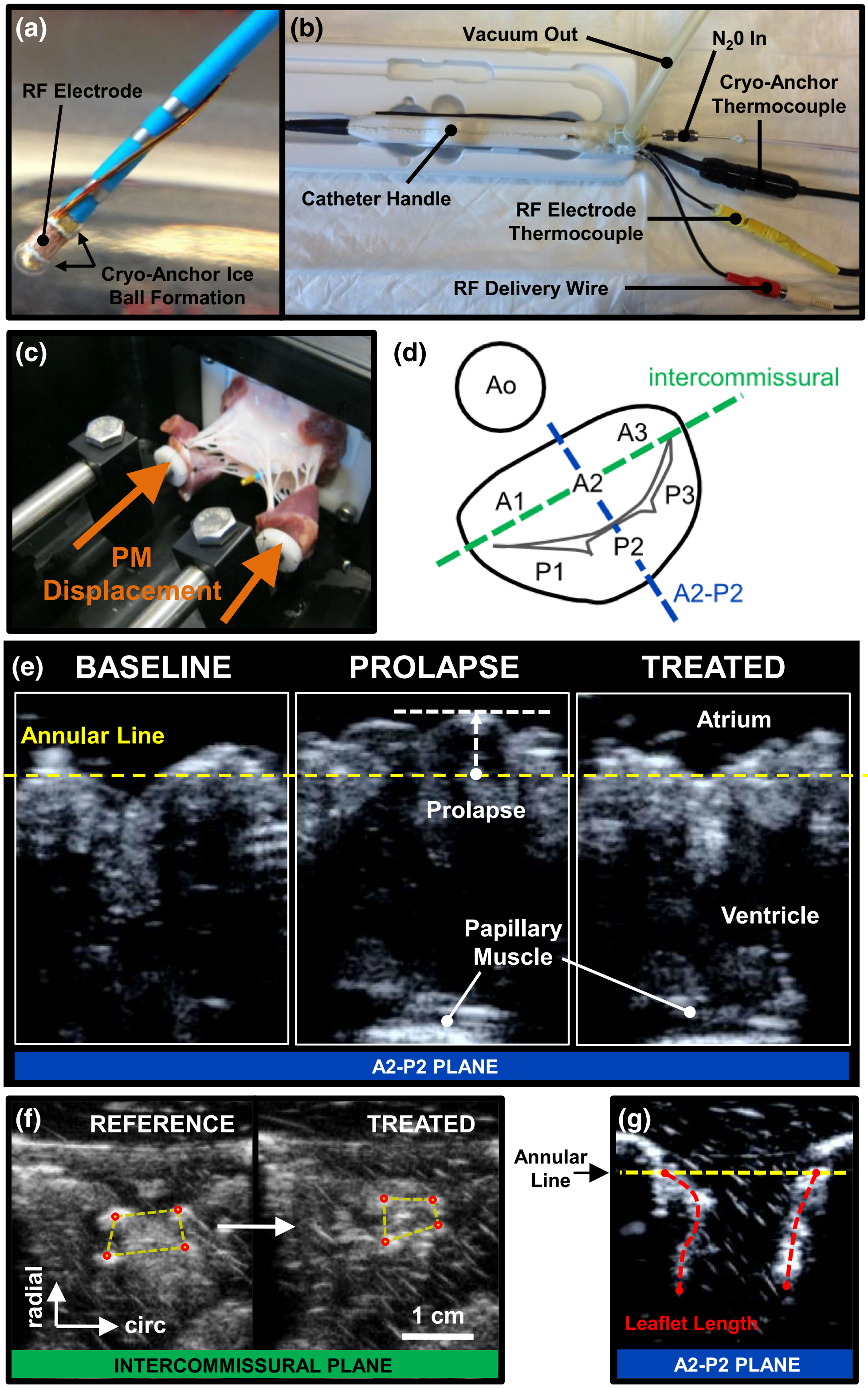

FIGURE 1. *Ex vivo* methods—(a) RFC catheter used in the *ex vivo* and *in vivo* studies, shown with the cryo-anchors active. (b) RFC catheter handle with inputs and outputs. (c) MV positioned into the left heart flow simulator with adjustable PM attachments (arrows). See Online Video 1 for leaflet cryo-anchoring and pre- and post-treatment leaflet motion. (d) Echocardiographic imaging planes used in the *ex vivo* study, surgical view. (e) Change in MV morphology following treatment with the RFC catheter, shown in the A2-P2 plane. Note the reduction of leaflet prolapse following treatment. See Online Video 2. (f) Markers placed on the AL were tracked in the intercommissural imaging plane. (g) Echocardiographic image of a porcine MV in the left heart flow simulator imaged in the A2–P2 plane with leaflet length measurements denoted in red.

from the four marker positions and averaged over three repeated measurements (MATLAB, MathWorks, Inc.).[2,12] After the first five ablations to the AL, the AL and posterior leaflet (PL) were both ablated up to 10 times, and post-treatment echocardiographic recordings were captured. AL and PL lengths were measured during mid-diastole, from the base of the annulus to the leaflet free edge, and averaged over three measurements (MATLAB) (Fig. 1g), while corresponding leaflet prolapse measurements were taken during mid-systole (Showcase Premier, Trillium Technology) (Fig. 1e).

In Vivo Investigational Protocol

Five consecutive studies in healthy pigs ranging in weight from 47 to 51 kg and 3 to 4 months old were carried out with a Vanderbilt University approved institutional animal care and use committee protocol to evaluate RFC catheter efficacy *in vivo*. On the day of surgery, anesthesia was induced with ketamine (2.2 mg/kg), telazol (4.4 mg/kg), xylazine (2.2 mg/kg), and atropine (0.04 mg/kg) *via* intramuscular injection, and isoflurane (1–3%) with O_2 was administered under endotracheal intubation for maintenance. No heparin or other anticoagulants were administered in this study. To prevent arrhythmias, animals were treated with an intravenous bolus loading dose of 2 mg/kg of amiodarone prior to the start of surgery and 1.2 mg/kg/h *via* intravenous drip for maintenance thereafter. Heart rate, rhythm, and blood pressures were monitored throughout the procedures.

A median sternotomy was performed and intervening fascial layers dissected to optimize visualization of the pericardium, heart and major blood vessels. An 18 gauge needle was utilized to cannulate the mid-to-distal anterior wall of the left ventricle through which a 0.035-inch J-wire was inserted for ventricular access. A 12 Fr short modified sheath was placed in the left ventricle (Figs. 2a and 2b) *via* an over-the-wire modified Seldinger technique followed by tandem removal of the wire and dilator. Sheath position on the left ventricular free wall and alignment with the MV apparatus was confirmed by direct echocardiographic guidance using an epicardial probe positioned superior to the access site on the pericardial–epicardial surface in an epicardial long-axis configuration. Fluoroscopy was available, if necessary, for sheath and catheter guidance but was used in only one animal in this study.

Prior to *in vivo* use, each RFC catheter used in this study was calibrated to determine the RF electrode temperature range that corresponded with tissue ablation temperatures between 65 and 100°C, a range that causes thermal shrinkage without significant impedance rise due to boiling or charring.[11] Due to the placement of the RF electrode thermocouple below the outer surface of the electrode, the proximity of the cryo-anchors to the RF electrode, and significant convective blood flow, the calibrated RF electrode temperature range can be expected to be significantly lower than corresponding leaflet ablation temperatures. Excised myocardium was placed in the left heart flow simulator near the MV orifice, and a thermocouple was placed directly below the RFC catheter electrode, approximately 1–2 mm below the myocardial surface. Myocardium was used due to ease of temperature measurement compared to MV leaflet tissue. For the RFC catheters used in this study, the target RF electrode temperature range was found to be 5–20°C, and RF power output was regulated to maintain this temperature range throughout the ablations.

The RFC catheter (10 Fr) was directed through the sheath and across the MV orifice under direct echocardiographic visualization. The AL was targeted specifically in this study because of the access route on the mid-to-distal anterior wall of the left ventricle. Once the RFC catheter tip was located in the vicinity of the AL, cryo-anchoring was obtained by intra-luminal delivery of nitrous oxide to securely cryo-anchor the catheter to the leaflet surface with a temperature of −50°C (Figs. 2c and 2d). No attempt was made to confine the RFC catheter to a specific location on the AL surface. Once cryo-anchoring was achieved, RF energy was delivered to the AL for 60 s in temperature-control mode (Figs. 2e, 2f). RF power output was regulated to maintain the measured RF electrode temperature within the calibrated range that was demonstrated to produce tissue ablation temperatures (>65°C) without significant impedance rise. Cryo-anchoring was ceased after 60 s of ablation, the RFC catheter tip was re-positioned, and the cryo-anchoring and ablation process was repeated up to 21 times for each animal.

In Vivo Study Evaluation Protocol

Echocardiographic examination of the MV and surrounding area was performed before and after RFC catheter treatment in long- and short-axis views of the heart. All echocardiographic imaging was performed epicardially using a custom-built scanner (Cephasonics, Santa Clara, CA) in B-mode with a 10 MHz linear array probe. Following catheter treatment and completion of the echocardiographic examination, each animal was euthanized according to protocol, and the heart was excised. At post-mortem, the entirety of the left heart, including the MV, ventricle, atrium and left ventricular outflow tract, was examined for signs of ablation. Gross leaflet morphology, as well as any

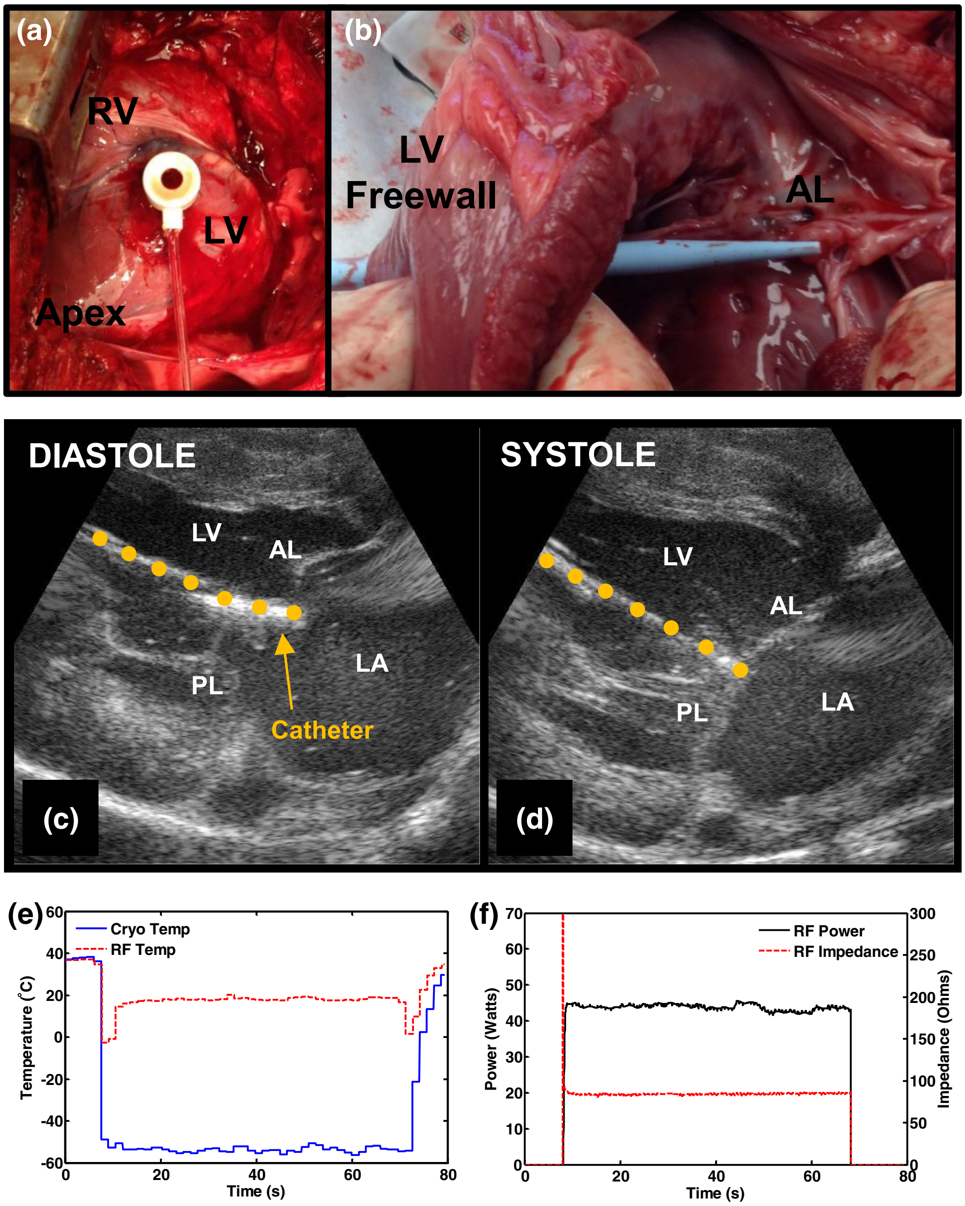

FIGURE 2. *In vivo* **methods—(a) Access to the MV was gained by placing a sheath midway up the left ventricular free wall, enabling direct access to the AL, shown post-mortem (b). Epicardial long-axis view of the RFC catheter cryo-anchored to the AL, while the PL moves freely. The diastolic view demonstrates the free PL (c), while the systolic view demonstrates that leaflet mobility is maintained, allowing valve closure (d). Dotted yellow line indicates the catheter shaft. See Online Video 3 for additional examples of cryo-anchoring. (e) Representative temperatures at the RF electrode (red, dash) and cryo-anchor (blue, solid) during one ablation. (f) Representative ablation power output (black, solid) and measured impedance (red, dashed) corresponding to temperatures measured in (e). LV: left ventricle. RV: right ventricle.**

off-target ablations, were noted. The belly region of each MV was then excised and mechanically tested to equibiaxial loads of 90 N/m in a biaxial mechanical testing device to assay for evidence of thermal alteration by fitting the mechanical loading curves to a Fung-type strain-energy model. Strain-energy measurements have previously been shown to provide a measure of thermal alteration and an estimate of resected leaflet area.[4,10] Afterwards, leaflets were fixed in 10% formalin before histological staining with picrosirus red to assess collagen structure for characteristic markers of thermal alteration.[4,15] Echocardiographic images of the AL in the long-axis view from pre- and post-treatment were analyzed offline to measure leaflet length during diastole (MATLAB). Leaflet length measurements were repeated three times per image frame over 3–6 different frames for a total of 9–18 measurements per leaflet and were averaged together.

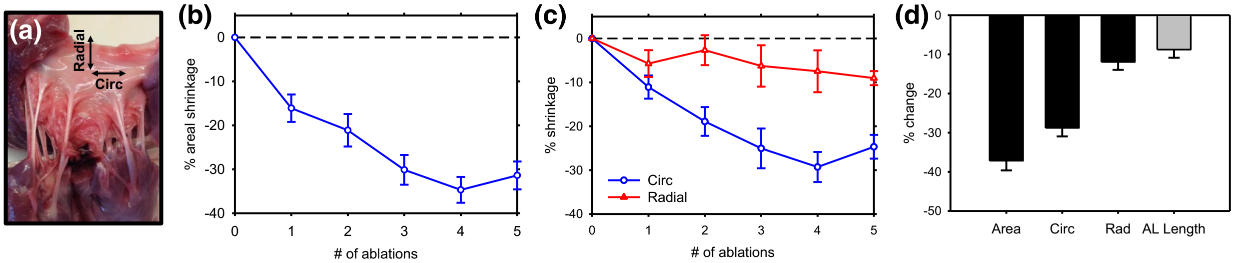

FIGURE 3. *Ex vivo* leaflet area measurements—(a) MV leaflet circumferential (circ) and radial directions. (b) Areal leaflet shrinkage over the course of 5 ablations within the 2 × 2 marker area, *n* = 6. (c) Corresponding directional shrinkage measurements in the circumferential (blue circles) and radial (red triangles) directions, *n* = 6. (d) Average final marker size reductions recorded in the intercommissural imaging plane (black bars) and corresponding leaflet length reduction measured in the A2–P2 imaging plane (gray bar), *n* = 9.

Statistical Analysis

All measurements are reported as mean ± standard error. One way ANOVA was conducted to examine statistical differences between groups with a follow-up Holm-Sidak method for multiple pairwise *t* tests at significance level $\alpha = 0.05$.

RESULTS

Ex Vivo Assessment

Maximum areal shrinkage within the marker region of interest was achieved after 4 ablations of 50 W for 90 s (Figs. 3a and 3b) and shrinkage was greatest in the circumferential leaflet direction (Fig. 3c). After 15 ablations, spread over the entirety of the AL, areal leaflet shrinkage was 38 ± 2.7%, circumferential shrinkage was 30 ± 2.3%, and radial was 12 ± 2.2% (Fig. 3d). For comparison, leaflet area, as measured by the area enclosed by tissue dye markers before and after experiments, was found to have shrunk by 32 ± 3.3 and 37 ± 5.9% in the AL and PL, respectively. Corresponding leaflet length reduction (9.2 ± 1.8%), measured on echocardiography in the A2–P2 imaging plane, was comparable to radial shrinkage. Overall, when leaflet length has been reduced by 9.2%, total leaflet area has decreased by 38%, which provides a quantitative target for determining leaflet resection efficacy in the *in vivo* study (Fig. 3d). PL length decreased by 12 ± 3%, and average leaflet lengths measured in the baseline, prolapse, and treated conditions are shown plotted in Figs. 4a and 4b. Leaflet prolapse decreased by 62 ± 17 and 50 ± 30% in the AL and PL, respectively (Figs. 4c and 4d).

In Vivo Efficacy

The RFC catheter was able to selectively adhere to the AL, and valve closure was minimally inhibited during cryo-anchor formation (Online Video 3). The maximum difference in heart rate during RFC catheter treatment from baseline averaged −2 ± 3.3 beats per minute, suggesting no adverse effects on cardiac function. Cryo-anchoring was successfully maintained throughout each ablation, and between 15 and 21 ablations were attempted on each animal for approximately 60 s with an average RF electrode temperature of 14°C (Table 1). Average RF power is reported as the power delivered to the RF electrode and is not representative of the total power delivery to the leaflet tissue. One animal (Animal #5) developed atrial fibrillation after 15 ablation attempts, likely due to an off-target ablation, and was successfully cardioverted to normal rhythm. Echocardiographic images taken of the AL post-treatment demonstrated apparent leaflet thickening, shortening of leaflet length (Figs. 5a and 5b), and altered leaflet motion throughout the cardiac cycle when compared to pre-treatment images (Online Video 4). Measurements of leaflet length on echocardiographic long-axis views indicated an average 13.3 ± 4.6% reduction in AL length following RFC catheter treatment, with leaflet length reductions exceeding the efficacy criteria established in the *ex vivo* study in 4 of 5 animals (Fig. 5c). Efficacy criteria was not met in Animal #2 due to insufficient power delivery to the leaflet and off-target ablations confined to one area in the posteromedial commissure (Table 1).

ALs treated with the RFC catheter demonstrated varying levels of char formation on the leaflet surface, coagulum formation in the annular tissue immediately above the leaflets, and apparent leaflet thickening (Fig. 6a; Table 1). Most off-target ablations were located around the posteromedial commissure in either the ventricle or atrium. Ablations located on the annulus immediately above the AL were not considered off-target. Strain energy values obtained for the $n = 5$ ALs treated in the pig study are shown plotted alongside estimates for resected leaflet area (Fig. 6b). Resected leaflet area estimates were obtained from previously published findings in leaflets treated in a

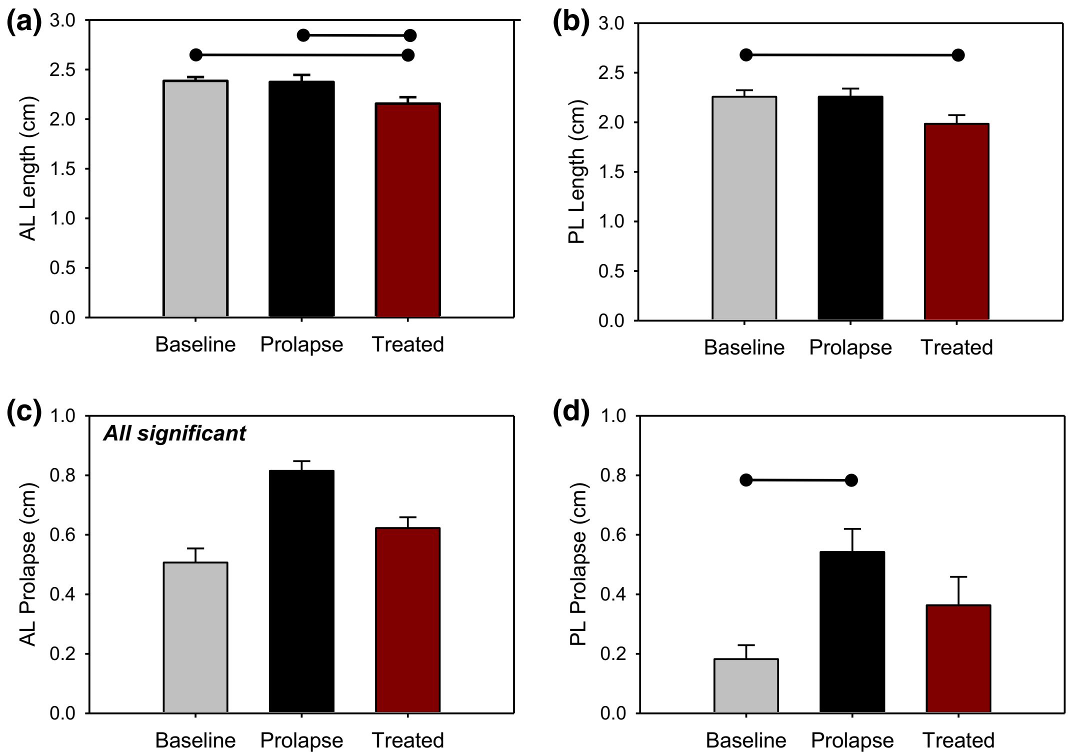

FIGURE 4. ***Ex vivo*** **length and prolapse measurements—(a, b) AL and PL length measured in the baseline and prolapse configurations and following treatment with the RFC catheter. (c, d) Corresponding reduction in prolapse of the AL (c) and PL (d) post-treatment. Lines above bars represent significant changes between groups ($p < 0.05$).** $n = 9$ **per group.**

TABLE 1. Average ablation output and post-treatment morphological assessment.

Animal #	Ablations attempted	Average duration (s)	Average power (W)	Average RF temp. (°C)	Off-target ablations	Morphologic changes
1	15	57 ± 2.5	50 ± 1.0	9.8 ± 0.4	2—Near posteromedial commissure on ventricular wall	Significant char in belly region Coagulum along annulus Thickening throughout
2	20	58 ± 1.8	50 ± 1.4	13 ± 0.6	1—Near posteromedial commissure in atrium above annulus	Small amounts of char Apparent thickening within 3–4 mm of free edge
3	21	52 ± 2.8	33 ± 2.0	14 ± 0.6	0—None could be identified	Significant char in A2 segment from annulus to leaflet free edge Coagulum along annulus Thickening throughout
4	20	58 ± 1.3	28 ± 1.5	19 ± 0.7	0—None could be identified	Moderate char, primarily in A2 segment from leaflet belly region to free edge Apparent thickening in leaflet central area
5	15	56 ± 2.8	28 ± 0.7	16 ± 0.8	2—Near posteromedial commissure on ventricular wall	Localized areas of leaflet thickening and light char on leaflet Coagulum along annulus near both commissures

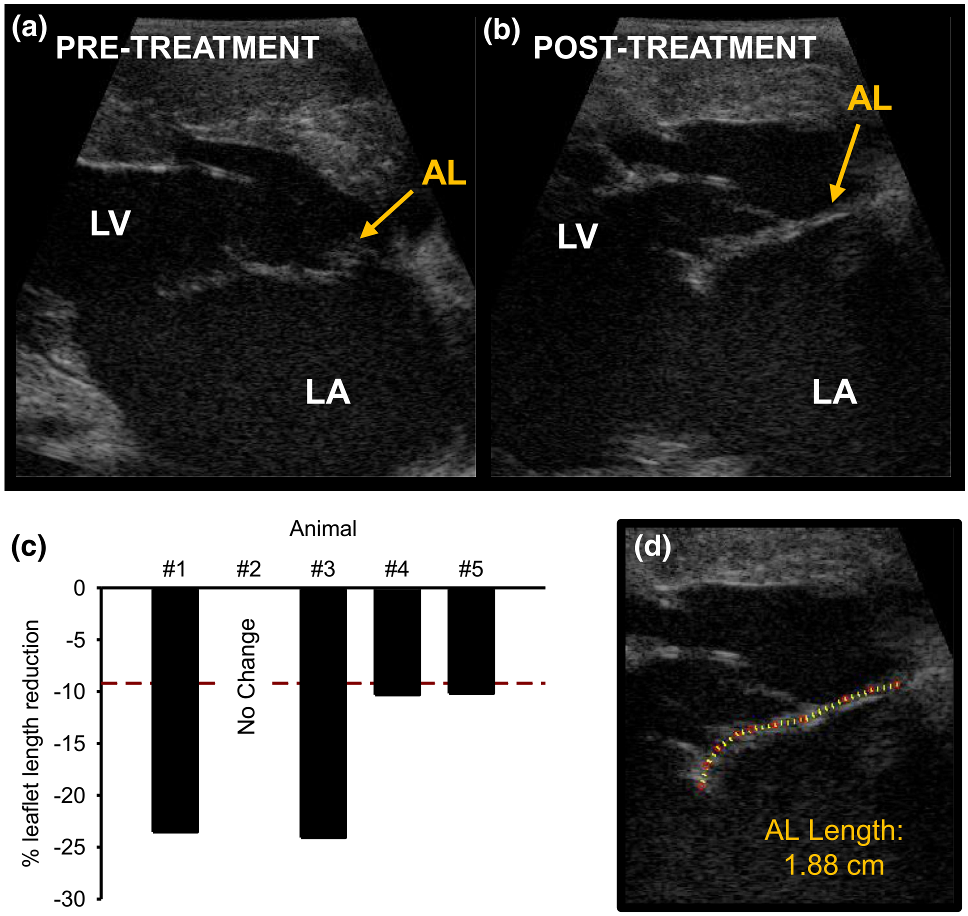

FIGURE 5. Post-treatment echocardiographic AL assessment—long-axis view of the AL before (a) and after (b) treatment with the RFC catheter. The post-treatment AL exhibits a characteristic increase in thickness and a decrease in radial length. See Online Video 4. (c) AL length reduction (% decrease) as measured on epicardial echocardiography following treatment with the RFC catheter. Dashed line indicates efficacy criteria established in the *ex vivo* study. (d) One representative leaflet length measurement (Animal #1).

static bench environment, and strain energy values greater than 10 N/m have been found to correlate with significant resected leaflet areas.[4] Three out of five of the leaflets positively tested for strain energy values that indicate significant leaflet resection. Additionally, picrosirius red staining confirmed that the collagen structure of the ALs had been thermally altered in four of five leaflets (Fig. 6c).

DISCUSSION

Establishment of Leaflet Resection Efficacy Criteria

As healthy animals without MR were used for this study, a quantitative measurement was needed to appropriately evaluate the ability of the RFC catheter to shrink—or resect—MV leaflet tissue. Thus, we used a two-part study to identify a measurement than can be used to assess the acute success of RFC catheter treatment in healthy animals: leaflet length measured on echocardiography.

First, leaflet area and directional length measurements were taken in a physiologic *ex vivo* environment. These measurements were recorded in the intercommissural imaging plane (Fig. 1d), which cannot be sufficiently visualized without the placement of markers on the leaflet surface. Next, corresponding leaflet length measurements in the A2–P2 imaging plane, which is an easily obtainable measurement *in vivo*, were taken for comparison. As AL length (A2–P2 plane) and radial marker length (intercommissural plane) were found to agree and because the relationship between radial and circumferential shrinkage was found to remain the same across samples, leaflet length reductions measured on clinical 2D echocardiograms can be used to estimate total leaflet area shrinkage in

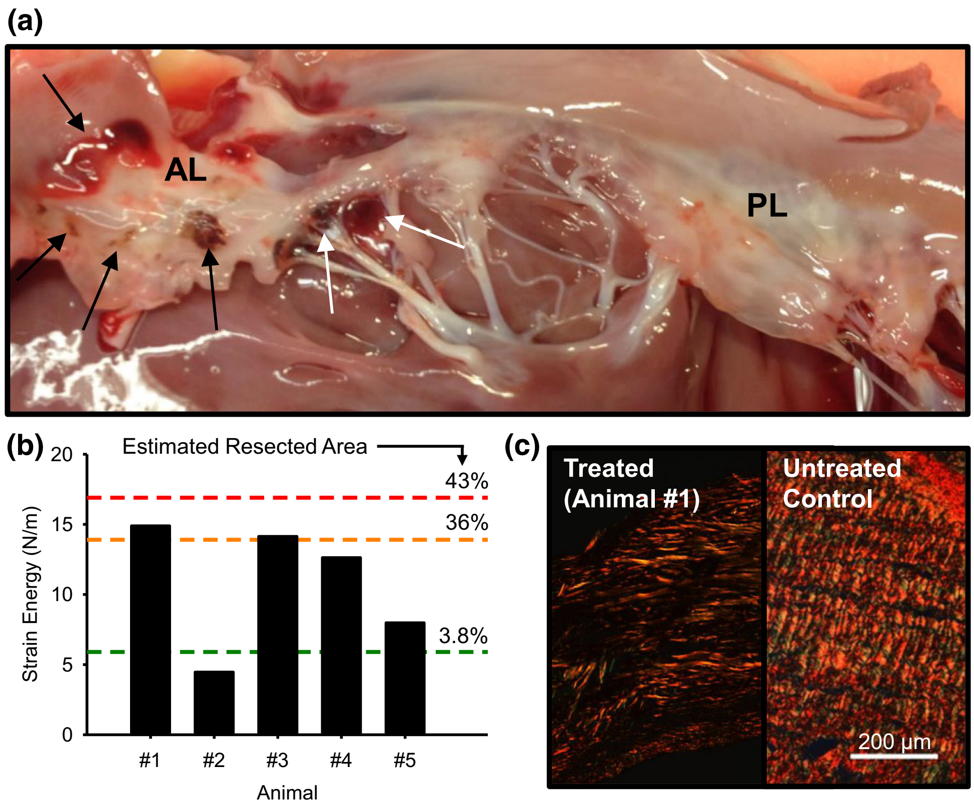

FIGURE 6. Post-treatment morphological, mechanical, and histological assessment—(a) MV leaflets post-mortem. The AL is shown partially excised on the left and exhibits significant char and coagulum on the leaflet surface and at the base of the annulus (black arrows). Off-target ablations were seen in the posteromedial commissure (white arrows). The PL has been left untreated and is shown for comparison. (b) Strain energy estimate on excised ALs following treatment with the RFC catheter. Values shown to the right are the corresponding estimates of resected leaflet area based on treated and untreated controls in a static bench environment.[2,4] (c) Representative picrosirius red stained section demonstrating the typical loss of collagen crimp following thermal shrinkage (Animal #1). An untreated control is shown on the right. Scale bar = 200 μm.

healthy animals. This difference in radial shrinkage (12%) and circumferential shrinkage (30%) is due to the preferred alignment of collagen with the circumferential direction and is consistent with previous *ex vivo* findings in a static environment.[2] Based on the *ex vivo* data, the target for *in vivo* RFC catheter treatment efficacy is approximately 10% reduction in leaflet length, which would indicate a total leaflet area reduction of approximately 40% (Fig. 3d).

A secondary component of the *ex vivo* study was treatment of the prolapse model, enabling large changes to MV morphology to be more easily appreciated (Fig. 1e). One hallmark of Barlow's disease is large, thickened, and billowing leaflets that prolapse significantly into the atrium during systole. In our *ex vivo* prolapse model, leaflet prolapse was reduced, on average, by over 50%. The large standard errors associated with the prolapse measurements (Figs. 4c and 4d) are likely an artifact of sample preparation (suturing of MV annulus). In some cases, leaflet prolapse was reduced by over 100% back to baseline.

RFC Catheter Treatment is Feasible In Vivo

RFC catheter leaflet resection was acutely successful in 4 of 5 animals according to the efficacy criteria established in the *ex vivo* study. Additionally, minor evidence of leaflet ablation was found in the unsuccessful animal, including small amounts of char formation on the leaflet surface and collagen thermal alteration visible on histology. This is the first study to demonstrate that MV leaflet area can be significantly reduced using a catheter-based strategy in a beating heart.

Selective administration of thermal shrinkage to each MV leaflet is essential in the RFC catheter treatment strategy. Depending on the severity and presentation of degenerative MV disease, enlarged and myxomatous leaflet tissue could be confined to a single segment of a single leaflet or be present throughout both leaflets.[7] The AL was specifically targeted in this study due to the access route, and the AL was successfully adhered to independently of free motion of the un-targeted PL. An alternative access route, closer to the apex, was originally attempted, and it was found that the PL could be similarly targeted for cryo-anchoring (Video 3).

The *ex vivo* study indicated that leaflet length reductions of greater than 10% of the original length correlated with a resected area of ~40%. For comparison, the average resected area of a typical PL quadrangular resection is 30%,[9] although it should be noted that quadrangular resection involves removal of an entire leaflet segment. The leaflet length reduction benchmark of 10% was achieved in 4 of 5 animals in the present study. Echocardiographic findings showed thickened and shortened leaflets post-treatment, characteristic of thermal alteration. Gross inspection of ALs post-mortem, picrosirius red staining, and mechanical testing, each verified successful RFC catheter treatment.

Study Limitations

Epicardial echocardiography, used in this study, provides some guidance of catheter position, but probe maneuverability was limited and difficult to achieve reliably, which may have led to some of the off-target ablations observed here. Off-target ablations occurred in 3 of 5 animals. Additionally, various amounts of char and coagulum formation were seen in all leaflets post-mortem, and tighter throttling of RF ablation power may reduce char formation in future studies.

The catheter access route used here was advantageous in that it allowed direct access to the MV leaflets with little need for catheter steering, but the RFC catheter is intended to be used percutaneously, and thus approach the MV atrially through a trans-septal course. Future animal studies will employ trans-esophageal echocardiography (TEE) to more consistently and reliably guide catheter placement and transmitral positioning for ablation. Precise locations on the AL surface were not targeted in the present study due to the epicardial imaging technique used. However, it is anticipated that specific leaflet segments can be targeted with appropriate image guidance in real time, such as with 3D-TEE. Future percutaneous studies will use a trans-septal route with an antegrade approach to the MV apparatus and utilize a steerable sheath, such as the Agilis™ NxT Steerable Introducer (St. Jude Medical), for more precise positioning. It is anticipated that the combination of a steerable sheath with 3D-TEE will reduce the likelihood of off-target ablations compared to epicardial echocardiographic guidance. Animal models of degenerative MV disease, such as small breeds of canine,[1] frequently develop degenerative MR late in life and are proposed to investigate the acute and long-term ability of the RFC catheter to reduce MR percutaneously. Long-term healing in response to thermal injury will be of particular interest. Small amounts of fibrosis and coagulation necrosis have previously been seen 6 weeks following RF ablation of the MV leaflets.[17]

The RFC catheter is intended as a non-surgical, catheter-based therapy to approximate surgical leaflet resection, which is a focal technique that removes entire enlarged or prolapsing leaflet segments. While the RFC catheter does not provide the complete removal of a specific leaflet segment, as in surgical leaflet resection, thermal ablation of the majority or entirety of the MV leaflet area can potentially restore leaflet size to that of a healthy valve and reduce or eliminate prolapsing leaflet segments. While there was no diseased animal model available for this study, *ex vivo* testing of RFC catheter treatment of the bi-leaflet prolapse model indicated that leaflet prolapse can be significantly reduced (Figs. 4c and 4d). However, our bi-leaflet prolapse model was limited in that leaflet size was normal and no MR was observed, which often arises due to the exaggerated clefts that form between enlarged leaflet segments. The extent to which percutaneous treatment with the RFC catheter can reduce MR in myxomatous valves is still unknown, but prior open-heart application of RF ablation in canines with MR has shown that significant reductions in regurgitant volume with ablation alone are possible.[17]

While RFC catheter treatment is not currently as precise as surgical leaflet resection, the RFC catheter eliminates the need for an open-chest procedure to restore normal leaflet size and to reduce leaflet prolapse. Ultimately, the RFC catheter is intended as a percutaneous addition to the MV armamentarium to be used in combination with other percutaneous devices to effect a complete closed-chest MV repair. With the many percutaneous annuloplasty and neochordae devices in development,[5,11,14] a complete closed-chest MV repair appears promising.

CONCLUSION

This study demonstrates the *in vivo* feasibility of a catheter-based alternative to surgical leaflet resection for repair of degenerative MV disease using a RF ablation and cryo-anchoring catheter. RF ablation with cryo-anchoring stability can resect up to 40% of MV leaflet area. The RFC catheter cryo-anchor can selectively target each leaflet individually under echocardiographic guidance, allowing for RF delivery to specific leaflet segments. Efficacy criteria established *ex vivo* was used to confirm that RFC catheter treatment can be monitored on 2D echo and to confirm that leaflet shrinkage was efficacious in healthy pigs. While further testing of safety and efficacy remain in animals, these initial *in vivo* results indicate that the RFC catheter is a potential percutaneous alternative to surgical leaflet resection.

ELECTRONIC SUPPLEMENTARY MATERIAL

The online version of this article (doi:10.1007/s10439-015-1494-1) contains supplementary material, which is available to authorized users.

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DISCLOSURES

None.

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