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Novel *In Vitro* Test Systems and Insights for Transcatheter Mitral Valve Design, Part I: Paravalvular Leakage

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Abstract

While transcatheter mitral valve (TMV) replacement technology has great clinical potential for surgically inoperable patients suffering from mitral regurgitation, no TMV has yet achieved regulatory approval. The diversity of devices currently under development reflects a lack of consensus regarding optimal design approaches. In Part I of this two-part study, a test system was developed for the quantification of paravalvular leakage (PVL) following deployment of a TMV or TMV-like device in pressurized, explanted porcine hearts (N=7). Using this system, PVL rate was investigated as a function of steady trans-mitral pressure (P), TMV shape, and TMV-annular oversizing, using a series of “mock TMV plug” devices. Across all devices, PVL was found to approximately trend with the *square* of P . PVL rates were approximately 0–15ml/sec under hypotensive pressure, 10–40ml/sec under normotension, and 30–85 ml/sec under severely hypertension. D-shaped devices significantly reduced PVL versus circular devices; however, this effect was diminished when the circular device was oversized to the annulus by 6mm inter-trigonal distance. In conclusion, this steady pressure, *in vitro* test system was effective to compare PVL performance across TMV-like designs. PVL exhibited complex dynamics in terms of its response to transvalvular pressure and TMV profile.

Keywords

Transcatheter mitral valve replacement; transcatheter mitral valve implantation; transcatheter; mitral valve; paravalvular leakage; in vitro; flow rate; test method; device-tissue interaction; medical device

Introduction

Hemodynamically significant mitral regurgitation (MR) affects 1.7% of the US adult population, including 9.3% over 75 years of age.²⁵ While surgical correction is often effective, nearly 50% of severe, symptomatic MR patients are denied for surgery due to operative risk.²² To address this population, many unique transcatheter mitral valve replacement (TMVR) devices are currently in development. TMVR takes inspiration from

the success of transcatheter aortic valve replacement (TAVR), which continues to grow rapidly in use since gaining initial FDA approval in 2011. To date, at least 10 transcatheter mitral valves (TMVs) have reached clinical trials, and at least 20 others are in preclinical testing.⁵ None has yet achieved regulatory approval.

One critical performance criterion for a successful transcatheter valve replacement is the prevention of paravalvular leakage (PVL) through effective sealing. In the TAVR setting, PVL has proven a persistent challenge that relates to both annulus-device congruence and procedure.^{9, 13, 31} PVL is widely predicted to present an even greater challenge post-TMVR than post-TAVR. One reason for this is biomechanical: the regurgitant mitral annulus is larger, less circular, more dynamic, and less rigid than the calcified, stenotic aortic annulus.^{7, 29} The mitral valve (MV) faces higher pressure gradients than the aortic valve, and the device landing zone is shorter. Together, these factors may challenge the TMV to generate sufficient radial force to seal against the native tissue around its full circumference. Another reason is experience: mitral PVL is significantly more lethal than aortic PVL after surgical replacement.⁶ When PVL occurs, its visualization and quantification under echocardiography is challenging, especially at mild or moderate levels.³⁰ Further, PVL correction in surgically inoperable patients is challenging. Although percutaneous PVL closure is an emerging field, it currently suffers from low reproducibility and from success rates reportedly as low as 60%.^{3, 28}

In limited publicly available information following animal and human trials, early evidence of PVL post-TMVR has validated these concerns.^{10, 15, 21, 32, 33} While the eventual incidence of PVL upon wider TMVR usage remains to be determined, it can be reasonably expected to exceed that observed in early, carefully selected patient cohorts. For example, Muller et al emphasized that the first 30 patients receiving Tendyne (Abbott Laboratories, Chicago, IL) “were selected to maximize the likelihood of procedural success.”²³ This approach is well-justified for preliminary trials. However, in order to minimize the occurrence of PVL post-TMVR in a wider patient population, it is clear that rigorous PVL test systems, as well as a robust understanding of TMVR PVL dynamics, will be necessary.

Reported TMV designs differ widely in many regards, including their mechanisms to establish a seal against PVL.²⁹ However, one very common aspect of sealing is the application of radial outward force against the MV annulus. Presently, little engineering research exists regarding the influences of fundamental TMV design parameters on resultant PVL. Two such parameters are stent profile and degree of annular oversizing. Although many reported TMV designs are circular, some are D-shaped. These include the Tiara (Neovasc, Richmond, Canada) and Tendyne, which are currently undergoing major clinical trials. Meanwhile, to our knowledge, no studies to date have systematically addressed best practices for annular oversizing. To this end, the present two-part study aimed to develop novel benchtop test systems pertinent to TMV design, and employ them to assess key implications of TMV stent shape and size. In Part I, presented herein, paravalvular leakage rates were studied in explanted porcine hearts, using mock TMV models manufactured across discrete shape-size combinations. In Part II,²⁷ the radial forces necessary for a TMV to expand the porcine MV annulus to the same shapes and sizes was quantified via a novel force transducer system.

Materials and Methods

Test Specimens

All testing was conducted on porcine hearts, acquired the same day from a local supplier (Holifield Farms, Covington, GA). These hearts were in rigor mortis; contracture during rigor mortis has been reported to generate 60–100% of the tension as in normal systolic contracture.^{14, 34} All test specimens were selected according to their mitral annular perimeter and inter-trigonal distance. Respectively, the targets for these dimensions were 108.4mm and 30mm, based on a preliminary assessment of the hearts obtainable from this source. In addition, valve area, septal-lateral diameter (D_{SL}), and inter-commissural diameter (D_{IC}) were measured. Diameters were defined according to current recommendations for analysis of computed tomography imaging for TMVR procedural planning.^{4, 24}

To prepare each heart for testing, all great vessels were removed, with the exception of the aorta, which was transected proximal to the brachiocephalic artery. Both atria were removed, taking care to leave approximately 15mm tissue intact superior to the mitral annulus (otherwise, the floppy left atrium limited optical access, which challenged accurate device positioning). The left and right main coronaries were carefully exposed close to the coronary ostia, and sealed with umbilical clamps. A custom, 0.25in hole saw was used to create a hole in the left ventricular apex. Flow ports were then installed in the apex and aorta (respective outer diameters: 0.5in and 1.0in). The custom apical port featured two components, as shown in Figure 1C. Each was 3-D printed from ABS plastic (Stratasys, Eden Prairie, MN), acetone vapor-polished, and threaded manually. After the inner component (which contained the flow channel) was passed through the apex, the outer component was screwed onto it until they tightly sandwiched a section of apical ventricular tissue.

Paravalvular Leakage Test System

The prepared heart was mounted within a flow loop, suspended in an inverted orientation (Figure 1). Flow was driven into the apex and out the aorta by a steady flow pump (12U596, Dayton Electric Manufacturing, Co., Lake Forest, IL). With the pump set to a constant 70% power (approximately 130 ml/sec output), a ball valve downstream of the aorta (R1 in Figure 1) could be adjusted to tune left ventricular pressure (LVP). Because the atrial surface of the MV was exposed to atmosphere, LVP was equivalent to the trans-mitral pressure gradient (ΔP). Under nominal conditions, the mitral valve closed effectively, with negligible leakage. A “bypass” upstream of the apex was equipped with another ball valve (R2 in Figure 1); this enabled a wider range of ΔP to be achieved. ΔP was monitored through a pressure probe in the aortic port (6199, Utah Medical Products, Midvale, UT). A pair of ultrasonic, in-line flow probes (ME25PXN, Transonic, Ithaca, NY) were positioned before and after the heart. The difference between their signals described the amount of leakage through the heart (i.e., through ports and/or the mitral orifice). This approach is distinct from previously reported studies that have used fluid columns to generate intracardiac pressure.²⁶ The current approach was selected because it enabled fast, easy, and accurate adjustments to LVP.

Mock TMV Plugs

For the present goal of comparing PVL rates as a function of TMV profile shape and size, devices termed “mock TMV plugs” were developed. These aimed to simulate real TMV-annulus interaction. Importantly, we emphasize that many previously reported TMVR frame constructs^{8, 29} feature a wide range of *additional* sealing features, such as leaflet hooks, atrial flanges, etc. The plugs were not intended to replicate any of these features, but rather, to isolate the contributions to sealing of outer stent profile.

Each plug featured three distinct regions, as shown in Figure 2. The first was a 20mm annulus-contacting region. This height was chosen to extend slightly beyond the height of the annulus itself. The second was a ventricular cage (with height 23mm and minimum outer diameter 15mm). The cage was required because, without it, the porcine mitral leaflets were long enough to “reach” around the plug and coapt. In this scenario, no fluid pressure ever reached the paravalvular perimeter and no PVL could occur. The ventricular cage transiently held the leaflets open while the plug was in place, creating an orifice through which LVP reached the paravalvular perimeter and enabling PVL to occur (Figure 2F-H). The dimensions of this cage were such that it repeatably held the leaflets apart but did not impinge on the ventricular wall. Note, another theoretical solution to this challenge would have been to simply cut away some portion of the leaflets. We opted against such an approach because (a) the leaflets may play a role in sealing along the annulus-contacting region, and (b) it would have caused the flow loop to drain between every acquisition. The third region of the plugs was a screw connection, to which a custom collection funnel could be attached.

Each device was 3D-printed from ABS plastic and acetone vapor-polished. The annulus-contacting region was wrapped with a polyester fabric skirt designed for use in cardiovascular implants (007831; C.R. Bard, Inc., Murray Hill, NJ). Eight devices were used, each with a unique profile (cross-sectional geometry). These included circular (C) and D-shaped (D) plugs, in four perimeter-matched sizes (Table 1). Sizes are referred to as +0, +2, +4, and +6, which indicates millimeters of oversizing relative to the native annulus, in terms of inter-trigonal distance (only size 30 annuli were selected). The ventricular cage geometry was constant across all plugs. Note, because the plugs were rigid, as opposed to the more deformable stents used in currently reported TMVs, they modeled *final* TMV outer stent profile post-implant (during systole, by virtue of the heart’s rigor mortis state). Although the degree of TMVR oversizing in eventual clinical practice remains unknown, we note that the +6 device constituted 21.4% oversizing by perimeter, while the largest Sapien 3 TAVR device (29mm; Edwards Lifesciences, Irvine, CA) is recommended to oversize a native annulus by no more than 10.7%, per the device Instructions For Use. Thus, based on currently available information, the +6 device provided a conservative worst-case scenario for oversizing.

Experimental Protocol

All data was recorded using hardware (cDAQ-9174 and accessories) and software (LabVIEW) from National Instruments (Austin, TX). After installing the heart in the PVL test system, the flow loop was filled with water and maintained at $37\pm 2^\circ\text{C}$ throughout all

testing. Pressure measurements were adjusted to account for the height difference between the pressure probe and the mid-annular plane. With $P=120\text{mmHg}$, the MV annular saddle height was measured with a ruler.

For each PVL data acquisition, a repeatable approach for plug positioning was used. With R1 open and R2 closed, and with a single operator holding the collection funnel, the mock TMV plug was positioned such that its A2 (mid-anterior) position aligned with the A2 position of the annulus. It was guided past the mitral orifice and into the LV, until the mid-planes of the annulus and of the device's skirt coincided. Finally, the device was gently rocked about its A2-P2 (i.e., septal-lateral) axis until the maximum possible P was achieved. The acquisition then entailed establishing each of five *approximate* P targets and maintaining each for eight seconds. In order, these were 60, 90, 120, and 150mmHg, and the maximum possible P , obtained by fully closing R1 and R2 (typically 160–200 mmHg). These targets were chosen based on ISO guideline 5840–3, which considers P levels of 60, 120, 150, and 180 as the peak pressure differentials across a closed mitral valve corresponding to hypotensive, normotensive, mild hypertensive, and severe hypertensive states, respectively.¹ These targets were intentionally only approximate, to provide more robust input data to the General Linear Model (GLM) used for statistical analysis. At every P level, the collection funnel served to catch the flow through the paravalvular orifice and guide it toward a spill tray.

To compensate for potential variation in deployment positioning, three acquisitions were collected for each of the eight mock TMV plugs. Acquisitions were in order of increasing size; the first shape tested for each size alternated between experiments. Note, two key actions were performed throughout each experiment. First, the epicardial surface of the heart was kept hydrated via a topical spray. Second, approximately every five minutes, a baseline recording was collected with no plug in place, to quantify incidental leakage through tubing connectors.

Data Processing and Statistical Analysis

Recorded data were processed using MATLAB (MathWorks, Natick, MA). All curve fitting and statistical testing were conducted using MATLAB or Minitab (Minitab Inc., State College, PA). The primary endpoints were the trans-mitral pressure gradients (P) established by adjusting the ball valves, and their corresponding paravalvular leakage rates (PVL). Five (P , PVL) data points were obtained from each acquisition. Each value was computed as a 4 second average. Incidental leakage, obtained from the baseline recordings, was de-trended from PVL data. PVL was calculated from the difference between *two* flow probe measurements, each with absolute accuracy of 4% (per manufacturer specifications). Thus, the theoretical worst case PVL uncertainty was 8% of the pump output (approximately 10ml). Owing to this error, PVL values were occasionally near zero or slightly negative after incidental leakage correction. To correct for this, in any experiments for which the minimum PVL recording was below 1ml/sec, *all* PVL samples from that experiment were offset uniformly, such that the minimum PVL recording became 1ml/sec.

To investigate significance of differences in PVL between device types, two complementary statistical methods were used. For the first method, each PVL observation, PVL_i , was converted to $PVL_{norm,i}$:

$$PVL_{norm,i} = \frac{\left(\frac{PVL}{(\Delta P)^2}\right)_i}{\left(\frac{PVL}{(\Delta P)^2}\right)_{mean}} - 1 \quad (1)$$

PVL_{norm} was the percent change in pressure-normalized leakage of each observation, *relative to the average value across all observations*. Results were reflective of the overall sealing performance of each device. For example, if PVL_{norm} for a given device type were 0.1, this would signify 10% more normalized leakage *across all pressures* using this device, as compared to the average device. The use of $(\Delta P)^2$, rather than ΔP , was based on the empirically observed quadratic relationship between PVL and ΔP (see Results). PVL_{norm} enabled comparisons between each device *as a whole*, rather than specific to a given pressure level; the effect of device size and/or shape on PVL_{norm} was evaluated via two-way ANOVA followed by Tukey's Honestly Significant Difference test.

For the second method, a GLM accounted for not only device shape and size, but also ΔP as a continuous, independent variable. This enabled generation of a 95% confidence interval for each device type at any exact ΔP of interest (namely 60, 90, 120, 150, and 180 mmHg, per ISO guideline 5840–3¹). Note, GLM assumes a linear relationship between the continuous independent and dependent variables. Given the empirically observed quadratic relationship between PVL and ΔP (see Results), this assumption was satisfied by using the *square root of PVL* as the dependent variable. Both the ANOVA and GLM were run first without interaction terms to assess main effects, then with all possible two-factor interactions.

Results

Test System Performance

Upon the installation of a fresh porcine heart having a competent MV, trans-mitral ΔP could be easily tuned. The fluid mechanics analog of Ohm's Law states that LVP (here equivalent to ΔP) could be adjusted by changing either the flowrate through the LV and/or the resistance to flow. The former required either increasing the pump speed or closing the bypass valve; the latter required closing the downstream valve.

Under nominal conditions (no mock TMV plug), ΔP was easily adjusted to between 40–215 mmHg, and held steady within 1–2 mmHg. When a TMV plug was deployed, the resulting leakage gaps lowered resistance to LV outflow and reduced the maximum achievable ΔP . Nevertheless, even amidst $PVL > 70 \text{ ml/sec}$, ΔP values $> 160 \text{ mmHg}$ were attainable. Across all experiments, the system sealed effectively: combined incidental leakage through apical, aortic, and other connections was $3.3 \pm 3.0 \text{ ml/sec}$ (this was detrended from PVL data, as noted above).

Test Specimens

Testing of each heart commenced 7–11 hours post-mortem. MV annular saddle height had mean $\pm$ SD 15.4 $\pm$ 1.6mm. Additional annular dimensions from the selected hearts are given in Table 2. Note, no differences were detected between the pre-procedural dimensions of these hearts and those tested in Part II.²⁷ The PVL experimental protocol caused the MV annulus to stretch plastically. On average, the PVL protocol caused 20.9% increase in valve perimeter, 58.6% increase in valve area, 32.7% increase in D_{SL} , and 18.0% increase in D_{IC} .

PVL response to P and TMV Profile

Across all samples, P ranged from 52–209 mmHg and PVL ranged from 1–88 ml/sec. The F- and p-values from the ANOVA and GLM are given in Table 3.

Based on the representative data given in Figure 3, it is apparent that changes to PVL in response to changes in P were non-linear. This was observed in real-time throughout testing, and was subsequently quantified across all acquisitions. Two sets of correlation coefficients were produced for each of the 168 acquisitions, using either linear or quadratic fits. For quadratic fits, a slope of 0 was enforced at P=0 (on the assumption that leakage rates would not decrease as pressure increased). Mean $\pm$ SD R^2 values for linear and quadratic fits were, respectively, 0.826 $\pm$ 0.111 and 0.905 $\pm$ 0.082 ($p<0.001$ by paired Student's t-test). Based on this empirical evidence, a quadratic relationship between P and PVL was assumed for all subsequent analyses.

To visualize the P vs. PVL relationship for each device, quadratic curves were fit to the data from each device in two ways. First, curves were fit to data collected with each device from *each heart separately* (56 total curves; Figure 4). For every device, mean R^2 (averaged across hearts) was >0.7 , indicating a clear and consistent relationship between pressure and leakage. However, the diversity in the curves within each subplot indicates variability in leakage dynamics among the seven hearts.

Next, curves were fit to data collected with each device *across all hearts* (8 total curves, each with a 95% confidence interval; Figure 5). Each curve exhibited $p<0.001$, demonstrating that a real relationship existed between pressure and leakage across hearts, despite the variability between hearts. Figure 5 is also useful to describe typical leakage ranges at pressures of interest. For example, at P=60mmHg, PVL rates were approximately 0–15ml/sec; at P=180mmHg, PVL rates were approximately 30–85 ml/sec. Although PVL differences between devices are apparent, further analyses were necessary to understand whether these differences were statistically significant.

The first of these analyses investigated annular sealing performance of each device, *independent of P*, via PVL_{norm} . The best- and worst-sealing devices were, respectively, D +6 (PVL_{norm} mean $\pm$ SD: -0.246 ± 0.481) and C+0 (0.244 ± 0.529). These devices also exhibited the single best-sealing (-0.958) and worst-sealing (2.299) observations, respectively. The ANOVA model (Figure 6) revealed that the D-shape offered significant reduction in PVL_{norm} versus the circular shape (-0.127 ± 0.523 vs. 0.133 ± 0.564 , $p<0.001$). Across shapes, increasing size also significantly reduced PVL_{norm} , but only when comparing the +6 size (-0.147 ± 0.521) to any smaller size (+0: 0.064 ± 0.563 , $p<0.001$ vs. +6; +2:

0.084 ± 0.547 , $p < 0.001$ vs. +6; +4: 0.008 ± 0.576 , $p = 0.012$ vs. +6). Shape and size were found not to interact significantly as a whole (Table 3). Yet, one notable finding from the interaction plot is that, whereas C+0, C+2, and C+4 were each inferior to 3 of the D-shaped devices, C+6 was non-inferior to every D-shaped device.

Finally, a GLM was used to assess differences in leakage due to device profile at five P levels of interest (Figure 7). Each 30mmHg increase in P significantly increased leakage: for any given device, no two 95% CIs from different P levels overlapped. Although most findings at each P level paralleled those found in terms of PVL_{norm} , some nuanced differences between P levels were detected. For example, at 60mmHg, no significant differences in leakage were detected between circular and D-shaped devices at matching sizes. But as P increased, the annular sealing benefits of the D-shape became more pronounced. At 180mmHg, *every* D-shaped devices exhibited significantly less leakage than the circular devices of matching size. Also, at hypertensive conditions only, C+0, C+2, and C+4 were each inferior to *every* D-shaped device.

Discussion

Although the potential for PVL post-TMVR has been demonstrated through early clinical experience,^{10, 15, 21, 32, 33} its eventual incidence upon wider use of TMVR remains to be determined. However, it is clear that PVL is (a) lethal in the TAVR setting, even when mild,¹⁷ (b) deadlier at the mitral than at the aortic position,⁶ (c) more challenging to prevent in the TMVR setting than the TAVR setting, due to numerous biomechanical disadvantages,^{2, 7, 29} (d) difficult to diagnose, especially when mild or moderate,³⁰ and (e) especially difficult to treat in surgically inoperable patients.^{3, 28} In light of the wide diversity of TMV designs currently under development, and the paucity of existing engineering data in this scenario, this study sought to develop and employ a test system to evaluate PVL rates as a function of simple parametric TMV models. Previously reported test systems have incorporated intact ventricles into *pulsatile* flop loops,^{16, 19} which have revealed valuable insights into valvular mechanics. Here, we utilized a *steady* flow loop, for improved alignment to commercial test methods and international standards for leakage assessment.

Based on ISO guideline 5840–3 for the testing of transcatheter heart valves,¹ 60, 120, 150, and 180 mmHg represented peak P for hypotensive, normotensive, and mildly/moderately hypertensive, and severely hypertensive patients, respectively. Using the *in vitro* test system employed presently, which utilized fresh, rigorized porcine hearts, it was routinely feasible to measure PVL at or near these pressures. Further, statistically significant differences were detectable among different profiles of mock TMV plugs, suggesting that this test system holds potential for early-stage TMV testing and design iteration. Use of explanted hearts, as compared to potential alternatives such as silicone or rigid acrylic models, offers advantages in terms of fine-scale anatomic details and realistic material properties. The continued development of this system, and others that similarly replicate critical aspects of the *in vivo* TMVR environment, may lessen reliance on animal testing for pre-clinical product development.

For all 8 mock TMV plugs, the relationship between P and PVL was empirically determined to obey an approximately quadratic relationship (Figures 3–5). This relationship can be understood by considering the leakage flowrate as the product of velocity and cross-sectional area, then considering the relationships between P and either velocity or area. Specifically, we propose that increasing P simultaneously increases *both* velocity *and* area, to an extent that could manifest the observed dynamics. Further analysis is presented in Supplementary Material.

As evident from Figure 4, even when holding device shape and size constant, the pressure-leakage curves varied substantially among hearts. Although some portion of this variability can be attributed to the manual TMV plug positioning approach, the remaining variability is attributable to variation between hearts. Despite the careful heart selection process according to key annular dimensions, some variability in these dimensions, and in others such as ventricular mass, leaflet size, and tissue properties, was inevitable. In this way, device testing with a series of explanted hearts offers a more robust representation of the diversity in patient anatomies than could be captured via a synthetic model.

The specific TMV parameters modeled in this work were stent shape (circular versus D-shaped) and size (+0, +2, +4, and +6 mm oversizing, relative to the native annulus' inter-trigonal distance). Significant differences in leakage were detected among these eight mock TMV plug profiles. Irrespective of pressure level, or of specific statistical method used, D-shaped plugs exhibited significantly reduced leakage. The superiority of the D-shaped devices was expected, as they more closely matched the shape of the native annulus. However, based on this study alone, it remains unclear which of two potential mechanisms actually explain this phenomenon. It is plausible that sealing due to radial force occurs *at the annulus alone*, and that the D-shaped profile's greater congruence with the annulus equates to more *consistent* radial force on the around the annular circumference. Alternatively, this sealing may occur at both the annulus and leaflets. In such a scenario, the D-shape may gain advantage merely by applying more *targeted* radial force at the commissural aspects of the annulus (i.e., where very little leaflet tissue exists). Part II of this study, which directly quantified the radial expansion forces associated with these circular and D-shaped stent profiles, offered further insights into this question.²⁷

TMV designers must make decisions about both outer profile shape and intended approach to sizing. A circular stent is likely simplest to develop, and is insensitive to rotational orientation during implantation. Yet, as demonstrated presently, a circular profile post-implant may be expected to encounter greater PVL. Therefore, one relevant question is whether the potential trade-offs of a circular shape in terms of PVL can be compensated for through greater oversizing. In the present work, D-shaped devices reduced mean normalized leakage by 12.7% versus average. C+0, C+2, and C+4 each *increased* mean normalized leakage by at least 8.7% versus average. C+6 reduced normalized leakage by 2.0% versus average. Thus, within the limitations of the test system used here, although the D-shaped plugs offered uniformly better sealing, the discrepancy was reduced by approximately half when C+6 was used (Figures 6–7).

We emphasize that as a design objective, the minimization of PVL does not exist in isolation. Use of greater annular oversizing in an effort to minimize PVL may conceivably introduce other challenges, such as more difficult delivery, risk of left ventricular outflow tract obstruction, or risk of conduction abnormalities. Eventually, improved understanding of these tradeoffs should inform best practices in terms of final stent profile post-implant. This notion is further discussed in Part II.²⁷

The following limitations to this study are noteworthy. First, water was used as the working medium. This was necessary due to the volume of fluid needed for each experiment (typically over 100 L). A fluid such as water-glycerine, whose viscosity can be matched to blood (i.e., 1.8-times higher than water), would be expected by basic fluid mechanics theory to uniformly reduce PVL rates. Thus, use of water provided a conservative worst case for leakage rates in a global sense, but should not impact relative differences between devices. Additionally, submersion of cardiovascular tissues in water, rather than isotonic saline, may promote tissue swelling.¹⁸ However, we conducted in-house compressive testing of annular tissue under conditions mirroring the present test setup, following submersion in either solution, and no significant effects were detected (Supplementary Material). It must also be acknowledged that the experimental procedure plastically stretched the annulus (Table 2). This was an inevitable consequence of mechanically loading non-living tissue. Due to the scarcity of hearts with target dimensions, each was tested across every oversizing level, in order of increasing size. This potentially exacerbated PVL at larger sizes. Finally, we note that these experiments were conducted using normal porcine hearts. The normal porcine model is an established and appropriate tool for both *in vitro* and preclinical *in vivo* testing of heart valve devices,^{11, 12, 20} but may differ from diseased human hearts in terms of anatomy and/or material properties.

Conclusions

The novel *in vitro* test system developed here was effective to discriminate among mock TMV plug devices, in terms of annular sealing against PVL. PVL rate varied significantly according to device shape, device size, and trans-mitral pressure gradient. Most notably, D-shaped devices significantly reduced PVL compared to circular devices.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations

C	Circular
D	D-shaped

GLM	General Linear Model
IC	inter-commissural
MV	mitral valve
MR	mitral regurgitation
LVP	left ventricular pressure
PVL	paravalvular leakage
SL	septal-lateral
TAVR	transcatheter aortic valve replacement
TMV	transcatheter mitral valve
TMVR	transcatheter mitral valve replacement
P	trans-mitral pressure gradient

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Figure 1.
Paravalvular Leakage test system. (A) Schematic diagram. R1, R2: ball valves; Q1, Q2: flow probes; P: pressure probe. (B) Fully-prepared heart. (C) Rendering of two-part apical port.

Figure 2.

(A) Schematic of a TMV. Green: TMV. Arrows: stent radial outward force applied to annulus. Dotted blue lines: atrial flange and leaflet grasping mechanisms, which have been reported in some TMV designs to further support sealing against PVL. LA: left atrium, LV: left ventricle, Ao: aorta. (B-E) Mock TMV plug renderings. (B) Side view. (a) Annulus-contacting region; (b) ventricular cage; (c) screw connection (threading not shown). (C) Top views of circular and D-shaped plugs. (D) Collection funnel. (E) Plug and funnel. (F-H) Schematic justification for the use of the ventricular cage. (I) All plugs as manufactured, with D+6 attached to the funnel.

Figure 3.

(A) Representative raw data from a single acquisition. Red lines: 4 second averages. (B) Representative processed data from a single acquisition, with the corresponding quadratic fit (a slope of 0 was enforced at $P=0$).

Figure 4.

P vs. PVL curve fits. Each curve is fit to all data points collected *from a single heart* with a single device. C: circular; D: D-shaped.

Figure 5.

P vs. PVL curve fits. Each black curve is fit to all data points collected *from all hearts* with a single device; red curves denote 95% confidence intervals. C: circular; D: D-shaped.

Figure 6. PVL_{norm} ANOVA results (means and 95% confidence intervals). Within each plot, pairs whose 95% confidence intervals do not overlap differ significantly ($p < 0.05$). Green line: mean normalized leakage across all samples.

Figure 7.

(PVL) GLM results, at five different ΔP levels (means and 95% confidence intervals). Pairs whose 95% confidence intervals do not overlap differ significantly ($p < 0.05$).

Table 1

Dimensions for annulus-contacting regions of mock TMV plugs.

Device-Annulus Sizing	Profile Perimeter	Associated Inter-trigonal Distance	Circular profile diameter	D-shaped diameter profile	
				SL	IC
+0	108.4	30	34.5	28.8	37.5
+2	116.2	32	37.0	30.8	40.2
+4	123.9	34	39.4	32.9	42.9
+6	131.6	36	41.9	34.9	45.5

Table 2

Geometric descriptors for the 7 tested hearts.

	Pre-procedure	Post-procedure
Inter-trigonal distance (mm)	30.0 ± 0	31.3 ± 1.6
Perimeter (mm)	105.9 ± 10.5	128.1 ± 7.8
Area (cm²)	7.5 ± 1.6	11.9 ± 1.9
D_{SL} (mm)	23.5 ± 4.4	31.2 ± 4.8
D_{IC} (mm)	35.0 ± 5.9	41.3 ± 3.4
D_{IC}/D_{SL}	1.54 ± 0.44	1.46 ± 0.34

Table 3

Omnibus results of statistical models.

		Factor	F	p
ANOVA (PVL _{norm})	<u>Main effects</u>	Shape	47.75	<0.001
		Size	7.70	<0.001
	Interactions	Shape:Size	1.39	0.245
	GLM (sqrt(PVL))	<u>Main effects</u>	Shape	56.44
Size			7.18	<0.001
P			1822.21	<0.001
Interactions		Shape:Size	2.16	0.091
		Shape: P	9.83	0.002
		Size: P	0.55	0.645