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Posterior Leaflet Augmentation in Ischemic Mitral Regurgitation Increases Leaflet Coaptation and Mobility

Arminder S. Jassar, MBBS^{1,2}, Masahito Minakawa, MD², Takashi Shuto, MD², J. Daniel Robb, MBBS², Kevin J. Koomalsingh, MD², Melissa M. Levack, MD², Mathieu Vergnat, MD², Thomas J. Eperjesi, BS², Benjamin M. Jackson, MD^{1,2}, Joseph H. Gorman III, MD^{1,2}, and Robert C. Gorman, MD^{1,2}

¹Department of Surgery, University of Pennsylvania, Philadelphia

²Gorman Cardiovascular Research Group, University of Pennsylvania, Philadelphia

Abstract

Background—Restoring leaflet coaptation is the primary objective in repair of ischemic mitral regurgitation (IMR). The common practice of placing an undersized annuloplasty ring partially achieves this goal by correcting annular dilation; however, annular reduction has been demonstrated to exacerbate posterior leaflet tethering. Using a sheep model of IMR, we tested the hypothesis that posterior leaflet augmentation (PLA) combined with standard annuloplasty sizing increases leaflet coaptation more effectively than undersized annuloplasty alone.

Methods—Eight-weeks after posterobasal myocardial infarction, 15 sheep with 2+ IMR underwent annuloplasty with either a 24mm annuloplasty ring (24mm group, n=5), 30mm ring (30mm group, n=5) or a 30mm ring with concomitant augmentation of the posterior leaflet (PLA group, n=5). Using 3D echocardiography, post-repair coaptation zone and posterior leaflet mobility were assessed.

Results—Leaflet coaptation length after repair was greater in the PLA group (4.1±0.3mm) and the 24mm group (3.8±0.5mm) as compared to the 30mm group (2.7±0.6mm, p<0.01). Leaflet coaptation area was significantly greater in the PLA-group (121.5±6.6mm²) as compared to the 30mm-group (77.5±17.0mm²) or the 24mm-group (92.5±17.9mm², p<0.01). Posterior leaflet mobility was significantly greater in the PLA group as compared to the 30mm, or the 24mm group.

Conclusions—PLA combined with standard-sized annuloplasty enhances leaflet coaptation more effectively than either standard-sized annuloplasty or undersized annuloplasty alone. Increased leaflet coaptation after PLA provides redundancy to IMR repair, and may decrease incidence of both recurrent IMR and mitral stenosis. Abstract Word Count = 230

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Address Correspondence to: Robert C. Gorman, MD, Gorman Cardiovascular Research Group, Glenolden Research Laboratory, University of Pennsylvania, 500 S. Ridgeway Avenue, Glenolden, PA 19036, Phone: 267-350-9614, Fax: 267-350-9627, gormanr@uphs.upenn.edu.

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Keywords

Animal Model; Echocardiography; Heart Failure Operations; Mitral Regurgitation; Mitral Valve Repair

Introduction

Approximately one million Americans suffer a myocardial infarction (MI) each year [1]. Ischemic mitral regurgitation (IMR) occurs in approximately 25% of patients with inferior MI and is present in 1.2–2.1 million Americans [2]. Laboratory [3,4] and clinical [5] studies have demonstrated that IMR is associated with changes in annular size, annular shape and leaflet tethering resulting in loss of leaflet coaptation and a regurgitant valve. Restoration of coaptation and valve competency is the primary objective of IMR repair. Under-sized ring annuloplasty reduces the annular size, thereby restoring leaflet coaptation and has emerged as the preferred surgical treatment of IMR [6]. Mid- and long-term results of mitral valve repair using this approach have been disappointing, with experienced centers reporting significant recurrence rates of moderate to severe MR in up to one-third of patients within 6 months of surgery [7–8].

In an effort to improve surgical results, sub-annular and leaflet techniques such as secondary chordal cutting [9], papillary muscle modification [10], and leaflet augmentation [11–12] have been proposed. While these techniques have been employed in patients, the putative effect of these procedures on leaflet and annular geometry and function has not been fully evaluated. We have previously demonstrated that leaflet augmentation increases leaflet curvature, and decreases leaflet tethering as compared to undersized annuloplasty [13]. In this study, we examine the effects of posterior leaflet augmentation on leaflet coaptation and mobility in an ovine model of posterolateral MI.

Material and Methods

Surgical Protocol

The study protocol was reviewed and approved by the University of Pennsylvania School of Medicine Institutional Animal Care and Use Committee and was in compliance with Guide for the Care and Use of Laboratory Animals (US National Institutes of Health publication No. 85-23, National Academy Press, Washington, DC, revised 1996).

Surgical protocol for infarct creation and valve repair has been previously described [13]. Briefly, 15 adult male sheep underwent right thoracotomy to allow ligation of the left circumflex coronary artery branches between the lateral and middle cardiac veins. Eight-weeks after infarction, animals were randomized to undergo one of the following procedures: 1) undersized (size 24) mitral annuloplasty ring placement (Carpentier-Edwards Physio annuloplasty ring, Edwards Life Science, Irvine, CA, n=5); 2) standard-sized (30 CE Physio) annuloplasty ring placement (n=5); 3) combination of size 30 annuloplasty ring (CE Physio) placement with concomitant posterior mitral valve leaflet augmentation using a patch of autologous pericardium (n=5). Valve repair was performed via a left thoracotomy using cardiopulmonary bypass and standard cardiac surgical techniques. For those animals undergoing leaflet augmentation, a piece of pericardium was harvested and maintained in a saline bath at room temperature until required. An incision was made in the posterior leaflet 2mm from and parallel to the annulus and extending from the mid-P1 to mid-P3 regions. All chordae tendineae were preserved and left attached to the anterior portion of the posterior leaflet. A pericardial piece was cut to 45mm in length and 10mm in width and its ends were tapered. Using 6-0 polypropylene, this patch was sutured into place, first along its annular

edge and then along the edge opposed to the cut edge of the main part of the leaflet (Figure 1). The chosen annuloplasty ring was implanted. Mean aortic crossclamp time was greater in the PLA group (112 ± 30 min) as compared to the 30mm group (53 ± 14 min, $p < 0.01$) and the 24mm group (62 ± 30 min, $p = 0.03$, ANOVA < 0.01)

Following acquisition of echocardiographic data (see below), animals were euthanized. The heart was excised, the LV was opened through the interventricular septum and a digital photograph was taken. All photographs were imported into an image analysis program (Image Pro Plus, MediaCybernetics; Silver Spring, MD) and computer assisted planimetry was performed to quantify the total leaflet area.

Echocardiographic Protocol and Analysis

Full-volume data sets of the mitral valve were acquired using real-time 3-dimensional echocardiography (rt-3DE) with a Philips X7-2t hand held probe (Philips, Bothell, WA). Epicardial echocardiography was performed prior to creating myocardial infarction at the initial operation, and subsequently at the time of valve repair before instituting cardiopulmonary bypass and again after valve repair, approximately 1 hour after separation from bypass. All rt-3DE studies were performed at an arterial systolic pressure of 150 mmHg. Severity of mitral regurgitation was determined semi-quantitatively by assessing the area of the regurgitant jet as a percentage of left atrial area in the apical four-chamber view. The following grading scale was used: grade 0=no MR; grade 1 < 20%; grade 2 = 20% to 40%; grade 3 = 40% to 60%; and, grade 4 > 60% [14].

Techniques of MV segmentation and modeling have been previously described [15]. Briefly, each data set was exported to an Echo-View (TomTec Imaging Systems, Munich, Germany) software workstation. All analysis was performed at mid-systole, which was defined as the frame midway between the first frame demonstrating closure of the mitral valve and the first frame demonstrating closure of the aortic valve. To delineate the mitral annulus, 36 annular points were interactively placed by orthogonal visualization of the mitral valve in 18 long-axis cross-sectional planes separated by 10° each. The anterior and posterior commissures (AC, PC) were defined as annular points at the junction between the anterior and posterior leaflets (middle of commissural region) and interactively identified.

Technique for coaptation analysis has been previously described [16]. Briefly, measurement planes were marked at 1-mm intervals along the entire length of the intercommissural axis (Figure 2A). In each two-dimensional (2D) plane, data points delineating anterior and posterior leaflets were traced across the atrial surfaces (Figure 2B) resulting in a 500–1000 point data set for each valve. For the coaptation tracing, meticulous care was taken to clearly identify the tip of both anterior and posterior leaflets immediately before coaptation (using previous frames), so that the highest (most atrial) and lowest (most ventricular) margins of the coaptation zone could be defined. These atrial and ventricular edges of the coaptation zone (actual area of overlap) were then marked interactively (Figure 2B) [16]. Coaptation length was defined as the actual extent of overlap between the two leaflets, and any leaflet tissue beyond the area of apposition of two leaflets was not included as coaptation.

The Cartesian coordinates of each data point, assigned to the annulus, anterior leaflet, posterior leaflet, or the coaptation surface were then exported to Matlab (The Mathworks, Inc, Natick, Mass). Using custom Matlab algorithms, the least squares plane of the data point cloud for the annulus was aligned to the x-y plane. The septum (S) was identified as the anterior horn of the annulus at the aortic valve. The lateral annulus (L) was located at the middle of the posterior annulus circumference. With the annular model transformed such that the commissures were aligned with the y-axis, the anterolateral (AL) and posteromedial (PM) annular points are the locations of maximal and minimal y-value. Septolateral

diameter (SL) was defined as the distance separating S and L. Commissural width (CW) was defined as the distance between the commissures. Mitral Transverse Diameter (MTD) was the widest diameter of the mitral valve and was calculated as the distance between AL and PM. Mitral annular area (MAA) was defined as the area enclosed by the 2D projection of a given annular data set onto its corresponding least squares plane. Mitral annular circumference (MAC) was defined as the total 3D length of the annulus [15]. To calculate the degree of annular under-sizing, the post-repair value of a given parameter was expressed as a percentage of its pre-repair value.

Coaptation area was defined as the area of overlap of the anterior and the posterior leaflets across the extent of the entire mitral valve. For coaptation area analysis, the 3D lengths of atrial and ventricular coaptation edges were determined. The 3D coaptation area was then determined by triangulating the sampling points and summing the areas of the individual triangles. Segmental coaptation areas were determined by dividing the valve into equal thirds along the intercommissural axis. To provide a 2D visual representation of the coaptation area the atrial and ventricular coaptation edges were projected on to a viewing plane orthogonal to the least squares annular plane passing through both commissures (Figure 2C) [16]. Length of leaflet apposition (coaptation length) was measured at 1mm intervals spanning the entire MV from anterior to posterior commissure and mean coaptation length was calculated.

Posterior leaflet mobility was measured as the difference in the angle subtended by the proximal (annular) portion of the leaflet surface to the line joining the posterior and anterior annulus during systole (Figure 3A) and during diastole (Figure 3B). Coaptation distance (Figure 3C) was calculated as the 2D projected distance of the point of coaptation from the anterior annulus as a percent of total anteroposterior annular diameter. This was repeated at every 1 mm interval along the intercommissural axis from commissure to commissure. Figure 3D represents coaptation distance in all 15 animals at baseline (i.e. prior to creation of myocardial infarction).

Statistical Analyses

Continuous parameters were compared using ANOVA with post-hoc testing. The central tendency of these measurements is presented as mean $\pm$ standard deviation. For all comparisons, $p < 0.05$ was considered significant. All statistical analyses were performed using Microsoft Excel (Microsoft Corp. Redmond, WA).

Results

Ejection Fraction and Mitral Regurgitation

Ejection fraction and severity of mitral regurgitation at 8 weeks post MI (pre-repair), and 1 hour post repair are presented in Table 1.

Annular Parameters

Annular parameters before and after repair are presented in Table 2. As expected, the annular size (mitral annular area, mitral annular circumference, commissural width, maximum transverse diameter) in the 24 mm ring annuloplasty group was smaller post-repair as compared to the 30mm, and the PLA group.

Coaptation Length and Coaptation Area

Pre-repair and post repair coaptation lengths and coaptation areas are presented in Table 3. Mean post-repair coaptation length across the intercommissural axis in each group is presented in Figure 4A. Figure 4B and 4C depict projection of the mean 2D coaptation area

on to the intercommissural axis, before (Figure 4B), and after repair (Figure 4C). Whereas the coaptation length was significantly increased in the 24mm group, there was no increase in the coaptation area, likely due to reduction in annular size. Both coaptation length and area are increased in the PLA group. It should be noted that the coaptation depth (atrial coaptation edge) in PLA group is greater (i.e. onset of coaptation is displaced towards the ventricle) as compared to the other two groups, especially towards the posterior aspect of the valve.

Mean patch area (for the PLA group) was $346.4 \pm 58.0 \text{ mm}^2$ and resulted in significantly larger post-repair posterior leaflet area in the PLA group ($760.3 \pm 117.7 \text{ mm}^2$) as compared to the 24mm ($549.4 \pm 128.3 \text{ mm}^2$), and 30mm ring annuloplasty groups ($565.0 \pm 130.3 \text{ mm}^2$, ANOVA $p=0.037$).

Coaptation Distance

Coaptation distance is depicted in Figure 5. It is quite evident that the coaptation line is displaced towards the posterior annulus after creation of MI in all three groups (compare Figure 3D and 5A,B,C). This pattern tends to be exacerbated after annuloplasty alone with either the 24 mm ring or the 30mm ring. In the 30mm ring + PLA group the coaptation distance is restored closer to a normal pattern (compare Figures 3D and 5D,E,F).

Posterior Leaflet Excursion Angle

Posterior leaflets are more mobile in the PLA group as compared to the 24mm, and 30mm groups (Table 4).

Comment

The primary component of a durable mitral valve repair is the restoration of an optimal area of leaflet coaptation [17]. The factors that influence coaptation are annular size, annular shape, and the amount of mobile leaflet tissue. Annular reduction for IMR improves leaflet coaptation by reducing annular size, but at the same time impedes coaptation by exacerbating posterior leaflet tethering [18]. It has been postulated that flat annuloplasty rings atrialize the commissures and can potentially exacerbate posterior leaflet tethering by increasing the distance between the posterior leaflet and the papillary muscles [16,19]. Annuloplasty rings have also been shown to decrease posterior leaflet mobility [20]. These factors result in a functionally unileaflet valve in IMR where the coaptation is displaced towards the posterior annulus [19,21]. Anterior mitral leaflet is almost completely flattened during systole, and achieves coaptation close to the plane of the annulus [19].

In the experiments reported here, we employed a clinically relevant ovine model of chronic IMR which results from both progressive annular dilatation and leaflet tethering, to compare leaflet coaptation and mobility after implantation of an undersized annuloplasty ring, or a standard sized ring, or a standard sized ring combined with extensive posterior leaflet augmentation. This study demonstrates that leaflet augmentation mitigated several of the above-listed negative effects of ring annuloplasty on leaflet and coaptation geometry. Leaflet augmentation not only increased the coaptation area when compared to either annuloplasty size alone, but also improved leaflet mobility (Table 4) and restored the inter-annular position of the coaptation line (Figure 5). The point of coaptation was also lower (towards LV) in animals with leaflet augmentation (Figure 4C). Leaflet augmentation achieved equivalent coaptation length (Figure 4A) while maintaining a significantly larger mitral annular orifice area as compared to the undersized group (Table 2), thus also reducing the risk of mitral stenosis. These data demonstrate the leaflet augmentation is not only beneficial for providing additional leaflet tissue for coaptation, but also effective for restoring posterior leaflet mobility and bileaflet function of the mitral valve. Using the same

experimental model, we have previously demonstrated that posterior leaflet augmentation reduces posterior leaflet tethering and increases leaflet curvature [13] (a surrogate for reduced valve stress). All these effects should theoretically act synergistically to improve repair durability.

The data reported here support the use of posterior leaflet augmentation as a useful adjunct to annular reduction in patients with IMR. However, the experiment that was conducted has several limitations that preclude direct extrapolation of our findings to clinical practice at this time. First, we did not perform long-term follow-up on our animals, so firm conclusions regarding improved durability are not warranted. It is quite possible that the major cause of recurrent IMR is progressive infarct-induced LV remodeling that is independent of any surgical intervention on the mitral valve. Secondly, although recurrence rates for IMR are high, there is clearly a large group of patients that are well-served with isolated undersized annuloplasty. Such patients would not benefit from the increased complexity and increased myocardial ischemic times that are required for leaflet augmentation procedures. Wide-spread application of complex adjunctive maneuvers to ring annuloplasty should await the development of imaging strategies that improve our ability to preoperatively identify patients at highest risk for recurrent IMR with ring annuloplasty alone.

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Abbreviations and Acronyms

AC	Anterior commissure
AL	Anterolateral annulus
CW	Commissural width
L	Lateral annulus (midpoint of the posterior annulus)
MAA	Mitral annular area
MAC	Mitral annular circumference
MTD	Mitral transverse diameter
PC	Posterior commissure
PLA	Posterior Leaflet Augmentation
PM	Posteromedial annulus
S	Septum (anterior horn of the annulus at the aortic valve)
SL	Septolateral dimension

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Figure 1.

Leaflet Augmentation: Posterior infarct involving the posterior papillary muscle (PPM) was created by ligating branches of the circumflex artery. A pericardial patch was sewn across the three scallops of the posterior leaflet (P1, P2, P3). AL= Anterior leaflet, Ao = Aorta, APM = Anterior papillary muscle, Septum = Interventricular septum (cut)

Figure 2.

Leaflet Segmentation Technique. Panel A demonstrates template of transverse cross-sections every 1 mm along intercommissural axis. Panel B demonstrates one of the 2D cross-sections represented by the white dashed line in panel A; the atrial surface of the mitral valve leaflets, and the coaptation zone is interactively marked (green curves). The most atrial coaptation point is marked with a white dot while the most ventricular coaptation point is marked with an X. Panel C is a schematic which demonstrates how the atrial and ventricular coaptation points are then projected onto a viewing plane orthogonal to the least squares annular plane passing through the commissures to construct a 2D representation of the coaptation zone. The white and red dashed lines are both within least squares annular plane in all three panels. AC = Anterior commissure, AML = Anterior mitral leaflet, AoV = Aortic valve, Coapt = Coaptation, LA = Left atrium, LV = Left Ventricle, LVOT = left ventricle outflow tract, PC = posterior commissure, PML = Posterior mitral leaflet

Figure 3.

Leaflet Mobility and Coaptation Distance: Panel A depicts mid (P2) portion of the mitral valve (post-repair with ring annuloplasty and leaflet augmentation) during systole, marks the angle between the line joining the anterior (A) and the posterior (P) annulus (green line) and the tangent drawn along the leaflet hinge point and the leaflet surface adjacent to it (red dashes). Panel B depicts the same region of the valve during diastole and θ' was the angle between the line joining the anterior and posterior annulus and the tangent drawn along the leaflet hinge point and adjacent leaflet surface. All values of θ and θ' above the interannular line (i.e. towards left atrium, LA) were assigned a positive value, and all values below (i.e. towards left ventricle, LV) were assigned negative values. Leaflet excursion angle was calculated as $(\theta - \theta')$. Coaptation distance (CD, Panel C) was calculated as the 2D projected distance of the anterior annulus to the plane of leaflet coaptation (black dashes). Coaptation distance was measured at 1mm interval along the intercommissural axis, from anterior commissure (AC) to posterior commissure (PC). To account for variability in valve size between animals, the coaptation distance and intercommissural distance were normalized, and depicted as percent.. Panel D depicts coaptation distance for the all 15 sheep prior to creation of infarct (i.e. normal mitral valve). Dashes represent standard error. Ao= Aorta, AoV = Aortic valve, LVOT = Left ventricle outflow tract, AL= anterior mitral leaflet, PL= posterior mitral leaflet

Figure 4.

Coaptation Length and Coaptation Area: Panel A depicts the post-repair coaptation length (overlap length) across the span of the entire mitral valve in the three groups. For purpose of depiction, the intercommissural position has been depicted as percent. Dashes represent standard error. Panel B depicts the 2D projected coaptation area (area of leaflet overlap) for the 30mm ring annuloplasty group (30mm), 24 mm annuloplasty group (24mm), and the 30mm ring annuloplasty + posterior leaflet augmentation group (PLA) 8 weeks after creation of the infarct (pre-repair). Panel C depicts the projected 2D area of leaflet overlap for the three groups after respective repair. In both panel A, and B, arrow heads indicate the atrial coaptation edge, and arrows indicate the ventricular coaptation edge. AC=Anterior commissure, PC=Posterior commissure

Figure 5.

Coaptation distance is depicted as percentage of the anteroposterior annular diameter across the entire mitral valve from anterior (AC) to posterior (PC) commissure prior to repair (Panel A, B, C) and after repair (Panel D, E, F) in the 30mm, 24mm, and the leaflet augmentation (PLA) groups respectively. Anterior = Anterior Annulus, Posterior = Posterior Annulus

Table 1**Ejection Fraction and Degree of Mitral Regurgitation**

	30 mm Ring (n=5)	24 mm Ring (n=5)	30 mm Ring + PLA (n=5)	P-value (ANOVA)
Ejection Fraction (%) (pre-repair)	31±2.2	31±2.2	29±2.2	0.299
Mitral Regurgitation (0–4 scale) (pre-repair)	2.6±0.9	2.6±0.5	2.6±0.9	1
Mitral Regurgitation (0–4 scale) (post-repair)	0.2±0.3	0.2±0.4	0.3±0.4	0.9

PLA=Posterior Leaflet Augmentation

Table 2**Annular Parameters**

Annular Parameter	30 mm Ring (n=5)	24 mm Ring (n=5)	30 mm Ring + PLA (n=5)	P-value (ANOVA)
Mitral annular area (pre) (mm ²)	656.1±102.4	656.4±79.7	746.4±168.9	0.43
Mitral annular area (post) (mm ²)	424.8±27.2 ^a	311.0±46.6 ^a	443.2±34.7 ^a	<0.01 ^{b,c}
% (Post/Pre)	65.8±9.4	47.9±8.5	61.6±12.2	0.04 ^c
Commissural Width (pre) (mm)	34.1±3.4	33.7±1.9	36.2±4.0	0.44
Commissural Width (post) (mm)	26.9±1.0 ^a	22.9±2.1 ^a	28.1±2.2 ^a	<0.01 ^{b,c}
% (Post/Pre)	79.3±4.8	68.2±8.4	78.4±11.0	0.1
Maximum Transverse Diameter (pre) (mm)	34.4±3.0	34.2±1.9	36.9±4.0	0.34
Maximum Transverse Diameter (post) (mm)	27.1±1.2 ^a	23.2±1.7 ^a	28.5±2.1 ^a	<0.01 ^{b,c}
% (Post/Pre)	79.0±3.3	68.0±6.8	78.2±11.0	0.078
Septolateral dimension (pre) (mm)	23.6±2.3	24.2±2.0	25.5±3.0	0.49
Septolateral dimension (post) (mm)	19.2±1.3 ^a	17.4±1.5 ^a	19.4±0.9 ^a	0.044 ^b
% (Post/Pre)	82.0±11.4	71.9±5.9	77.0±8.2	0.23
Annular Circumference (pre) (mm)	96.7±5.8	95.4±4.5	101.8±9.9	0.36
Annular circumference (post) (mm)	76.2±2.3 ^a	64.6±4.7 ^a	79.1±5.7 ^a	<0.01 ^{b,c}
% (Post/Pre)	79.0±4.5	67.8±5.4	78.3±8.6	0.03 ^{b,c}

PLA=Posterior Leaflet Augmentation, pre=pre-repair, post=post-repair

^a p<0.05 for pre vs. post-repair

^b p< 0.05 for 24mm ring vs. 30mm + PLA

^c p< 0.05 for 24mm ring vs. 30mm ring

Table 3

Pre- and post-repair Coaptation Length and Coaptation Areas

	30 mm Ring (n=5)	24 mm Ring (n=5)	30 mm Ring + PLA (n=5)	P-value (ANOVA)
Coaptation Length - Pre-Repair (mm)	2.0±0.4	2.3±0.8	2.0±0.3	0.69
Coaptation Length - Post- Repair (mm)	2.7±0.6	3.8±0.5 ^a	4.1±0.3 ^a	<0.01 ^{b,c}
Coaptation Area - Pre-Repair (mm²)	75.2±13.8	91.6±37.6	79.4±19.3	0.59
Coaptation Area – Post- Repair (Total) (mm²)	77.5±17.0	92.5±17.9	121.5±6.6 ^a	<0.01 ^{b,d}
Coaptation Area – Post-Repair (Segmental) (mm²)				
A1-P1	22.3±9.3	28.5±4.1	35.3±5.9	0.03 ^b
A2-P2	31.5±11.4	38.0±9.0	49.4±6.8	0.03 ^b
A3-P3	23.7±5.0	25.9±8.6	36.8±5.5	0.02 ^{b,d}

A1,2,3=Anterior leaflet segments from anterior to posterior commissures, P1,2,3=Posterior leaflet segments from anterior to posterior commissures, PLA=Posterior Leaflet Augmentation

^a p<0.05 for pre-repair vs. post-repair comparison within each group

^b p<0.05 for 30mm ring vs. 30mm ring + PLA

^c p<0.05 for 30mm ring vs. 24mm ring

^d p<0.05 for 24mm ring vs. 30mm ring + PLA

Table 4

Leaflet Excursion Angles

	30 mm Ring (n=5)	24 mm Ring (n=5)	30 mm Ring + PLA (n=5)	P-value (ANOVA)
Pre-Repair (degrees)	46.5±10.6	38.6±18.9	48.6±12.2	0.52
Post-Repair (degrees)	51.8±13.1	54.0±10.7	102.4±8.7 ^a	1.15E-05 ^{b,c}

PLA=Posterior Leaflet Augmentation

^a p<0.05 for pre vs. post-repair^b p<0.05 for 30mm ring vs. 30mm ring + PLA^c p<0.05 for 24mm ring vs. 30mm ring + PLA