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An Ex Vivo Model of Left Ventricular Dilation and Functional Mitral Regurgitation to Facilitate the Development of Surgical Techniques

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ABSTRACT

Objective: Functional mitral regurgitation (FMR) is a common sequelae of myocardial ischemic disease. It results from annular dilation and outward rotation of the posterior papillary muscle. Different surgical techniques are under investigation for the treatment of FMR. However, an ex vivo model of FMR would be valuable to develop and compare the effect of techniques on the geometry of the left ventricle and the correction of FMR.

Methods: FMR was induced in explanted ovine hearts (n = 12) by manual dilation of the mitral annulus or by posterior papillary muscle repositioning with a patch. Left ventricular dimensions were measured. Mitral regurgitant volume (MRV) was measured in a continuous flow system.

Results: Annular dilation significantly increased MRV from 93.0 ± 110.4 to 472.2 ± 211.8 mL/min (P = 0.031), and the patch increased it from 37.8 ± 55.2 to 365 ± 189.6 mL/min (P = 0.031), with no significant differences between the 2 groups. When both techniques were applied, MRV significantly increased to 1383.5 ± 567.0 mL/min (P = 0.0005). The left ventricular sphericity index decreased from 3.25 ± 0.7 to 2.34 ± 0.6 (P = 0.0025) after application of the patch. The posterior papillary muscle was displaced after patch placement, following an outward rotation.

Conclusion: This ex vivo model reproduces annular dilation and outward rotation of the posterior papillary muscle, which are both present during FMR after ischemic myocardial disease. This model could be used to evaluate and compare interventions to treat FMR.

INTRODUCTION

Functional mitral valve regurgitation (FMR) is defined as systolic retrograde flow from the left ventricle into the left atrium in the presence of a structurally normal mitral valve [Enriquez-Sarano 2009]. Functional mitral regurgitation is the consequence of annular dilatation, tethering of the papillary muscle, and dysfunction of the left ventricular wall resulting in leaflet malcoaptation. Functional mitral regurgitation occurs as a consequence of dilative cardiomyopathies or postischemic events [Ahmed 2009].

Thirty percent of patients with a myocardial infarction will develop FMR as a complication of their ischemic disease [Levine 2005; Enriquez-Sarano 2009]. Retrospective studies have correlated the presence of increasing severity of FMR with decreasing survival [Levine 2005].

Surgical options consist of annular (restrictive mitral valve annuloplasty), valvular (leaflet augmentation, edge-to-edge clip), and subvalvular techniques (chordal cutting and repositioning of the papillary muscle), and remodeling of the left ventricular wall [McCarthy 2001; Messas 2001; Takagaki 2001; Fukamachi 2004; Levine 2005; Alfieri 2010]. The success of combinations of techniques attempted together confirms that surgery should include additional procedures apart from annuloplasty to enhance the effectiveness and durability of the repair [Isomura 2003; Alfieri 2010]. Those treatments need to be tested in animal models before being implemented in human patients. Animal models with ischemia require development of ventricular dysfunction and left ventricular remodeling to induce FMR. Animal models are associated with different degrees of morbidity and mortality. The variability of the animal models makes the comparison of the different techniques for the treatment of FMR difficult.

Richards et al [Richards 2009] developed an ex vivo model of mitral valve regurgitation with manual dilation of the mitral annulus. However, because this model did not reproduce left ventricular dilation it could not be used to evaluate surgical techniques to treat FMR. Ex vivo models reproducing annular dilation and tethering of the papillary muscles by placing the mitral valve apparatus in a test chamber have been developed [Katoh 1999; Poglajen 2010]. However, because only the mitral valve apparatus was used and not the complete left ventricle and atrium, those models could not be used to test different techniques for the treatment of FMR.

The purpose of this study was to develop an ex vivo model of FMR that reproduces left ventricular remodeling with tethering of the papillary muscles and dilation of the mitral valve annulus and preserves the left ventricle and atrium for testing of different surgical procedures.

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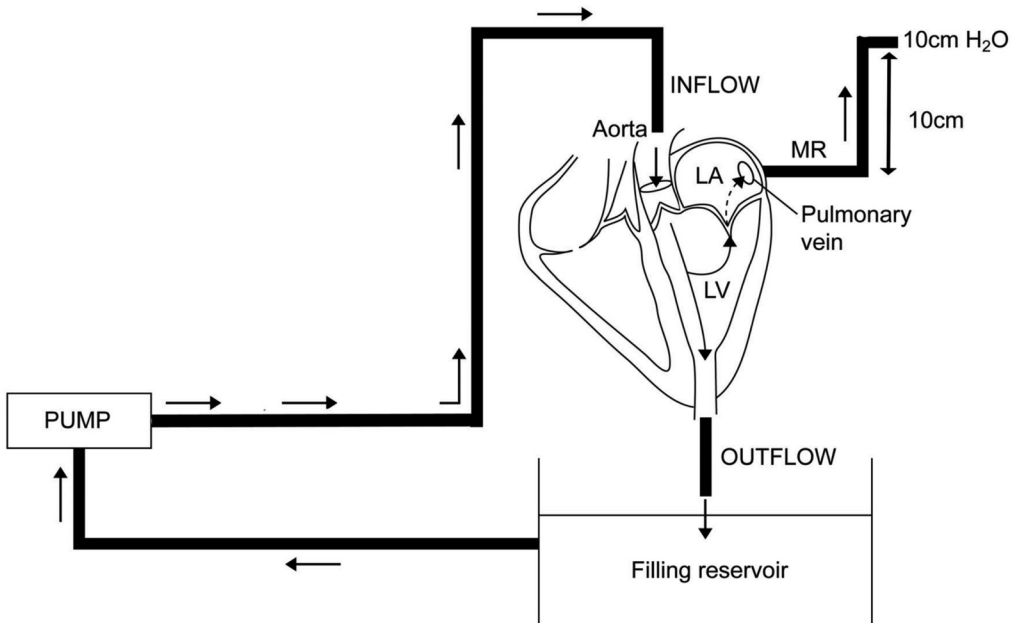

Figure 1. Schematic of the system component to establish a continuous flow in a retrograde fashion the left ventricle. Mitral regurgitation was measured 10 cm above the level of the mitral valve annulus.

MATERIALS AND METHODS

Hearts were acquired from adult Dorsett sheep (70 ± 5 kg) with no detectable abnormalities. Experimentation was carried out within 48 hours after harvesting. Hearts were kept in saline and stored at 40C.

The pericardium was removed and the aorta isolated and trimmed to a length of approximately 5 cm. The left and right coronary arteries were sutured closed at the base of the aorta. The aortic valves were removed to facilitate retrograde flow through the heart. The aorta was cannulated with a 3/8-inch cannula (Figure 1). The cannula was secured with a nylon tie around the aorta. It was connected to a pump to induce retrograde inflow into the left ventricle and atrium. The flow through the pump could be adjusted. A 1/4-inch cannula was then placed into the pulmonary vein.

Mitral regurgitation was induced with annular or left ventricular dilation and annular and left ventricular dilation. Annular dilation was produced by stretching of the annulus in both the transverse and anterior-posterior directions until an increase in anterior-posterior distance was evident, and mitral regurgitation was induced as described by Richards et al [Richards 2009]. The stretching of the annulus was performed while the heart was mounted within the continuous flow system. The dilation was performed incrementally until mitral regurgitation was visible. The annulus was carefully manipulated to avoid damage to the annular wall and mitral leaflets and rupture of the chordae. This necessitated the use of slow, constant, manual expansion.

Left ventricular dilation was achieved by suturing a patch of ovine diaphragm around the isolated papillary muscle (Figure 2). The posterior papillary muscle was isolated with an incision that was first performed 2 cm distal to the coronary groove in the posterior part of the ventricle between the second and third obtuse branches of the circumflex coronary artery. The incision was extended around the posterior papillary muscle with direct visualization of the chordate toward the apex of the heart to allow outward rotation of the papillary muscle. The posterior papillary muscle was then attached only to the apex of the heart by a 2-cm strip of myocardium at the heart apex and by the chordate. A crescent-shaped strip of ovine diaphragm, measuring 5 cm at its widest point, was then sutured along the incised ventricular wall, causing the papillary muscle to move away from in the annular plane, with consequential apical displacement of the posterior leaflet. It was sutured using a continuous mattress suture pattern with 4-0 suture material.

Seven ultrasound digital crystals (Sonometrics, London, Canada) were implanted at predetermined loci within the left ventricular chamber and annulus (Figure 3). The crystals were tunneled in the myocardium to the endocardium. Positioning of the crystals was based on prior studies investigating the geometric changes of the remodeled heart [Enriquez-Sarano 2009]. The ultrasound crystals were connected to a data acquisition system (Sonosoft; Sonometrics, London, Canada).

A microtip high-fidelity pressure transducer (Millar, Houston, TX, USA), inserted via a pulmonary vein and into the left ventricle, was used to measure the pressure (mmHg)

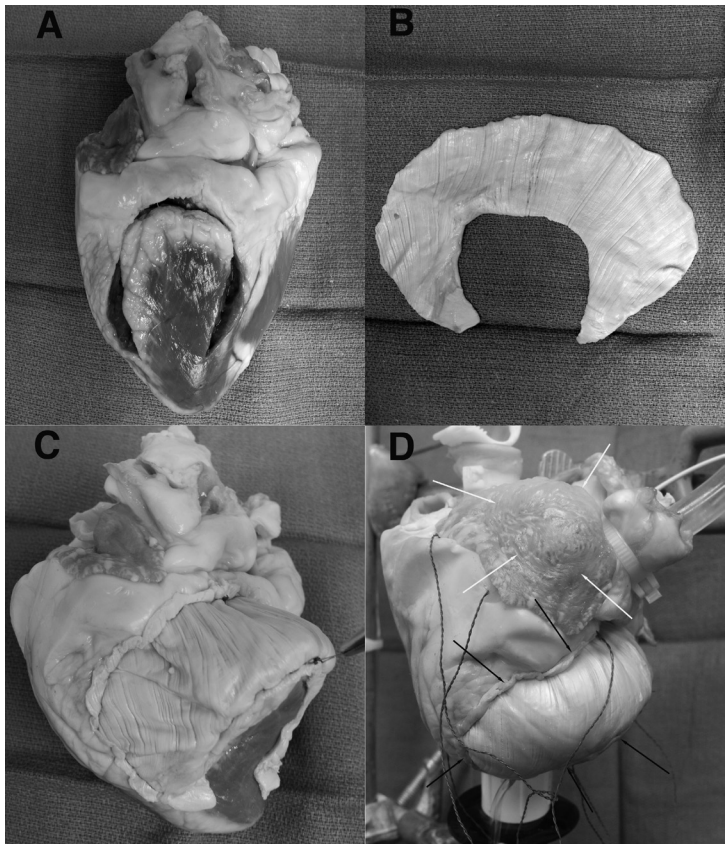

Figure 2. A, Incisional isolation of the posterior papillary muscle. B, Crescent-shaped patch made of ovine diaphragm is prepared. C, The patch of pericardium has been sutured with simple continuous sutures, allowing displacement of the posterior papillary muscle. D, Instrumented heart mounted within the continuous flow system. Sonomicrometer crystals have been implanted. The patch of diaphragm is pictured bulging when the left ventricular pressure reached 100 mmHg. The left atrium is distended because of mitral regurgitation.

within the left ventricle. Left ventricular pressure was maintained at 100 mmHg during the experiment by adjusting the flow of the pump. Mitral regurgitant flow was measured as the flow through the 1/4-inch cannula in the pulmonary vein with a trans–mitral-valve gradient of 90 mmHg (Figure 1).

The left ventricular sphericity index was defined as the ratio of the left ventricular long axis to the short axis. It was calculated at baseline, after each procedure, and with both treatments applied. Annular surface area was measured as a function of the mitral annular commissure-commissure dimension (C-C) and mitral annular septal-lateral dimension (S-L) ($\pi \times$ C-C $\times$ S-L).

Because the mitral regurgitant volume and the distances measured with crystals were not normally distributed, all data were reported as median and range. A Kruskal-Wallis test was used to compare mitral regurgitant volume between treatment groups. A Wilcoxon signed-rank test was used to compare match pairs to evaluate the effects of each treatment from baseline. A *P* value less than 0.05 was considered significant.

RESULTS

Twelve hearts were included in the study after an initial pool of 44 hearts. Twenty-one hearts were excluded from the study because of inadvertent mitral valve damage during dilation of the annulus. The mitral valve leaflets were either torn or the chordae damaged. Seven hearts were excluded because of transection of primary chordate. Three hearts were excluded because they could not maintain pressure within the left ventricle (leakage at suture line), and 1 papillary muscle was inadvertently avulsed during the testing.

The measured functional mitral valve regurgitant flow increased significantly from 93.00 ± 110.40 to 472.20 ± 211.80 mL/min ($P = 0.031$) after dilation of the annulus. It increased from 37.80 ± 55.20 to 365.00 ± 189.60 mL/min ($P = 0.031$) after placement of the patch. The amount of regurgitation was not significantly different after annular dilation or application of the patch ($P = 0.67$). Compared to baseline, dilation of the annulus and application of the patch significantly

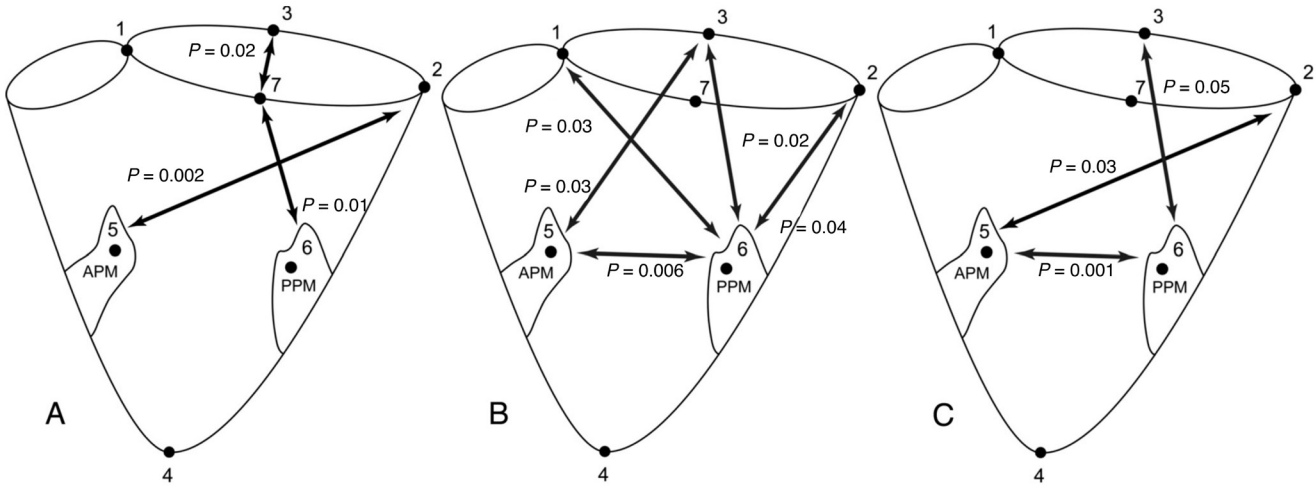

Figure 3. A, Geometric modifications due to annular dilation. B, Geometric modifications due to posterior papillary muscle distraction by epicardial patch application. C, Geometric alterations after both treatments. Significant increases noted in all reported dimensions. Distance 1-2 = mitral annular septal-lateral dimension; distance 3-7 = mitral annular commissure-commissure dimension. APM indicates anterior papillary muscle; PPM, posterior papillary muscle.

increased functional mitral valve regurgitant flow (1383.50 ± 567.00 mL/min, P = 0.0005). Dilation of the annulus and application of the patch significantly increased mitral regurgitant flow compared to only the patch (P = 0.001) and only annular dilation (P = 0.002).

Dilation of the annulus resulted in a significant augmentation of the commissure-commissure dimension of the annulus (P = 0.02). It also resulted in augmentation of the distance from the anterior papillary muscle tip to the lateral annular wall (P = 0.002) (Table 1 and Figure 3A). Annular dilation significantly increased the distance from the posterior papillary muscle to the anterior commissure of the mitral annulus (P = 0.01). Annular dilation increased the annular surface area from baseline (750.80 ± 284.20 to 964.60 ± 333.50 mm2) (P = 0.059). The left ventricular sphericity index decreased from 3.3 ± 0.8 at baseline to 3.2 ± 1.0 postannular dilation (P = 0.84).

After placement of the patch on the left ventricle, the distance of the posterior papillary muscle to the mitral annular septum significantly increased (P = 0.03), as did the distance to the lateral wall of the mitral annulus (P = 0.04) (Table 1 and Figure 3 B). The distance from the posterior papillary muscle to the posterior commissure of the mitral annulus was also significantly increased (P = 0.02). Application of the patch significantly increased the distance from the anterior papillary muscle tip to the posterior commissure of the mitral annulus (P = 0.03). Application of the patch also moved the papillary muscles apart (P = 0.006). Annular surface area was not significantly different from baseline (919.60 ± 379.80 to 788.20 ± 206.50 mm2) after application of the patch, (P = 0.47). The left ventricular sphericity significantly decreased from 3.30 ± 0.70 to 2.34 ± 0.60 after application of the patch (P = 0.031). The left ventricular sphericity index was not significantly different after applying the patch or dilating the annulus (P = 0.11).

When both treatments were applied, the distance between the anterior papillary muscle and the lateral wall of the mitral annulus was significantly increased (P = 0.03), as well as the distance between the posterior papillary muscle and the posterior commissure of the mitral annulus (Table 2 and Figure 3 C). The distance between the 2 papillary muscles was also significantly increased (P = 0.001). The annular surface area was not significantly increased, from 827.50 ± 325.30 to 1005.20 ± 355.00 mm2 after annular dilation and application of the patch (P = 0.27). The sphericity index significantly decreased, from 3.30 ± 0.70 at baseline to 2.50 ± 0.60 (P = 0.0015) after application of the patch and dilation of the annulus.

DISCUSSION

Application of patch in the left ventricle around the posterior papillary muscle induces mitral regurgitation by remodeling the left ventricle and outward rotation of the posterior papillary muscle. Mechanical dilation of the mitral valve annulus also induced mitral regurgitation in a similar fashion. The patch and the annular dilation interact to increase the amount of regurgitation when both techniques are cumulated.

The size of the patch can be varied depending on the amount of dilation needed. In this study the size of the patch of diaphragm was chosen to ensure maximal apical displacement of the papillary muscle. Once applied, the limiting factor for further apical leaflet tethering was only the chordae themselves. When the left ventricle was under pressure the chordae were stretched to and were tethered on the valve leaflet to induce FMR. None of the chordae tendinae were damaged during preparation. Annular dilation was applied

Table 1. Geometric Measurements at Baseline, after Either Annular Dilation or Patch Application*

Geometric Measurements, mm	Baseline (n = 6)	Treatment (Annular Dilation) (n = 6)	*P*	Baseline (n = 5)	Treatment (Patch) (n = 5)	*P*
Annular S-L	31.1 ± 9.4	34 ± 8.0	0.27	27.9 ± 8.3	24.3 ± 4.7	0.22
Annular C-C	30.6 ± 5.6	35.8 ± 8.4†	0.02	40.3 ± 5.7	41.2 ± 6.6	0.87
1-5	45.8 ± 5	45 ± 10.1	0.84	46.4 ± 5.3	56.3 ± 6.7	0.1
2-5	39.2 ± 5.4	43.1 ± 7.3†	0.002	42.3 ± 3.9	42.7 ± 2.3	0.87
3-5	44.1 ± 5	48.2 ± 7.5	0.09	49.1 ± 3.4	66 ± 10.3†	0.03
4-5	70.8 ± 8.4	73.2 ± 5.3	0.32	66.6 ± 9.4	74.6 ± 6.8	0.1
7-5	34.3 ± 4.8	36.1 ± 2.9	0.21	34.9 ± 6.7	34.1 ± 6.1	0.7
1-6	55.7 ± 2.7	54.9 ± 5.5	0.72	59.4 ± 3.8	68.8 ± 7.3†	0.03
2-6	36.2 ± 4.9	37.4 ± 5.2	0.1	40.8 ± 5	59.1 ± 18.2†	0.04
3-6	39.3 ± 6.7	39.3 ± 6.1	0.96	37.7 ± 5.4	69.3 ± 14.7†	0.02
4-6	51.6 ± 6.6	54.2 ± 5.2	0.08	51 ± 8.6	46.5 ± 6.5	0.09
5-6	29.6 ± 9	30.53 ± 7.9	0.47	31.2 ± 4.7	43.5 ± 4.5†	0.006
7-6	49.25 ± 6	61.5 ± 9.9†	0.01	53.8 ± 5.5	80.5 ± 23.3	0.07
3-4	90.4 ± 9	91.9 ± 10.7	0.57	96.9 ± 14	106 ± 14.5	0.43
Left ventricular pressure	100 ± 0.1	100 ± 0.1	0.36	100 ± 0.2	100.3 ± 0.1	0.36

*Numbers 1 to 7 refer to crystal implanted in the heart as described in Figure 3. Also as an example 1-5: is the distance between the crystal 1 and 5.
†Denotes significant measurement.

with simple mechanical distraction of the annulus without damaging the mitral valve leaflets or the chordae [Richards 2009]. Therefore in this model the leaflet apparatus and the chordae were left intact.

Our model is associated with an outward rotation of the posterior papillary muscle and an augmentation of the diameter at the level of the papillary muscles. It has been shown in various animal models and in clinical cases that the outward rotation of the papillary muscle with increased sphericity of the heart is an important component of the development of functional mitral valve regurgitation [Nielsen 1999; Otsuji 2001]. Otsuji et al [Otsuji 2001] showed in 2 different animal models the correlation between the tethering distance and mitral regurgitation orifice area. Tibayan et al [Tibayan 2003] highlighted the importance of the displacement of the posterior papillary muscle in chronic ischemic mitral regurgitation. In our study, with and without annular dilation, the mitral regurgitation was a direct result of increased tethering force on the valve, measured as the increased distance of the posterior papillary muscle from the anterior and posterior annulus, as used in clinical studies in which tethering lengths are measured using echocardiography [Ciarka 2010].

Annular dilation alone induced significant mitral regurgitation in this in vitro model. The annular dilation we achieved was not the 25% increase in anterior-posterior annular distance as recommended in an in vitro porcine model by Richards et al [Richards 2009]. The commissure-to-commissure diameter (3-7) increased by 19% in our study. Sheep hearts were used in this model instead of pig hearts. It has been previously reported that sheep have shorter mitral valve leaflets with smaller coaptation surface areas than humans [Gorman 1997]. Because pig hearts have an anatomy closer to human hearts, this anatomical difference may have contributed to the induction of mitral regurgitation by annular dilation alone in this in vitro model.

Table 2. Geometric Measurements at Baseline and After Application of Both Annular Dilation and Posterior Papillary Muscle Repositioning (Patch Placement)*

Geometric Measurements, mm	Baseline (n = 11)	Treatment (Annular Dilation) (n = 11)	*P*
Annular S-L	29.7 ± 8.6	30.6 ± 4	0.75
Annular C-C	35 ± 7.4	43.3 ± 13.4	.08
1-5	45.3 ± 5	45.8 ± 8.2	.8
2-5	40.6 ± 5.4	44.0 ± 5.7†	.03
3-5	46.4 ± 4.9	51 ± 18.6	.38
4-5	68.9 ± 8.7	69.6 ± 11.7	.85
7-5	34.6 ± 5.4	38.6 ± 9.8	.25
1-6	57.4 ± 3.6	58.9 ± 9.8	.59
2-6	38.3 ± 5.3	40.7 ± 8.2	.22
3-6	38.6 ± 5.9	53.6 ± 20.2†	.05
4-6	51.3 ± 7.2	56.4 ± 13.1	.3
5-6	30.3 ± 7	40.3 ± 8.5†	.001
7-6	51.3 ± 6	54.1 ± 14	.55
3-4	93.3 ± 11.4	94 ± 12.4	.85

*Numbers 1 to 7 refer to crystal implanted in the heart as described in Figure 3. Also as an example 1-5: is the distance between the crystal 1 and 5.
†Denotes significant measurement.

This model is in a continuous and static flow system. Nonpulsatile models have been used in the past to evaluate functional mitral valve regurgitation [Katoh 1999; Poglajen 2010]. However, pulsatile flow could be used as described in Richards et al [Richards 2009] if the impact of the dynamics of the mitral leaflets on FMR are to be studied. In our model we established retrograde flow through the aortic root because we were using a continuous flow. A pulsatile flow could be established as described by Richards et al [Richards 2009] through the apex of the left ventricle. Our model, like the model described by Richards et al [Richards 2009], used a heart that was not supported by any perfusate, therefore the experiment has to be conducted rapidly after collection of the heart from a fresh cadaver. The suturing of the patch was performed with a simple continuous pattern with a 4-0 monofilament suture. The amount of leakage at the suture line was very minimal, and a pressure of 100 mm Hg could be maintained in the left ventricle. However, only 1 experiment can be performed on each heart because tearing of the suture line occurs when too many manipulations are performed. Therefore different transmitral pressure gradients could not be evaluated.

Left ventricular dilation responsible for FMR can be recreated in an ex vivo model by the displacement the left posterior papillary muscle. The combination of displacement of the posterior papillary muscle and annular dilation increased the amount of mitral regurgitation. Our model mirrors the modified geometry of the left ventricle, as well as annular dilation, without modifying the mitral leaflets or chordae. Consequently, the technique used to treat FMR, including reshaping of the left ventricle, can be tested utilizing this model.

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