THESIS

DEVELOPMENT OF AN IN VITRO MODEL OF FUNCTIONAL MITRAL VALVE REGURGITATION

Submitted by

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ABSTRACT

DEVELOPMENT OF AN IN VITRO MODEL OF FUNCTIONAL MITRAL REGURGITATION.

Functional or ischemic mitral regurgitation (FMR) is a common sequelae to various cardiomyopathies which result in altered cardiac geometry secondary to ventricular remodeling. The causative architectural changes are typified by apical tethering of the mitral valve leaflets by ventricular dilation and papillary muscle repositioning, and is usually accompanied by annular dilation and mitral valve leaflet malcoaptation. The resultant mitral regurgitation (MR) and consequent volume overload contributes to further ventricular remodeling and perpetuation of the clinical scenario. The present mainstay of surgical therapy involves annular undersizing with the use of annuloplasty rings. However, surgical interventions thus far have been limited by numerous shortcomings and inconsistent results emphasizing the need for continued research into the mechanics of FMR correction.

In this study a novel invitro model of FMR utilizing explanted ovine hearts was introduced as a tool for investigating the mechanism of FMR and determining strategies aimed at correction. In the first phase of model development FMR was induced by either annular dilation or papillary muscle repositioning in a static flow system. Both techniques were individually able to significantly increase the regurgitant volume from baseline (annular dilation: baseline 15.5ml/10s to 78.7 ± 35.3 ml/10s, p=0.02, patch: baseline7.6ml/10s to 67.4 ± 30.4 ml/10s, p=0.02) with no significant differences between the two groups and a marked increase in regurgitant volume noted when both techniques were applied together (p=0.0001). The devised technique of papillary muscle

displacement by patch placement successfully recreated the outward rotation and increased LV sphericity (baseline: 3.25±0.7, patch: 2.34±0.6, p=0.0025) observed clinically. For the second phase of the study the developed model was investigated in a pulsatile flow system with FMR induced by posterior papillary muscle displacement only. A timed, positive pressure valve pump with a set rate of 80 simulated beats/min and approximate flow rate of 6L/min was used and procured results even more pronounced than that recorded for the static flow system (static flow system MR vol: 67.4±30.4ml/10s vs. pulsatile flow system MR vol: 310.5 ± 86.6ml/10s).

The final investigation involved subjection of the developed FMR model to geometric modifications aimed at correcting MR in the pulsatile flow system. Attempts were made to correct the modeled papillary muscle displacement until the regurgitant volume was eliminated/minimized and the associated LV dimensions measured. The results showed that correction of the apical tethering of the chordae was sufficient to significantly reduce MR volume (patch: 310±86.6ml/10s vs. displacement correction: 16.1 ± 23.7ml/10s, p=0.0001) despite failure to return to baseline dimensions.

In the developed model, which has been demonstrated to be amenable to both pulsatile and static flow systems, annular dilation and posterior papillary repositioning were both able to individually induce FMR and significant increases in regurgitant volume was noted once the two techniques were combined. The role of posterior papillary muscle repositioning in the correction of this disease was emphasized. The developed model provided evidence for the possibility of FMR elimination by geometric

alterations beyond restoration of baseline/pre-disease dimensions with direct clinical implications to the surgical treatment of affected patients.

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To my family; my mother, my sisters Colleen and Leslie and my uncle Samuel Thornhill for their prayers, support and unlimited encouragement; mere words cannot suffice.

And finally and foremost to the One whose name is above every other name, the great I am that I am, who makes a way in the wilderness and rivers in the desert: thank you Father!

DEDICATION

I would like to dedicate this work to my mother, Lynette Pouching. She saw this day long before it was a reality, she always believes in my dreams.

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INTRODUCTION

BACKGROUND

What is functional mitral regurgitation?

Functional, ischemic or secondary mitral valve regurgitation can be defined as systolic retrograde flow from the left ventricle into the left atrium in the presence of a structurally normal mitral valve.[7] This somewhat peculiar aetiology of mitral regurgitation (MR) can be noted as a consequence of annular dilatation and resultant leaflet malcoaptation despite normal leaflet motion. This scenario is classified as Type Ia according to Carpentier's classification of mitral regurgitation. Functional mitral regurgitation (FMR) can also be the direct result of restricted leaflet motion due to excessive leaflet tethering, classified as Carpentier Type IIIb[41]and occurs as a consequence of dilative cardiomyopathies or post ischemic events.

What is the significance of this disease?

Every 34 seconds an American suffers a heart attack, of these persons 30% will develop FMR as a complication of their ischemic disease.[7] Of the persons eventually progressing to chronic heart failure, 35-50% will concurrently experience FMR.[42] It has also been estimated that FMR is likely to affect the majority of heart failure patients as a preterminal or terminal event.[43] Retrospective studies have correlated the presence of increasing severity of FMR with decreasing survival.[42]

What is the Pathophysiology of FMR?

This condition is characteristic of cardiomyopathies resulting in altered ventricular geometry such as secondary to ischemic disease or dilated cardiomyopathy.[8] The resultant physiologic cascade is a compensatory mechanism to optimize cardiac performance and restore forward cardiac output. Cellular and molecular events orchestrate architectural changes of the LV as the body attempts to maintain an adequate stroke volume. The volume overload results in eccentric cardiac hypertrophy and dilation due to increased length of individual myocardial fibers with minor increases in wall thickness.[17] The transcriptional response of the myocardium results in the reexpression of fetal myosin heavy chains isoforms (less contractile ability) along with fetal cardiac genes responsible for the production of growth hormone, proto-oncogenes and natiuretic peptides.[3] Cardiac fibroblasts, the most numerous cell type of the myocardium (60-70%), differentiate into myofibroblasts which not only directly respond to mechanical stretch and ischaemia but also to the onslaught of the various hormonal and proinflammatory cytokines increased in the remodeling heart. These cells then secrete more proinflammatory cytokines and growth factors and contribute to increased proliferation and extracellular matrix turnover. [4,5] These responses contribute to increased ventricular accommodation allowing increased loading volume and improved ejection fraction, however, workload is increased.[13]

Xia et al in a murine model noted that "Fibrotic remodeling of the ventricle is initially associated with marked hypertrophy without ventricular enlargement or systolic functional impairment, followed by the development of chamber dilation and systolic

dysfunction".[6] It is this LV dysfunction which marks the graduation from a variable asymptomatic period to the onset of clinical signs of pulmonary hypertension due to increased LV filling pressure.[13] There is also reduced forward output along with continuing dilation. Interventions must be made before this decompensatory phase[10] which is associated with a poor prognosis even post-correction.[11] LV dysfunction is a significant predictor of hospital mortality which is increased in these patients vs those without ischemic mitral valve regurgitation.[13, 14.] Surgery before the onset of irreversible ventricular dysfunction is crucial for a favorable outcome.[13] Severe cases of LV dysfunction and papillary muscle asynchrony may also require cardiac resynchronization therapy.[22,32]

The described LV alterations give rise to apical and posterior displacement of the papillary muscles with displacement of the posterior papillary muscle having the most significant impact on the development of FMR. [2] There is resultant tethering of the mitral valve leaflets, with/without annular dilation; the end result being incomplete mitral leaflet closure and consequent mitral regurgitation.

How can this disease be corrected?

The solution to functional mitral valve regurgitation has been somewhat of an enigma as it continues to affect millions worldwide. Despite years of research, clinical trials and the advent of numerous surgical interventions, a consensus on the best approach for treatment remains not only elusive but also the source of much debate and controversy.

The numerous surgical interventions in existence attest to the relative inadequacies of each of the available techniques (Table 1). Annuloplasty, mitral valve repair or mitral valve replacement are used routinely in patients with functional MV regurgitation[10] though replacement is frowned upon due to the increased intra and post-operative risk.[31] Leaflet extension to increase the effective surface area has also attempted with some success.[39] Undersized annuloplasty is currently the gold standard of treatment. Annular dilation which can be present in both ischemic and dilative cardiomyopathy results in less leaflet surface area available for coaptation and a regurgitant jet is formed,[17] hence the relative effectiveness of correction of annular geometry with undersized mitral annuloplasty.[18] This technique is regarded as a safe procedure, [45] and once successful, is believed to be able to reverse the maladaptive LV remodeling.[15] However, this approach underlies the debate of the relevance of a valvular solution to a ventricular problem and is further belied by poor long-term outcomes [40] and the reoccurrence of mitral regurgitation which is associated with a poor prognosis and suboptimal quality of life for these patients. [9] MR has been found to reoccur in as much as 30 % of patients within 1 yr[40] and 72 % by 5yr follow-up after corrective annuloplasty.[44] Investigations into the mechanism of MR reoccurrence post annuloplasty show the persistence of leaflet tethering after annuloplasty alone [47] highlighting the necessity of combining this with some other complementary procedure. [2,48]

Frequently overlooked is the importance of correcting subvalvular dimensions of dilated ischemic or non-ischemic hearts. The importance of LV geometry on the function of the mitral valve has been investigated using various in vitro[16,33] and in vivo[26,29] studies. The effectiveness of techniques aimed at reducing the tethering of the chordae

tendinae due to papillary muscle displacement[19,25] implicate the need for the concurrent use of both annular and subvalvular approaches.[2] Alternatively, strategic transection of strut chordae can also relieve tethering and correct regurgitation.[38] The outcome of ventriculoplasty for severely dilated ventricles supports the proposed benefit of a dual approach by the significant clinical functional improvements noted post correction.[20] Devices that change the shape of the dilated ventricle,[34,36] or reposition both the annulus and papillary muscles,[35] have been proposed. Passive ventricular support devices have also been investigated, their goal: to limit or even reverse the remodeling process of the failing heart.[23,24,37] They have also been found to improve the LV function of non-ischemic dilated cardiomyopathies.[30] The success of combinations of techniques attempted together[12] confirms that surgery should include additional procedures apart from annuloplasty to enhance the effectiveness and durability of the repair.[46]

Table 1: Existing surgical techniques for the amelioration of FMR.

Undersized flexible ring annuloplasty
Suture annuloplasty
Annular cinching
Selective valve replacement
Uniform chordal sparing valve replacement
Posterior leaflet patch extension
Transection of secondary chordate
Papillary muscle repositioning
Papillary muscle sling[19]

Infarct plication

Cardiomyoplasty

Passive containment with ventricular support devices

Surgical ventricular restoration (ventriculoplasty/ventriculectomy)

Present research limitations

For the medical community to remain relevant to an aging society research must be aimed towards an exhaustive understanding of the impact of the geometric changes present in the remodeling heart on the development of mitral regurgitation. This insight is the fundamental basis for the evaluation of strategies for correction. Pertinent research utilizing animal models are limited by cost, feasibility and hampered by anatomical differences among species.[21] Studies involving human patients have been limited by a lack of randomized control and prospective studies to help ascertain the best surgical treatment.

REVIEW OF EXISTING MODELS

In vivo models

Numerous canine and ovine in vivo models have been used in an attempt to recreate chronic heart failure (CHF) of which FMR can be a sequelae. Rapid ventricular pacing, coronary occlusion by ligation/microembolization and volume overload by aortic banding are some of the most commonly employed means of inducing CHF. The animal subject is then maintained for a minimum of 6 weeks, depending on the technique used, while FMR develops as a result of ensuing cardiac remodeling. Mitral regurgitation is

monitored by echocardiographic studies for the development of moderate to severe MR. Subjects that develop FMR are then used in subsequent surgical procedures investigating corrective techniques.

Rapid ventricular pacing, introduced as early as 1986 by Armstrong et al.[55], involves the implantation of a pacemaker allowing the heart rate of the animal to be controlled by the investigator. Heart rates are chronically increased, with rates as high as 250 for dogs and 230 for sheep[53], and has also been used with leporine and porcine subjects. This CHF model has successfully reproduced the global remodeling hallmark of FMR as well as the neurohormonal and hemodynamic characteristics of human DCM. However, the pathogenesis of the changes remains unknown, this model never develops the typical cardiac hypertrophy seen in CHF, and reverts within 1-3 weeks of cessation of pacing.[53,56,57]

Numerous insights into the physiology of FMR and the effectiveness of novel corrective interventions have been achieved with use of rapid ventricular pacing in vivo studies. For example, the Coapsys device aimed at correcting FMR was investigated in a canine rapid ventricular pacing in vivo model before being sent to clinical trials.[51] Likewise in 2011, a six-month terminal ovine rapid ventricular pacing model was used by Raman et al. to investigate the efficiency of the latest version of the BACE (Basal Annuloplasty of the Cardia Externally) device, a new intervention aimed at eliminating FMR without the need for cardiopulmonary bypass. Positive results led to a promising human clinical trial.[54]

Methods of inducing volume overload have also been applied to create CHF models. Arteriovenous shunts have been used to cause eccentric ventricular hypertrophy and FMR, however, the unreliable development of MR has limited the use of this in vivo model in FMR investigations.[58] Aortic or mitral valve compromise has also been used as a method of volume overload induction of CHF for pharmacological studies.[59]

Another commonly employed in vivo model involves the induction of myocardial ischemia in animal subjects to create CHF and subsequent FMR, and may be the closest to the clinical scenario of FMR in ischemic cardiomyopathy. Coronary artery ligation or microembolization has been explored in sheep and dogs. The collateral coronary circulation in dogs has made this a sometimes unreliable and expensive venture in canine studies[58], whereas the ovine counterpart has been used in various studies investigating the effect of infarct location on MR development.[60]

Tibayan et al. successfully used an ovine model of induced posterior-inferior coronary infarction by ligation to investigate the effect of various approaches to correcting FMR[2] supporting the merit of in vivo models. However, these models are not without shortcomings; after an extensive critique of the existing large animal (dogs, sheep, calves) in vivo models of CHF by Schmitto et al. in 2009[49] they presented a novel approach to a more reproducible CHF model in 2010. Their approach to inducing chronic heart failure bypassed a common problem of the heart's tendency to hypertrophy and develop compensatory hyperkinesis of adjacent unaffected regions as opposed to the global remodeling which is required for the development of FMR. They were able to

achieve this by several strategically placed, transmural, coronary ligations spanned over the LV.[52]

Other approaches for experimentally inducing CHF include transmyocardial electric shock, increasing LV afterload by aortic banding and the use of cardiotoxic agents such as snake toxins or chemotherapeutic agents.[58] These in vivo models have not been utilized in more recent FMR studies.

In vivo FRM models are all riddled with short comings. Firstly, the chronic nature of FMR limits their feasibility. After the induction phase subjects have to be kept and frequently monitored for extended periods of time; not only is this labor intensive but can also be very costly. Losses are incurred when only a fraction of the initial subjects make it to the final stages of the experiment as frequently happens. For example, in the aforementioned in vivo study by Tibayan et al., in which coronary ligation by a method outlined by Llaneras et al.[61] was used, an initial test group of 26 sheep dwindled to 10 animals surviving the CHF induction process and developing adequate mitral regurgitation for inclusion into the study.[2] The requirements of the intervention to be analyzed also impacts on the choice of in vivo model that should be used. Multiple invasive procedures requiring cardiopulmonary bypass not only increases the risk and poses ethical concerns but also demands specialist surgical expertise/post-op care. A detailed look at the relative pros and cons of the available large animal models by Schmitto et al. is presented in Table 2.

Table 2: Advantages and disadvantages of various animal models by Schmitto et al.[49]

Model	Animal	Advantage	Disadvantage
Coronary microembolization	Dogs, sheep, calves	- Closed-chest - Clinical correlation	- Time consuming - Technically demanding - Expensive
Coronary ligation	Dogs, sheep, pigs	- easy to perform - complete vessel occlusion - Clinical correlation	- Opened-chest - Adhesions - High mortality - High incidence of arrhythmias
Rapid Pacing	Dogs, sheep	- Closed-chest - Technically easy	- No clinical correlation - Reversible when pacing is stopped - Lack of long-term stability - Pathomechanisms of this model is still unclear - No hypertrophy - No collagen increase
Volume overload	Dogs, sheep, pigs	- Clinical correlation exists - Closed-chest	-Time consuming
Toxic	Dogs, sheep, pigs	-Closed chest	- Repetitive operations are necessary - Time consuming - Systemic side effects - Only rare clinical correlation

In vitro models

In vitro models of FMR have also taken many approaches. Reanimation of explanted mammalian hearts with the use of cardioplegic solutions has been used in exploratory physiologic studies as an introduction to their possible future application to surgical cardiac disease and repair investigations. [28] While a working heart model of FMR would be advantageous, this is not presently available.

The developed in vitro models of FMR thus far have been largely ex situ investigations using excised mitral valves and subannular apparatus frequently used by Yoganathan et al.. The mitral apparatus is mounted onto a device that allows the manipulation of the annular dimensions as well as the papillary muscle positioning allowing extrapolation of the results to the clinical scenario.[50,63,64] Considering the complexity of the physiology of FMR, and how much remains to be discovered, these models, while insightful, should be interpreted with caution, bearing in mind that the results are not reflective of the intact heart as an organ.

In 2009 an in situ, in vitro model facilitating mitral valve repair by Richards et al. was successful at presenting a computerized pulsatile system using intact, explanted porcine hearts.[1] This model combined annular dilation with mitral valve/chordal damage and repair with restrictive annuloplasty. It allowed visualization of the degree of MR via echocardiography and endoscopy.

Another promising advancement, presented by Wenk et al. in 2010, was the development of the first finite element model of the LV inclusive of the MV.[62] With the

use of MRI data obtained from a sheep with an induced MI by coronary ligation and subsequent FMR, specialized software was used to reconstruct digital replications of the LV. This technology is proposed as a minimally invasive tool that can be used in future investigations of FMR repair strategies.

Need for an in vitro model of FMR:

The need for the development of an in vitro model that would allow the accurate reproduction of the architectural changes evident in the failing human heart still persists. This model can then be used to investigate the ability of each of the various techniques to effectively eliminate mitral regurgitation. Combinations of existing techniques can be explored and the impact of these procedures on cardiac geometry can be measured and compared. Existing in vitro models necessitate the use of adjunct prefabricated machinery and computerized pumps[1] or involve complex reanimations requiring cardioplegic solutions,[27,28] limiting the use of these models. At present the need for a model to address Carpentier Type I or Type IIIb mitral valve regurgitation remains unmet.

Chronic functional mitral valve regurgitation is a disease of the elderly, when severe it is usually accompanied by complications making any surgical intervention high risk. The development of an effective minimally invasive strategy to safeguard the future these patients is imperative. The exploitation of an in vitro model is the first step to bridge this divide by firstly determining the most effective approach to correcting this problem and translating this knowledge to further work involving minimally invasive techniques.

RESEARCH OBJECTIVE

The aim of this study is to develop an in vitro model of FMR that accurately reproduces tethering of the mitral leaflets by the papillary muscles with consequent mitral regurgitation. The developed model could then be used for the evaluation of interventions aimed at the correction of Carpentier Type I/Type IIIb mitral valve regurgitation.

We hypothesized that displacement of the left posterior papillary muscle and dilation of the mitral annulus would induce FMR in vitro.

We proposed that the developed model should:

1. represent an accurate depiction of the architecture of the failing heart including:

    - annular dilation
    - papillary muscle displacement with resultant tethering and apical tenting of the valve leaflets

2. exhibit mitral regurgitation under pressures within the physiologic range, with evidence of a regurgitant jet.

3. allow for the quantitative assessment of mitral regurgitation.

4. permit investigation of the regurgitation mechanism e.g. tethering severity etc.

5. allow the application of surgical interventions and the assessment of the effect of the technique on mitral regurgitation.

6. provide results easily reproducible by other investigators.

The major research questions were:

1. Can we induce FMR by displacement of the left posterior papillary muscle and dilation of the mitral annulus?

2. Would the developed technique for displacement of the left posterior papillary muscle be capable of increasing LV sphericity?

3. Can FMR be reproduced by leaflet tethering/annular dilation alone?

4. What are the geometric changes necessary to eliminate/reduce FMR once established in the model?

CHAPTER 2

DEVELOPMENT OF A STATIC IN VITRO MODEL OF FMR

HYPOTHESES

The first phase of this study sought to investigate the conditions under which FMR could be produced in explanted, normal ovine hearts. We hypothesized that:

- manual stretching of the mitral annulus of normal ovine hearts mounted in a continuous flow system would successfully reproduce the FMR that occurs secondary to annular dilation.
- a novel technique of posterior papillary muscle isolation and displacement in normal ovine hearts mounted in a static flow system would successfully reproduce the FMR that occurs secondary to ventricular remodeling.
- combining annular dilation and posterior papillary muscle displacement in normal ovine hearts mounted in a static flow system would significantly increase FMR volume.

EXPERIMENTAL DESIGN and MATERIALS AND METHODS

Hearts (n=50) were acquired from adult Dorsett sheep 70kg±5 and were all of similar size with no detectable abnormalities. Experimentation was carried out as soon after harvesting as possible, not exceeding five days. Hearts were kept in saline and stored between 37 and 41°F. We hypothesized that the implemented method of producing

FMR would provide a reproducible process for initiating FMR from normal explanted hearts applicable to in vitro studies.

Heart Preparation

The pericardium was removed and the aorta isolated and trimmed to a length of approximately 5cm. 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.

Mapping of LV geometry

Sonomicrometer crystals were implanted at predetermined loci within the left ventricular chamber and annulus (Fig 2). This was done by tunneling the crystals through the myocardium from the exterior of the heart to the endocardium. Positioning of the crystals was based on prior studies investigating the geometric changes of the remodeled heart.[2] The distances measured reflect papillary muscle positioning and dimensions of the annulus.

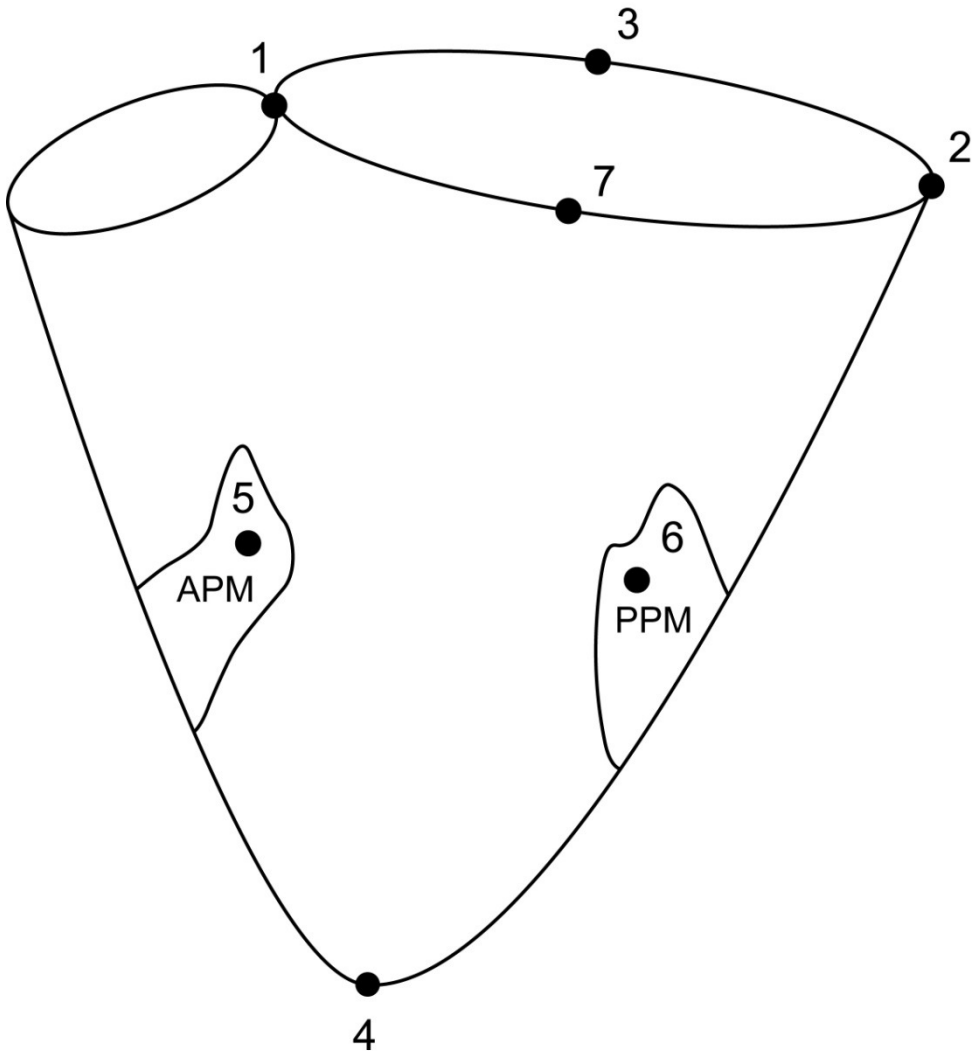

Fig 1: Schematic of sonomicrometer crystal positioning

7 crystals implanted

1-2=mitral annular septal-lateral dimension

3-7=mitral annular commissure-commissure dimension

APM=Anterior papillary muscle

PPM=Posterior papillary muscle

Ventricular Pressure

A Millar high fidelity pressure transducer, inserted via a pulmonary vein and into the left ventricle, was used to measure the pressure (mmHg) within the ventricular chamber. Pressures were limited to within the physiologic range; typically measurements at 100mmHg were used. The transducer allowed continuous instrumentation of the model for the real-time evaluation of pressure changes.

Description of heart cannulation

The prepared heart was mounted within the static flow system via the aorta which was attached to an input source for retrograde inflow into the ventricular chamber (Fig1). The aorta was secured around the inflow conduit with the use of a nylon tie. A cannula (1/4" Tygon tubing) was then placed into the pulmonary vein and kept at the level of the vein. Outflow from the left atrium (mitral regurgitation) via this cannula could be easily collected and measured.

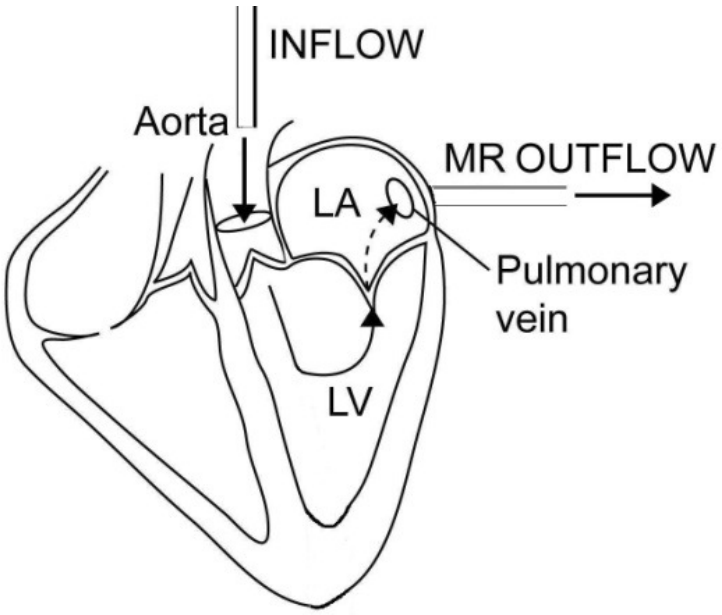

Fig 2: Layout of continuous flow model. Inflow via the aorta, outflow from the LA via the pulmonary vein equivalent to MR.

Determination of MR Volume

Under physiologic pressure retrograde outflow from the atrium was equivalent to regurgitant volume. Atrial outflow was collected and measured over a 10s interval.

Baseline measurements

Once mounted within the continuous flow system, inflow into the LV was initiated until attaining a LV pressure of 100mmHg and the inflow rate closely monitored to ensure that pressure was sustained. With the use of a stopwatch, 10 seconds of outflow from the LA via the pulmonary cannula was collected and measured.

Induction of FMR

Measurements were made at baseline after which FMR was induced either by annular dilation or posterior papillary muscle displacement. After quantification of FMR volume the second procedure (annular dilation or papillary muscle displacement) was applied and measurements made; each heart eventually received both annular dilation and displacement of the posterior papillary muscle.

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 FMR induced. The stretching of the annulus was performed while the heart was mounted within the continuous flow system. The dilation was performed incrementally until FMR was visible. The annulus was carefully manipulated to avoid damage to the annular wall, mitral leaflets or rupture of the chordae. This necessitated the use of slow, constant, manual expansion.

Posterior papillary muscle displacement was achieved by suturing a patch of ovine diaphragm around the isolated papillary muscle. During the application of the patch the heart was dismounted from the flow system. To isolate the posterior papillary muscle an incision was first performed 2cm distal to the coronary groove in the posterior part of the ventricle between the second and third obtuse branches if 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 (Fig 3). The posterior papillary muscle was then only attached to the apex of the heart by a 2cm 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 and consequential apical displacement of the posterior leaflet. It was sutured using a continuous mattress suture pattern with 4-0 suture material.

The size of the applied patch of diaphragm was chosen to ensure maximal apical tethering of the chordate. Once applied the limiting factor for further apical leaflet tethering was no longer the myocardium but the chordae themselves, so that once the hearts were subjected to volume inflow the chordate were stretched to capacity. None of the chordae tendinae were damaged during preparation.

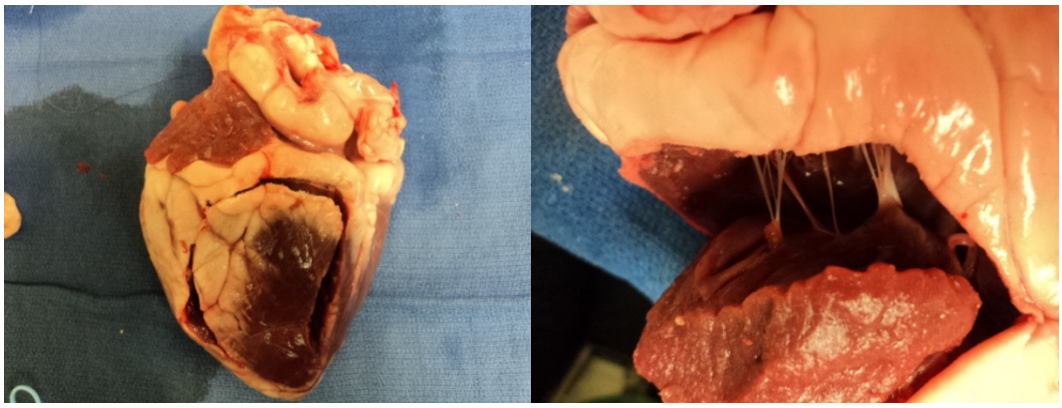

Figure 3: Isolation of the posterior papillary muscle with intact chordae tendinae

The prepared hearts were then remounted within the static flow system and re-measured (Fig 4).They were classified based on which treatment was performed first; either annular dilation or posterior papillary muscle repositioning by patch placement.

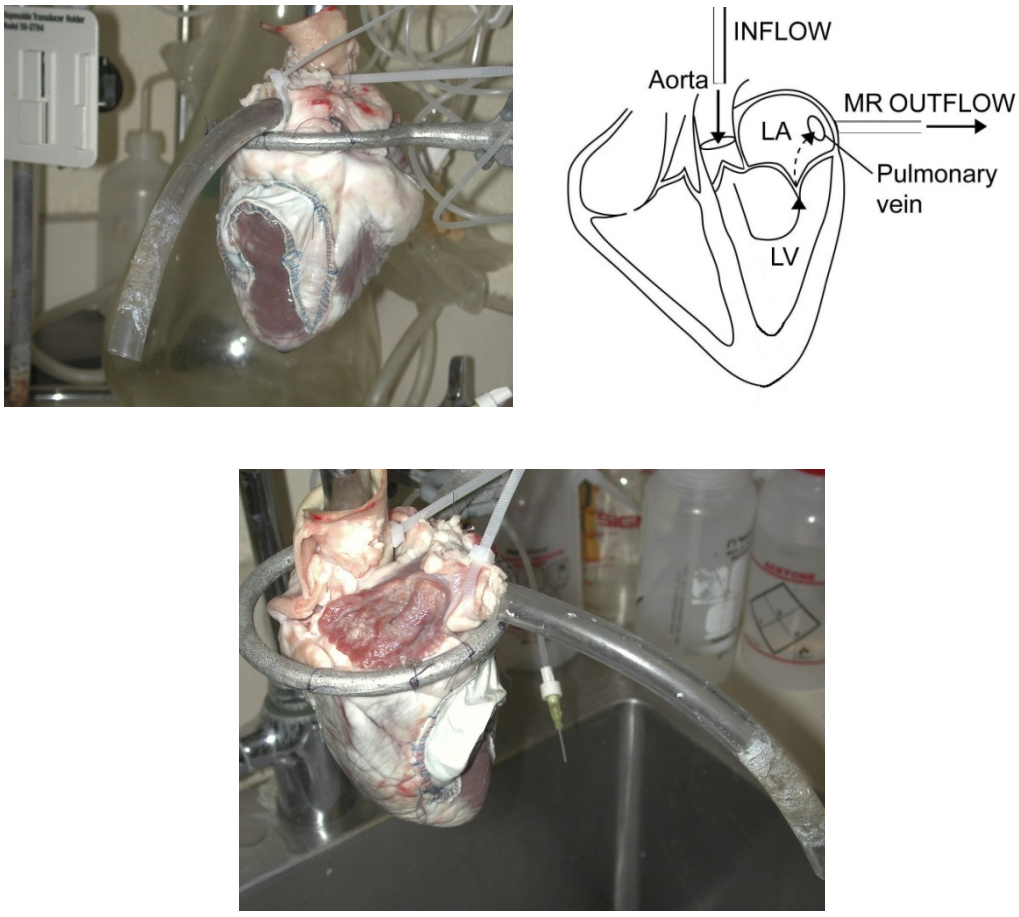

Fig 4: Static inflow system. A prepared heart treated by posterior papillary muscle distraction by patch application is mounted within the system.

Finally, the technique not used in the prior preparation was then applied so that each heart eventually displayed both annular and ventricular dilation, and measurements retaken.

Calculations:

The ratio of LV long axis to short axis (Sphericity Index) was calculated for each of the hearts at baseline, after each procedure and with both treatments applied. Annular surface area was measured as a function of the intercommisural and septo-lateral annular distances.

Statistical analysis:

All data were reported as means ± standard deviation. Repeated measurements analysis of variance (ANOVA) for multiple comparisons was performed while matched t-tests were used to compare the individual treatments applied to the model.

RESULTS

A total of 12 hearts were included in the study after an initial pool of 50 hearts. The majority of eliminations were made after the development of iatrogenic MR during annular dilation during which the annulus would often tear or secondary chordae rupture. This was a somewhat difficult process with n=21 hearts 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. Hearts were also lost during isolation of the posterior papillary muscle (n=7) by accidental transection of primary chordate. An initial investigation involved the use of 6 hearts for technique development

and another four hearts developed aberrations (e.g. papillary muscle rupture once pressurized) further discussed in subsequent sections.

Functional mitral valve regurgitant flow measured over 10 seconds increased significantly from baseline after either dilation of the annulus (from 15.5ml/10s to 78.7 ± 35.3 ml/10s, p=0.02) or application of the patch (from 7.6ml/10s to 67.4 ± 30.4 ml/10s, p=0.02). The amount of regurgitation was not significantly different after annular dilation or application of the patch (p=0.42). Dilation of the annulus and application of the patch significantly increased functional mitral valve regurgitant flow from baseline (256.80 ± 102.8 ml/10s, p=0.0001).

Geometric modifications due to annular dilation or the application of the patch are reported in Table 3. Dilation of the annulus resulted in a significant augmentation of the commissure-commissure dimension of the annulus (p=0.02). It also resulted in an augmentation of the distance from the anterior papillary muscle tip to the lateral annular wall (p=0.002) (Fig 5). Annular dilation had no effect on the positioning of the posterior papillary muscle beyond increasing the distance from this papillary muscle to the anterior commissure of the mitral annulus (p=0.01). Annular dilation increased annular surface area from baseline 750.81±284.19 mm^2 at baseline to 964.59±333.48, (p = 0.059). Left Ventricular sphericity index after annular dilation alone was not significantly increased from 3.25±0.7 at baseline to 3.22±0.97, (p=0.67).

Table 3: 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
LV Pressure	100±0.1	100±0.1	0.36	100±0.2	100.3±0.1	0.36

S-L=mitral annular septal-lateral dimension

C-C=mitral annular commissure-commissure dimension

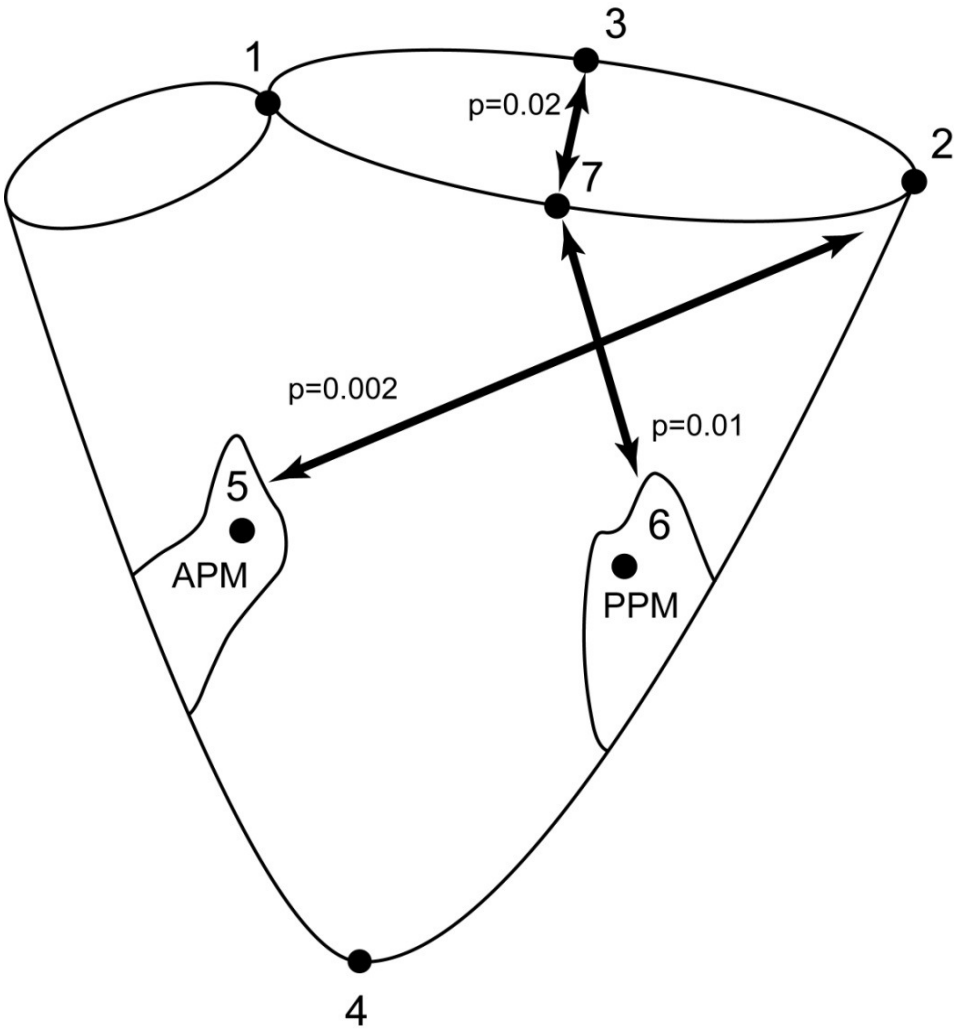

Figure 5: Geometric modifications due to annular dilation. Significant increases noted in all highlighted dimensions.

Placement of the patch resulted in significant alteration of the positioning of the posterior papillary muscle in relation to the mitral annulus (Fig 6). The distance of the posterior papillary muscle to the mitral annular septum significantly increased (p=0.03) and the lateral wall of the mitral annulus (p=0.04). 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) (Table3). Annular surface area was not significantly different from baseline 919.59±379.77 mm^2 at baseline to 788.2 ±206.48mm^2 after application of the patch, (p = 0.47) (Fig3). Left ventricular sphericity significantly increased from 3.25±0.7 at baseline to 2.34±0.6 after application of the patch, (p=0.0025).

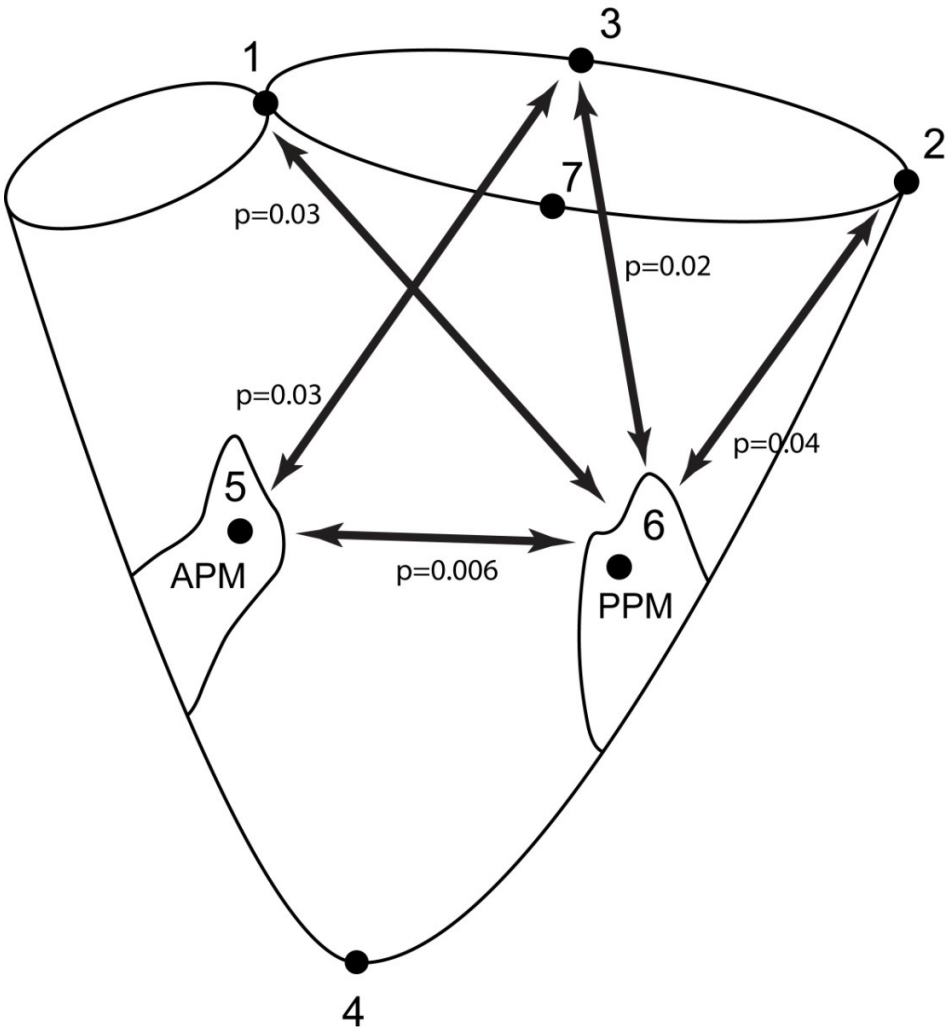

Figure 6: Geometric modifications due to posterior papillary muscle distraction by epicardial patch application. Significant increases noted in all highlighted dimensions.

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 (Fig7, Table 4). The distance between the two papillary muscles was also significantly increased (p=0.001). Annular surface area was not significantly increased from 827.53±325.33mm^2 at baseline to 1005.22±355.01mm^2 after annular dilation and application of the patch, (p = 0.27). There was a significant increase in left ventricular sphericity from 3.25±0.7 at baseline to 2.5±0.6 with both treatments (p=0.0014).

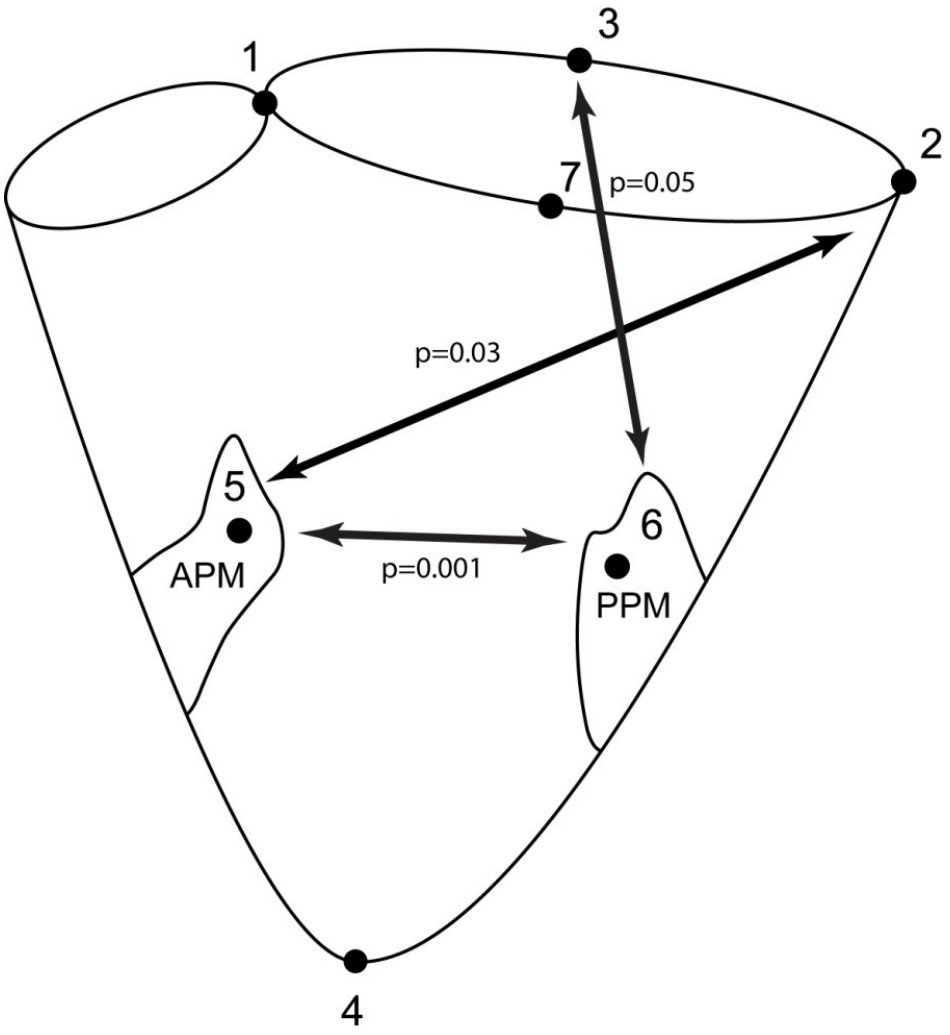

Figure 7: Geometric alterations after both treatments. Significant increases noted in all highlighted dimensions.

Table 4: Geometric measurements at baseline and after application of both annular dilation and posterior papillary muscle repositioning (patch placement).

Geometric measurements (mm)	Baseline n=11	Both treatments 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	0.08
1-5	45.3 ± 5	45.8 ± 8.2	0.8
2-5	40.6 ± 5.4	44.0 ± 5.7*	0.03
3-5	46.4 ± 4.9	51 ± 18.6	0.38
4-5	68.9 ± 8.7	69.6 ± 11.7	0.85
7-5	34.6 ± 5.4	38.6 ± 9.8	0.25
1-6	57.4 ± 3.6	58.9 ± 9.8	0.59
2-6	38.3 ± 5.3	40.7 ± 8.2	0.22
3-6	38.6 ± 5.9	53.6 ± 20.2*	0.05
4-6	51.3 ± 7.2	56.4 ± 13.1	0.3
5-6	30.3 ± 7	40.3 ± 8.5*	0.001
7-6	51.3 ± 6	54.1 ± 14	0.55
3-4	93.3 ± 11.4	94 ± 12.4	0.85

DISCUSSION

The annular and left ventricular dilatation characteristics of functional/ischemic mitral regurgitation were reproduced in this in vitro study. Ventricular dilation was modeled by the attachment of a patch on the left ventricular wall and resulted in significant displacement of the posterior papillary muscle; manual stretching of the annulus produced the annular dilation. Application of these treatments resulted in FMR in sheep cadaver hearts.

Functional mitral valve regurgitation can result from annular dilatation, tethering of the papillary muscle because of severe dilation of the left ventricular free wall, and left ventricular dysfunction.[68,69,70,71] As a consequence of the mitral insufficiency, the leaflets themselves can be modified.[72,73] It has been demonstrated that left ventricular dilation with displacement of the papillary muscles and augmentation of the sphericity index is the most important factor for the development of functional mitral valve regurgitation.[2, 38, 42, 69, 70, 74, 75] Also, failure of undersized mitral annuloplasty has been shown to result from persistent tethering of the mitral leaflets by the posterior papillary muscle.[77]

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 with or without annular dilation. LV sphericity increased in the developed model which is another index of LV remodeling. 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.[69, 50, 76] Otsuji et al[69] showed in two different animal models the correlation between the tethering

distance and mitral regurgitation orifice area. Tibayan et al[2] 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 MR was a direct result of increased tethering force on the valve measured as 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.[77] The distance of the posterior papillary muscle to the lateral annulus (2-6) was not significantly increased when the patch was applied with annular dilation because the annular dilation moved the lateral annular wall outwardly which is confirmed by the distance between the anterior papillary muscle and the lateral annulus (2-5) significantly increasing. Functional mitral valve regurgitation developed in this model independent of dilation of the annulus, this supports prior findings that MR occurs independent of annular dimensions.[78]

Annular dilation alone was able to induce 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.[1] The commissure to commissure diameter (3-7) increased by 19% in our study and explanted sheep hearts were used in this model instead of pigs. It has been previously reported that sheep have shorter mitral valve leaflet with smaller coaptation surface area than humans.[79] This anatomical difference may have contributed to the induction of mitral regurgitation by annular dilation alone in this in vitro model.

Annular dilation has been shown to be present in several studies. [80, 70] The importance of annular dilatation in the development of IMR has remained unclear,

especially since MR has been found to recur in patients post restrictive/undersize annuloplasty[81] adding emphasis to the role of the underlying, and ongoing, ventricular remodeling.[40] Because the mitral valve leaflets have a surface area of at least twice the surface of the mitral annulus surface area it precludes the development of MR.[78,82] The developed model has been able to isolate annular dilatation without concurrent ventricular abnormalities to investigate the contribution of this factor to FMR.

The combination of annular dilation with ventricular remodeling resulted in a significant augmentation of mitral regurgitation. It is important to note that the amount of mitral regurgitation was similar regardless of whether the ventricular dilation or annular dilation was applied first. This observation implies that both components have to be present in order to induce severe mitral regurgitation and both components interact together. It does not seem to matter what phenomenon happened first.

The reported findings are of the model in a static flow system. Non-pulsatile models have been used in the past to evaluate functional mitral valve regurgitation.[83] They have also been used to study the effect of edge to edge repair on functional mitral valve regurgitation.[84] However, pulsatile flow would be required if the impact of the dynamics of the mitral leaflets on FMR is to be studied.

CHAPTER 3

CONVERSION OF THE DEVELOPED STATIC FLOW MODEL OF FMR TO A PULSATILE SYSTEM.

HYPOTHESES

The developed FMR model was investigated within a pulsatile flow system for the next phase of the study. We hypothesized that the results of the static FMR model could be reproduced in a pulsatile flow system with similar results.

EXPERIMENTAL DESIGN and MATERIALS AND METHODS

Pulsatile system design

Experimental design and heart preparation remained the same as previously described. Explanted ovine hearts were attached to a timed, positive pressure, pulsatile, valve pump via an inflow cannula (1/2 inch Tygon tubing) into the aorta, the pump was set to replicate a heart rate of 80 beats/min and a maximum flow rate of approximately 6 L/min. Opening of the pump valve allowed the inflow of water into the LV via the fixed cannula, increasing LV volume and pressure toward maximal pressurization (systole) until closure of the valve terminated LV inflow, allowing LV pressure and volume to fall (diastole) until the valve reopened again. Outflow from the LA via a pulmonary vein cannula (1/4 inch Tygon tubing) allowed the collection of MR volume at 10 and 20cmH_2O as variations of LA preload, all recorded measurements in this study were taken at 10cmH_2O. An apical outflow port (1/2 inch Tygon tubing) served as an escape for the pump stroke volume, preventing over pressurization of the LV, this volume was collected in a filling reservoir which was then pumped back to the pulsatile valve for

another simulated cardiac cycle (Fig 8). Cannula attachments were fastened using a combination of suturing and nylon ties.

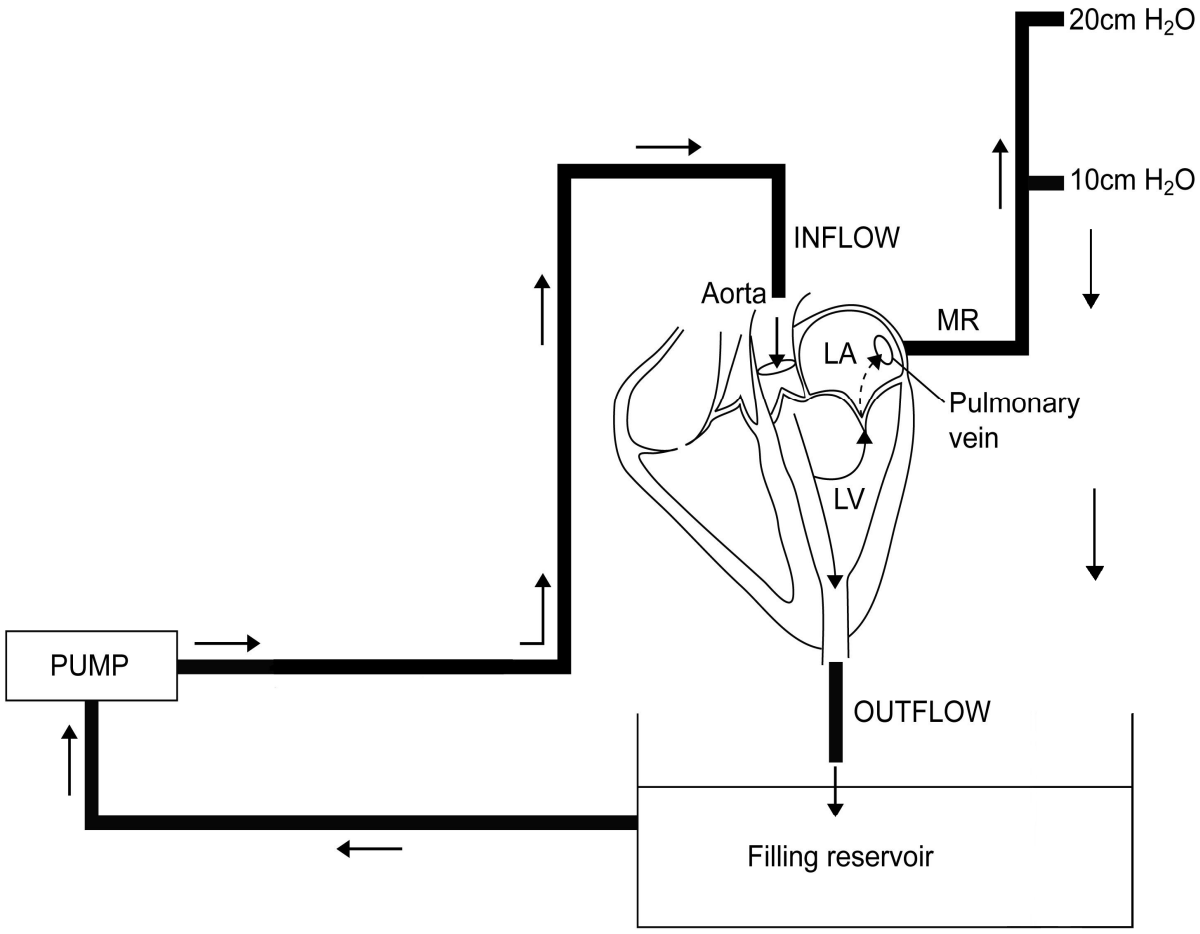

Figure 8: Pulsatile flow system

Induction of FMR

An epicardial patch isolating the posterior papillary muscle was applied as previously described. Annular dilation was not performed for this phase of the study.

Calculations:

The Sphericity Index (ratio of long axis to short axis) was calculated for each heart.

Statistical analysis

All data were reported as means ± standard deviation. Repeated measurements analysis of variance (ANOVA) for multiple comparisons was performed while matched t-tests were used to compare the individual treatments applied to the model.

RESULTS

The sphericity index decreased in the pulsatile model (2.3 ± 0.8) as compared to the sphericity found in untreated hearts at baseline in the earlier study (3.2 ± 0.7). Mitral regurgitation was 310.5 ± 86.6ml/10s (Table 5).

DISCUSSION

The previous study showed the successful duplication of key components crucial to any model of FMR; namely LV dilation evidenced by increased chamber sphericity accompanied by MR under physiologic LV pressures. Validation of the previously tested model in a pulsatile system required the confirmation of the presence of these desired features.

LV sphericity index showed a marked decrease from values found in untreated hearts similar to what was previously shown. Additionally, identical increases in LV sphericity were found in both studies (2.34±0.6 vs. 2.3±0.8 in this study). This highlights the consistency of the model, with reliable and predictable increases in LV sphericity attained post patch application.

The significance of infarct location was highlighted by the effect of the position of the placement of the epicardial patch which affected the development of MR. If the upper border of the patch was too ventral there would be no MR, despite massive apical displacement of the isolated posterior papillary muscle and the absence of annular dilation. This was found to be due to the effect of the prevailing balance of trans-myocardial forces at systole (peak pressure). The transmitral force, important in controlling mitral valve closure was actually increased by the epicardial patch at this level, resulting in an unintentional annuloplasty despite severe tethering. However, this was not the case once the upper margin of the patch was placed just ventral to the coronary sinus, which resulted in the presented MR featured in the developed model. This peculiarity can be paralleled to the clinical quandary currently revolving around the impressive initial results of mitral annuloplasty which deteriorates with time as global LV remodeling progesses[40] or as the myocardium undergoes further papillary muscle stiffening, loss of contractility or papillary muscle remodeling.[67]

When subjected to pulsatile flow distinct regurgitant output from the LA was noted, further confirming the efficiency of the model since this is not expected from normal hearts subjected to inflow via the aorta. In fact, a marked increase from what was previously found under static flow was noted (67.4±30.4ml/10s vs. 310.5 ± 86.6ml/10s in the pulsatile study). Since all other factors remained the same, the increased regurgitant volume can be only attributed to the pulsatile flow, giving some insight to the effect of mitral valve dynamics on regurgitant volume.

CHAPTER 4

INVESTIGATION OF THE GEOMETRIC ALTERATIONS NECESSARY FOR THE TERMINATION OF FMR IN A PULSATILE SYSTEM.

HYPOTHESES

The next phase of the study investigated the geometric alterations necessary to correct or reduce the induced FMR. We hypothesized that:

- repositioning of the displaced posterior papillary muscle would be sufficient to eliminate FMR in a pulsatile system.
- movement of the papillary muscles toward the annulus would correct FMR.

EXPERIMENTAL DESIGN and MATERIALS AND METHODS

System design

Experimental design, heart preparation and materials and methods remained the same. An epicardial patch isolating the posterior papillary muscle was applied as before. A timed, positive pressure, valve pump was used in this study, replicating a heart rate of 80 beats/min and a maximum flow rate of approximately 6 L/min as previously described.

Correction of FMR

Hearts with patch application were secured within the pulsatile system and subjected to aortic inflow to attain maximal LV pressures of 100mmHg. Measurements of mitral regurgitation via the pulmonary vein cannula were made for a 10 second

duration. The heart was then manually compressed with pressure placed directly over the posterior papillary muscle in an effort to reposition it towards the mitral annulus. Adjustments in positioning were made until the regurgitant outflow was as small as possible, sonomicrometer crystal positioning was then used to measure geometric alterations.

Calculations:

The Sphericity Index (ratio of long axis to short axis) was calculated for each heart.

Statistical analysis

All data were reported as means ± standard deviation. Repeated measurements analysis of variance (ANOVA) for multiple comparisons was performed while matched t-tests were used to compare the individual treatments applied to the model. Simple regression was used to assess linear relationships between variables.

RESULTS

The hearts, with displaced posterior papillary muscles via patch application, displayed increased sphericity and mitral regurgitation confirming that the validity of the FMR model. The regurgitant flow significantly decreased and was almost eliminated after externally compressing and repositioning the displaced papillary muscle (310±86.6ml/10s with patch vs. 16.1 ± 23.7ml/10s after manual correction, p=0.0001) (Table 5).

At peak LV pressures it was found that correction of FMR by posterior papillary muscle repositioning via manual external compression resulted in significant alteration of the positioning of both the posterior and anterior papillary muscles, and also decreased the distance between the septal and lateral annular walls($p=0.002$). The distance between the anterior papillary muscle and the lateral wall ($p=0.0001$), as well as, the anterior ($p=0.02$) and posterior commissures ($p=0.0007$) of the mitral annulus were significantly increased during this correction. This process also displaced the anterior papillary muscle away from the apex ($p=0.05$). The posterior papillary muscle moved away from both the septal ($p=0.1$) and lateral walls ($p=0.0001$) of the mitral annulus (Fig 9).

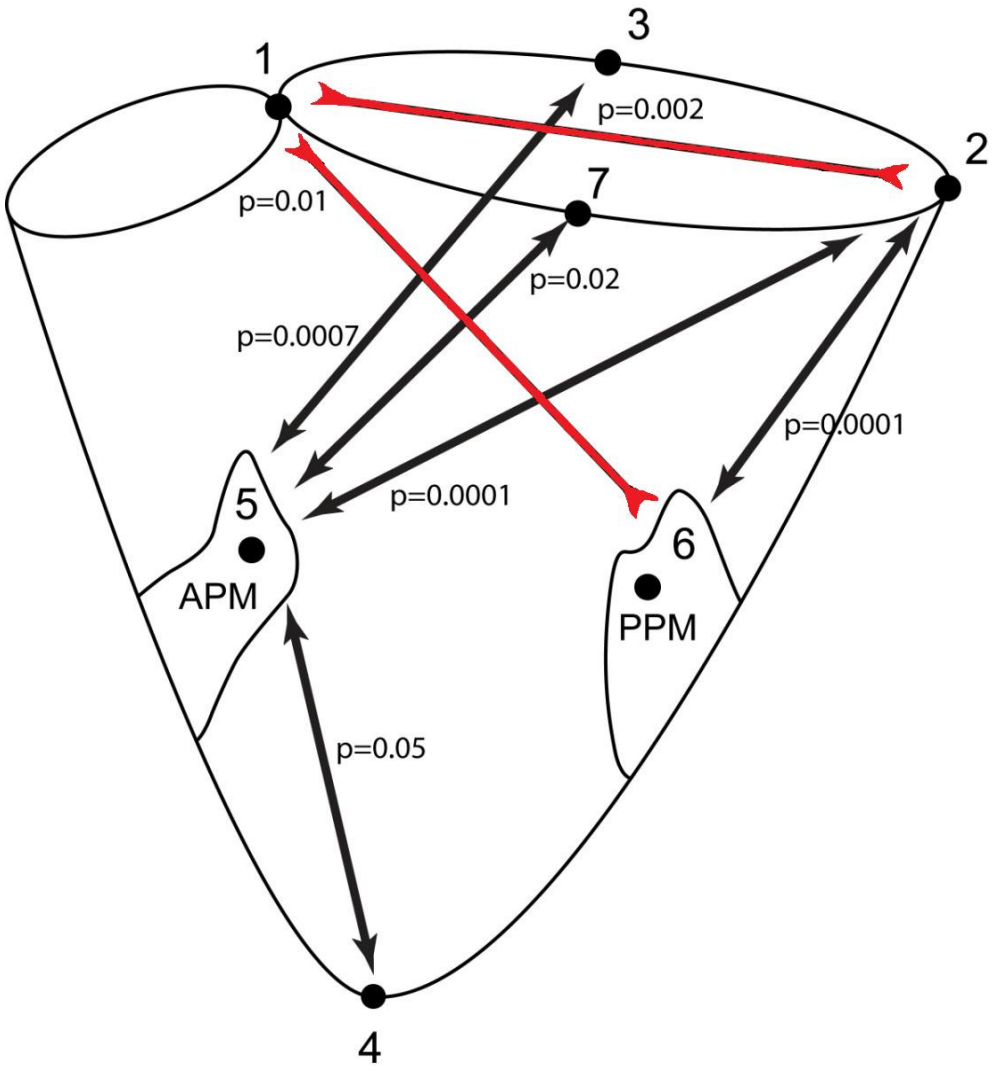

Figure 9: Geometric alterations after manual correction of FMR during maximal LV pressure. Red arrows indicate decreased dimensions, all other dimensions were increased.

Manual correction significantly increased LV pressure (100.2±0.6 to 137.8±28mmHg, p=0002) and concurrently decreased LV volumes (63.2±24.5 to 21.8±21.7, p=0.0008). At minimum LV pressures increased distances between the anterior papillary muscle and the lateral (p=0.0012) and posterior (p=0.0005) annular wall was noted after manual correction. Likewise the distance between the posterior

papillary muscle and the lateral annular wall was also increased (p=0.003) (Table 6, Fig 10). This treatment also increased LV pressure (13.7±5.2 to 24.7 ± 14.1mmHg, p=0.01) and decreased LV volume (60 ± 23.4 to 22.7 ± 28.5ml, p=0.0007).

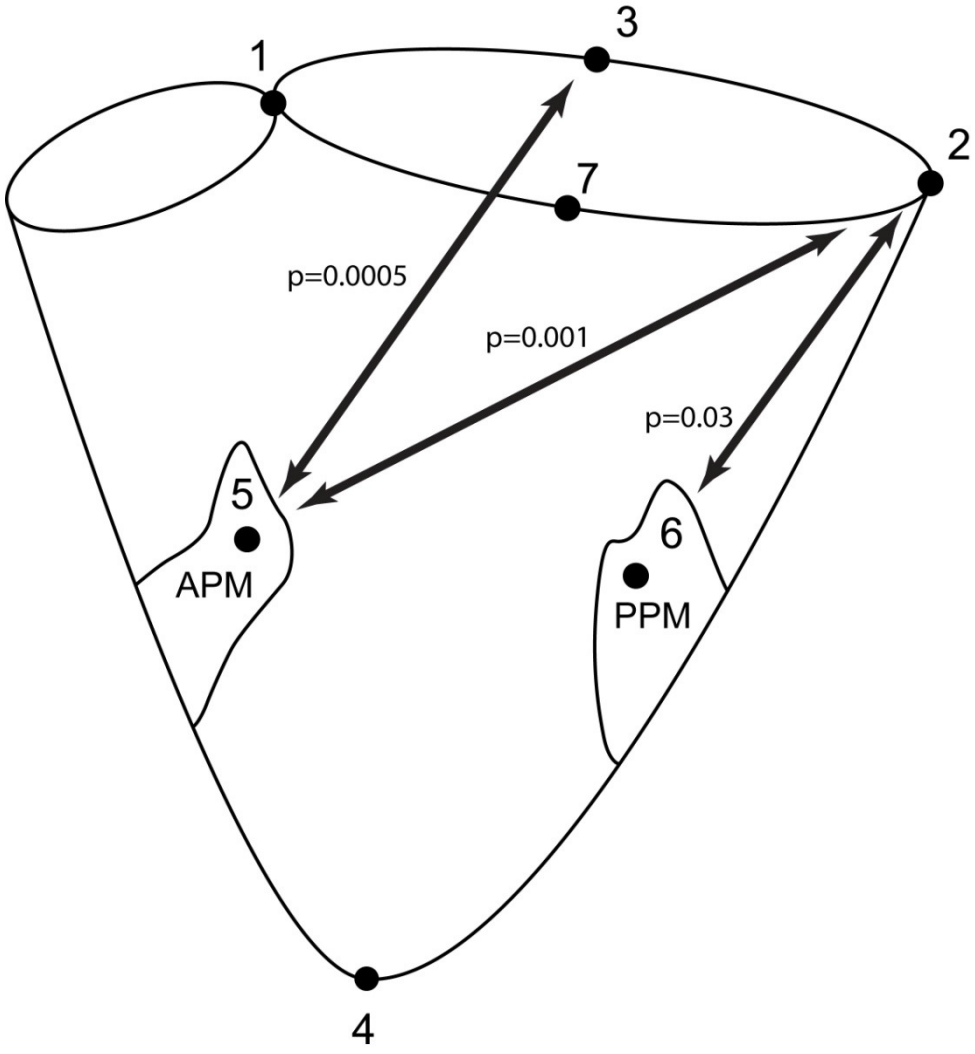

Figure 10: Geometric alterations after manual correction of FMR during minimal LV pressure: significant increases in all dimensions shown.

Table 5. Geometric measurements at maximal LV pressure at baseline, and after manual correction.

Geometric measurements (mm)	Baseline n=14	Treatment (Man corr) n=14	P
Annular S-L	37.5 ± 7.1	22.7 ± 9.7*	0.002
Annular C-C	37.4 ± 7.1	40.1 ± 16.7	0.56
1-5	53.2 ± 11.5	54 ± 12	0.63
2-5	45.6 ± 5.1	53.1 ± 5.7*	0.0001
3-5	54 ± 7.8	59.3 ± 8*	0.0007
4-5	59.9 ± 9.6	63.9 ± 9.2*	0.05
7-5	44.4 ± 7.7	48.1 ± 9.5*	0.02
1-6	66.3 ± 5.7	59.3± 7.9*	0.01
2-6	35.11 ± 8.6	45.23 ± 7.5*	0.0001
3-6	50.1 ± 8.5	50.8 ± 6.9	0.73
4-6	45± 5.2	44.8 ± 3.9	0.87
5-6	41 ± 8.2	43.9 ± 10.5	0.22
7-6	57.3 ± 4.5	56.9 ± 9.3	0.86
3-4	86.3 ± 7	88.5 ± 11.6	0.44
LV Pressure	100.3 ± 0.6	137.8 ± 28*	0.0002
LV Vol	63.2 ± 24.5	21.8 ± 21.7*	0.0008
LV Sphericity	2.3 ± 0.8	2.2 ± 0.7	0.6
Regurgitation vol	310.5 ± 86.6	16.1 ± 23.7*	0.0001

Table 6. Geometric measurements at minimal LV pressure at baseline, and after manual correction.

Geometric measurements (mm)	Baseline n=14	Treatment (Man corr) n=14	P
Annular S-L	36.8 ± 8.1	20.1 ± 8.3	0.0009
Annular C-C	36.9 ± 15.4	34.2 ± 8.7	0.5
1-5	50.4 ± 11.6	52.2 ± 11.3	0.36
2-5	44.3 ± 5.9	51.4 ± 3.6*	0.0012
3-5	50.5 ± 8.3	57 ± 8.3*	0.0005
4-5	59.3 ± 12.8	63.5 ± 9.8	0.06
7-5	44.5 ± 9.5	45.8 ± 7	0.33
1-6	61.1 ± 11	58.8 ± 6.7	0.5
2-6	34.3 ± 8.4	41.4 ± 4.7*	0.003
3-6	47.8 ± 7.5	48.5 ± 5.6	0.7
4-6	45.7± 7.5	43.3 ± 3.9	0.28
5-6	38.9 ± 10.4	37.9 ± 8.6	0.57
7-6	51.8 ± 8.4	56.4 ± 8.8	0.07
3-4	83.8 ± 9.3	89.3 ± 8.2	0.1
LV Pressure	13.7 ± 5.2	24.7 ± 14.1*	0.01
LV Vol	60 ± 23.4	22.7 ± 18.5*	0.0007
LV Sphericity	2.4 ± 1	2.5 ± 0.9	0.8

DISCUSSION

In this study the termination of mitral regurgitation was attempted by manual correction/repositioning of the displaced posterior papillary muscle. Measurements of LV dimensions during this treatment provide insight to which dimensions are important for the elimination of FMR with clinical implications. The findings underscore the significance of the outward rotation of the posterior papillary muscle to the development of FMR and shows that the combined repositioning of the anterior papillary muscle towards the mitral annulus, along with the return of the posterior papillary muscle toward the annular septum are important for the elimination of MR. Conclusions from investigations aimed at determining the best approach to the correction of this disease have revolved around the need to progress beyond annular undersizing via restrictive annuloplasty toward subvalvular surgical interventions.

The difficulty of separating the relative contributions of LV closing force versus the influence of LV dimensions on FMR persists in this study. LV pressure has an important role in the closure of the mitral valve during systole. Patients with CHF and FMR experience the joint effect of reduced mitral valve closing force, as well as, apical leaflet tethering due to muscular dysfunction/asymmetry/remodeling. Similarly, in this study manual correction of MR causes a consequential rise in LV pressure which along with the LV dimensional changes diminishes MR volume.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

The developed in vitro model of FMR successfully reproduced MR as a direct consequence of annular dilation and posterior papillary muscle displacement, under simulated physiologic conditions, in both static and pulsatile systems. According to our search it is the first, intact, in vitro model featuring the hallmarks of FMR applicable to ischemic MR, as well as, dilative cardiomyopathies.

The results of this study have confirmed that the LV dilation and increased sphericity responsible for FMR can be recreated in an in vitro investigation by the displacement the left posterior papillary muscle. Annular dilation, without any other alterations, was also able to produce MR, while not a common clinical problem, this scenario has been found typically in poorly controlled, female hypertensives.[65]

This study has exposed the possibility of correcting FMR by geometric alterations that do not necessarily restore original LV and mitral annular dimensions. This implies that a compromise is likely acceptable when surgical correction of FMR is undertaken. Future investigations correlating the degree of MR mitigation with varied geometric modifications are warranted. In this study LV dimensions were measured at the point of MR minimization, however, incremental geometric changes associated with the degree of MR reduction would be another informative approach.

During the creation of the model various unexpected insights were gleaned. Firstly, this model has allowed relative isolation of the factors implicated in FMR. It is

widely held that LV dysfunction, reduced mitral valve closing force, annular deformation, increased LV sphericity and apical leaflet tethering post-infarction/DCM, each play a role in the development of FMR. The relative contribution of each of these factors is difficult to ascertain since their effects cannot be isolated clinically and overlap of their roles is unavoidable. This model has been able to focus on individual factors in isolation, confirming the work by many authors on the role of the posterior papillary muscle in FMR, the ability of annular dilation to produce FMR in the absence of increased LV sphericity, the relevance of the use of the distance of the septal annulus to the papillary muscle tip[66] as a measure of valve tethering. Testing was able to be carried out under physiologic pressures somewhat removing the effect of LV pressure or reduced closing force.

In support of the widely held belief that correction of sub-valvular LV dimensions is crucial to the correction of FMR, during testing of the model it was found that MR could be completely terminated from an abundant flow volume by incomplete detachment of the posterior papillary muscle from the LV wall toward the annulus as would sometimes happen when the model was tested to failure. This movement corrected the apical leaflet tethering and relieved the leaflet restriction, highlighting the significance of correcting papillary muscle displacement to successfully treat this disease (Fig 11).

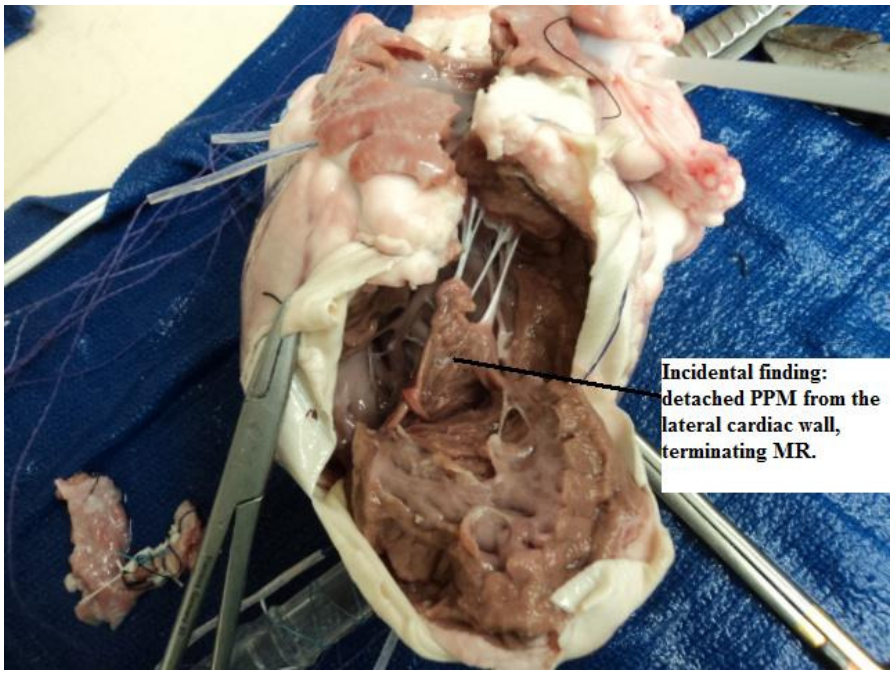

Figure 11: Termination of FMR by papillary muscle detachment and movement toward the annulus.

The in vitro nature of the developed model caused some limitations. A significant limitation of this study was an unavoidable departure from physiologic cardiac hemodynamics. In the cardiac cycle filling of the LV occurs during diastole and LV output is a function of LV systole. However, in the developed pulsatile system peak LV pressures occurred during LV filling, contrary to the physiologic scenario. Consequently, the mechanics of FMR in this in vitro study was characterized by peak regurgitant flow rates coinciding with peak LV pressures during LV filling. The impact of annular contraction on MR dynamics was also lost in this in vitro study. Otherwise quantifiable hemodynamic parameters of in vivo studies with the use of pressure loops etc, was precluded in this in vitro investigation.

Additionally, accurate comparison of the induced MR volume with MR severity guidelines used clinically has not be feasible. Although the developed model is amenable to echocardiographic studies with ERO (effective regurgitant orifice area) and PISA (proximal isovelocity surface area) calculations possible, these investigations have been outside the scope of this study and are proposed for future investigations of the model.

The developed FMR model has a limitless scope of possible applications. This model can be used as a novel tool for device testing and validation, analysis and comparison of the efficacy of various surgical correction techniques, as well as, for the practice of corrective strategies, investigation of device/technique failure or as pre-in vivo testing support for new interventions. The results thus far attest to the robustness of this in vitro model in the investigation of the physiology FMR and whets interest for further analyses such as: the role of LA afterload in MR development, an investigation of FMR as a function of the degree of leaflet tethering or LV sphericity, the amount of MR attained after the displacement of both papillary muscles compared to just the posterior/anterior solely, echocardiographic studies for MR quantification and clinical comparisons and the possibilities go on.

REFERENCES

1. Richards A, Cook R, Bolotin G, Buckner G. A Dynamic System to Facilitate the Development of Mital Valve Repair Techniques. *Ann. Biomed. Eng.* 2009; 37(4):651-660.

2. Tibayan F, Rodriguez F, Langer F et al. Annular or subvalvular approach to chronic ischemic mitral regurgitation ? *J. Thorac. Cardiovasc. Surg.* 2005; 129(6):1266-1275.

3. Depre C, Davies P, Taegtmeyer H. Transcriptional Adaptation of the heart to Mechanical Unloading. *Am J Cardiol.* 1999; 83:58H-63H.

4. Porter K, Turner N. Cardiac fibroblasts: At the heart of myocardial remodeling. *Pharmacology and Therapeutics.* 2009; 123:255–278.

5. Venkatachalam K, Venkatesan B, Valente A, Melby P, Nandish S, Reusch J, Clark R, Chandrasekar B. S, WISP1, a Pro-mitogenic, Pro-survival Factor, Mediates Tumor Necrosis Factor (TNF) stimulated Cardiac Fibroblast Proliferation but Inhibits TNF Induced Cardiomyocyte Death. *J Bio Chem.* 2009; 284(21):14414–14427.

6. Xia X, Lee K, Li N, Corbett D, Mendoza L, Frangogiannis N. Characterization of the infammatory and fibrotic response in a mouse model of cardiac pressure overload. *Histochem Cell biol*. 2009; 131(4):471-81.

7. Sarano M, Akins C, Vahanian A. Mitral Regurgitation. *Lancet*. 2009; 373:1382-1394.

8. Ahmed M, McGiffin D, O'Rourke R, Dell'Italia L. Mitral Regurgutation. *Curr Probl Cardiol*. 2009; 34:93-136.

9. Maisano F, Vigano G, Calabrese C, Taramasso M, Denti P, Blasio A, Guidotti A, Alfieri O. Quality of life of elderly patients following valve surgery for chronic organic mitral valve surgery. *Eur Heart J*. 2009; 36:261-266.

10. Bonow et al. ACC/AHA VHD Guidelines: 2008 Focused Update Incorporated. *Circulation*. 2008; 118:e523-e661.

11. Tahta SA, Oury JH, Maxwell JM, Hiro SP, Duran CM. Outcome after mitral valve repair for ischaemic mitral valve regurgitation. *J Heart Valve Dis*. 2002; 11:11-8.

12. H. Suma, Tanabe H, Uejima T, Isomura T, Horii T. Surgical Ventricular Restoration combined with mitral valve procedure in end stage ischemic cardiomyopathy. *Euro J of Cardio-thorac Sur,* 2009; 36:280-285.

13. Carabello B. The Pathophysiology of Mitral Regurgitation. *J Heart Valve Dis*. 2000; 9:600-608.

14. Siepelt R, Schoendube F, Vasquez-Jimenez J, Doerge H, Voss M, Messmer B. Combined mitral valve and coronary artery surgery: Ischemic vs non-ischemic mitral valve disease. *Eur J Car-Thorac Sur*. 2001; 20:270-275.

15. Onorati F, Santarpino G, Marturano D, Rubino A, Pasceri E, Zinzi S, Mascaro G, Cristodoro L, Renzulli. Successful surgical treatment of chronic ischemic mitral regurgitation achieves left ventricular reverse remodeling but does not affect right ventricular function. *J Thorac Cardiovas Sur*. 138:341-51, 2009.

16. Espino D, Shepherd D, Buchan K. Effect of mitral valve geometry on valve competence. *Heart vessels.* 2007; 22:109-115.

17.Carabello B. The relationship of left ventricular geometry and hypertrophy to left ventricular function in valvular heart disease. *J Heart Valve Dis* 1995; 4:S132-139.

18. Romano M, Bolling S. Mitral Valve Repair as an Alternative Treatment for Heart Failure Patients. *Heart Failure Monitor.* 2003; 4:7-12.

19. Hvass U, Tapia M, Baron F, Pouzet B, Shafy A. Papillary Muscle Sling: A New Functional Approach to Mitral Repair in Patients With Ischemic Left Ventricular Dysfunction and Functional Mitral Regurgitation. *Ann Thorac Surg.* 2003; 75:809-11.

20. Bove T, Belleghem Y, Vanberplas G, Caes F, Francois K, De Backer J, De Pauw M, Van Nooten G. Short-term systolic and diastolic ventricular performance after surgical ventricular restoration for dilated inschemic cardiomyopathy. *Eur J Car-Thorac Sur*. 2009; 35:995-1003.

21. Hongo M, Ryoke T, Ross J. Animal Models of Heart Failure. Recent Developments and Perspectives. *Trends Cardiovasc Med.* 1997; 7:161-167.

22. Chan J, Amirak E, Zajjar M, Flather M, Pepper J, Punjabi P. Ischemic Mitral Regurgitation:In Search of the Best Treatment for a Common Condition. *Progress in Cardiovascular Diseases.* 2009; 51:460-471.

23. Sabbah H. The Cardiac Support Device and the Myosplint: Treating Heart Failure by Targeting Left ventricular Size and Shape. *Ann Thorac Surg.* 2003; 75:S13-9.

24. Mann D, Ackar M, Jessup M, Sabbah H, Starling R, Kubo S. Rationale, Design, and Methods for a Pivital Randomized Clinical trial for the Assessment of a Cardiac support Device in Patients With New York Health Association Class III-IV Heart Failure. *J Cardiac Failure.* 2004; 10:185-191.

25. Hung J, Guerrero L, Handschumacher M, Supple G, Sullivan S, Levine R. Reverse Ventricular Remodeling Reduces Ischemic Mitral Regurgitation. Echo-Guided Device Application in the Beating Heart. *Circ.* 2002; 106:2594-2600.

26. Kaplan S, Bashein G, Sheehan F, Legget M, Munt B, Li X, Sivarajan M, Bolson E, Zeppa M, Arch M, Martin R. Three-Dimensional echocardiographic assessment of annular shape changes in the normal and regurgitant mitral valve. *Am Heart J.* 2000; 139:378-87.

27. Chinchoy E, Soule C, Houlton A, Gallagher W, Hjelle M, Laske T, Morisette J, Iaizzo P. Isolated Four-Chamber Working Swine Heart Model. *Ann Thorac Surg.* 2000; 70:1607-14.

28. Hill A, Laske T, Coles J, Sigg D, Skadsberg N, Vincent S, Soule C, Gallagher W, Iaizzo P. In Vitro Studies of Human Hearts. *Ann Thorac Surg.* 2005; 79:168-77.

29. Sadeghpour A, Abtahi F, Kiavar M, Esmaeilzadeh M, Samiei N, Ojaghi S, Bakhshandeh H, Maleki M, Noohi F, Mohebbi A. Echocaardiographic evaluation of mitral geometry in functional mitral regurgitation. *J Cardio thorac Sur.* 2008; 3:54.

30. Lembcke A, Dushe S, Enzweiler C, Kloeters C, Weise T, Hermann K, Hamm B, Konertz W. Passive external cardiac constraint improves segmental left ventricular wall motion and breduces akinetic area in patients with non-ischemic dilated cardiomyopthy. *Eur J Car-Thorac Sur.* 2004; 25:84-90.

31. Milano C, Daneshmand M, Rankin J, Honeycutt E, Williams M, Swaminathan M, Linbald L, Shaw L, Glower D, Smith P. Survival Prognosis and surgical Management of Ischemic Mitral Regurgitation. *Ann Thorac Surg.* 2008; 86:735-44.

32. Kron J, Aranda J, Miles W, Burkart T, Woo G, Saxonhouse S, Sears S, Conti J. Benefit of cardiac resynchronization in elderly patients: results from the Multicenter InSync Randomized Clinical Evaluation (MIRACLE) and Multicenter InSync ICD Randomized Clinical Evaluation (MIRACLE-ICD) trials. *J Interv Card Electrophysiol.*2009; 25:91-96.

33. Espino D, Shepherd D, Buchan K. Effect of mitral valve geometry on valve competence. *Heart Vessels.*2007; 22:109-115.

34. Takagaki M, McCarthy P, Ochiai Y, Dessoffy R, Doi K, Vidlund R, Mortier T, Schweich C, Fukamachi K. Novel Device to Change Left Ventricular Shape for Heart Failure Treatment: Device Design and Implantation Procedure. *ASAIO Journal.* 2001; 47:244-248.

35. Inoue M, McCarthy P, Popovic Z, Doi K, Schenk S, Nemeh H, Ootaki Y, Kopcak M, Dessoffy R, Thomas J, Fumamachi K. The Coapsys device to treat functional mitral regurgitation: In vivo long-term canine study. *J of thorac and cardiovas sur.*2004; 127:1068-77.

36. McCarthy P, Takagaki M, Ochiai Y, Young J, Tabata T, Shiota T, Qin J, Thomas J, Mortier T, Schroeder R, Schweich C, Fukamachi K. Device-based change in left ventricular shape: A new concept for the treatment of dilated cardiomyopathy. *J of thorac and cardiovas sur.* 2004; 127:1068-77.

37. Sabbah H, Sharov V, Gupta R, Mishra S, Rastogi S, Undrovinas A, Chaudhry P, Todor A, Mishima T, Tanhehco E, Suzuki G. Reversal of Chronic Molecular and Cellular Abnormalities Due to Heart failure by Passive Mechanical Ventricular Containment. *Circ Res, 2003;* 93:1095-1101.

38. Messas E, Guerrero L, Handschmacher M, Conrad C, Chow C, Sullivan S, Yoganathan A, Levine R. Chordal Cutting: A New Therapeutic Approach for Ischemic Mitral Regurgitation. *Circulation,* 2001; 104:1958-1963.

39. Varennes B, Chaturvedi R, Sidhu S, Côté A, Li Pi Shan W, Goyer C, Hatzakorzian R, Buithieu J, Sniderman A. Initial Results of Posterior Leaflet Extension for Severe Type IIIb Ischemic Mitral Regurgitation. *Circulation*, 2009; 119:2837-2843.

40. Hung J, Papakostas L, Tahta S, Hardy B, Bollen B, Duran C, Levine R. Mechanism of Recurrent Ischemic Mitral Regurgitation After Annuloplasty:
Continued LV Remodeling as a Moving Target. *Circ, 2004 ;* 110:SII85-II90.

41. Carpentier A, Chauvaud S, Fabiani JN, et al. Reconstructive surgery of mitral valve incompetence: ten-year appraisal. *J Thorac Cardiovasc Surg*, 1980;79:338-48.

42. Levine R, Schwammenthal E. Ischemic mitral regurgitation on the threshold of solution: from paradoxes to unifying concepts. *Circulation* 2005;112:745-58.

43. Hunt SA et al, Current status of cardiac transplantation. JAMA 1998;280(19):1692-1698.

44. Gelsomino S, Lorusso R, De Cicco G, Capecchi I, Rostagno C, Caciolli S, Romagnoli S, Da Broi U, Stefàno P, Gensini GF. Five-year echocardiographic results of combined undersized mitral ring annuloplasty and coronary artery bypass grafting for chronic ischaemic mitral regurgitation. *Eur Heart J.* 2008; 29(2):231-40.
45. Di Salvo T, Acker M, Dec G, Byrne J. Mitral valve surgery in advanced heart failure. *J Am Coll Cardiol*. 2010; 26;55(4):271-82.

46. Alfiere O, De Bonis, M. Mitral valve repair for functional mitral regurgitation: is annuloplasty alone enough?*Current Opinion in Cardiology,* 2010; 25:114-118.

47. Lee AP, Acker M, Kubo SH. Mechanisms of recurrent FMR after MV repair in non ischemic DCM. *Circulation* 2009;119:2606-14.

48. Hirokuni Arai. What is the Optimal Surgical Therapeutic target in Functional Ischemic MR: Annulus, Chord, Ventricle or Papillary Muscle? *Annals of Thoracic and Cardiovascular Surgery*,2009;15:141-143.

49. Schmitto J, Mokashi S, Lee L, Laurence R, Schotola H, Coelho-Filho O, Rajab T, Kwong R, Bolman III M, Quintel M, Cohn L, Chen F. Large Animal Models of Chronic Heart Failure (CHF). J of Sur Res, 2011; 166: 131–137.

50. Nielsen SL, Nygaard H, Fontaine AA, Fontaine AA, Hasenkam JM, He S, Yoganathan AP. Papillary muscle misalignment causes multiple mitral regurgitant jets: an ambiguous mechanism for functional mitral regurgitation. J Heart Val Dis; 1999:8(5):551-64.

51. Inoue M, McCarthy PM, Popović ZB, Doi K, Schenk S, Nemeh H, Ootaki Y, Kopcak MW Jr, Dessoffy R, Thomas JD, Fukamachi K. The Coapsys device to treat functional mitral regurgitation: in vivo long-term canine study. J Thorac Cardiovasc Surg,2004;127(4):1068-1076.

52. Schmitto J, Mokashi S, Lee L, Laurence R, Schotola H, Coelho-Filho O, Rajab T, Kwong R, Bolman III M, Quintel M, Cohn L, Chen F. A Novel, innovative ovine model of Chronic Ischemic Cardiomyopathy induced by Multiple Coronary Ligations. Art Org,2010;34(11):918-922.

53. Yarbrough W, Spinale F. Large animal models of congestive heart failure: A critical step in translating basic observations intoclinical applications. J Nucl Cardiol 2003;10:77-86**.**

54. Raman J, Jagannathan R, Chandrashekar P, Sugeng L. Can we repair the mitral valve from outside the heart? A novel extra-cardiac approach to functional mitral regurgitation. Heart, Lung and Circulation 2011;20:157–162.

55. Armstrong P, Stopps T, Ford S, de Bold A. Rapid ventricular pacing in the dog: pathophysiologic studies of heart failure. Circ,1986;74:1075-1084.

58. Hongo M, Ryoke T, Ross J. Animal models of heart failure Recent developments and perspectives. Trends Cardiovasc Med 1997;7:161-167.

59. Tsutsui H, Spinale F, Nagatsu M. Effects of chronic beta-adrenergic blockade on the ventricular and cardiocyte abnormalities of chronic canine mitral regurgitation. J Clin Invest 1994;93:2639.

60. Gorman J, Gorman R, Plappert T, Jackson B, Hiramatsu Y, John-Sutton M, Edmunds L. Infarct size and location determin development of mitral regurgitation in the sheep model. J Thorac Cardiovasc Surg 1998;115:615-622.

61. Llaneras M, Nance L et al. Large animal model of ischemic mitral regurgitation. Ann Thorac Surg, 1994;57:432-439.

62.Wenk J, Zhang Z, Cheng G et al. First finite element model of the left ventricle with mitral valve: Insight into ischemic mitral regurgitation. Ann Thorac Surg 2010;89:1546-1554.

63. Votta E, Vismara R, Redaelli A, Arcobasso L, Maisano F, Alfieri O, Fiore G. Response of two annular prostheses to functional mitral regurgitation main determinants: An invitro evaluation. ASAIO J 2010;56:491-496.

64. Erek E, Padala M, Pekkan K, Jimenez J, Yalcinba Y, Salihoglu E, Sarioglu T, Yoganathan A. Mitral Web-A new concept for mitral valve repair:Improved engineering design and in vitro studies. J of Heart and Vas Dis 2009;18:300-306.

65. Glower D, Bashore T, Harrison K, Wang A, Gehrig T, Rankin S. Pure annular dilation as a cause of mirtal regurgitation: A clinically distinct entity of female heart disease. J of Heart and Val Dis2009;18:284-288.

66. Otsuji Y, Levine R, Takeuchi M, Sakata R, Tei C. Mechanaism of inschemic mitral regurgitation. J of Cardiol 2008;51:145-156.

67. Magne J, Pibarot P, Dumesnil J, Senechal M. Continued global LV remodeling is not the sole mechanism responsible for the late recurrence of ischemic mitral regurgitation after restrictive annuloplasty. J Am Soc Echocardiogr 2009;22:1256-1264.

68. Timek T, Dagum P, Lai D, Laing D, Daughters G, Ingels N, Miller D. Pathogenesis of mitral regurgitation in tachycardia-induced cardiomyopathy. *Circulation.* 2001; 104(12 Suppl 1):I47-I53.

69. Otsuji Y, Handschumacher MD, Liel-Cohen N, Tanabe H, Jiang L, Schwammenthal E,Guerrero JL, Nicholls LA, Vlahakes GJ, Levine RA. Mechanism of ischemic mitral regurgitation with segmental left ventricular dysfunction: three-dimensional echocardiographic studies in models of acute and chronic progressive regurgitation. *Journal of the American College of Cardiology.* 2001;37(2):641-648.

70. Tibayan FA, Rodriguez F, Zasio MK, Bailey L, Liang D, Daughters GT, Langer F, Ingels

NB, Jr., Miller DC. Geometric distortions of the mitral valvular-ventricular complex in chronic ischemic mitral regurgitation. *Circulation.* 2003;108 Suppl 1:II116-121.
71. Levine R, Hung J, Otsuji Y, Messas E, Liel-Cohen N, Nathan N, Handschumacher M, Guerrero L, He S, Yoganathan A, Vlahakes G, Gus J. Mechanistic insights into functional *mitral* regurgitation. *Current cardiology reports. 2002*, 4(2):125-129.

72. Chaput M, Handschumacher M, Tournoux F, Hua L, Guerrero L, Vlahakes G, Levine R. Mitral leaflet adaptation to ventricular remodeling: occurrence and adequacy in patients with functional mitral regurgitation. *Circulation.* 2008;118(8):845-852.
73. Dal-Bianco JP, Aikawa E, Bischoff J, Guerrero JL, Handschumacher MD, Sullivan S, Johnson B, Titus JS, Iwamoto Y, Wylie-Sears J, Levine RA, Carpentier A. Active adaptation of the tethered mitral valve: insights into a compensatory mechanism for functional mitral regurgitation. *Circulation.* 2009;120(4):334-342.

74. Ahmad R, Gillinov M, McCarthy P, Blackstone E, Apperson-Hansen C, Qin J, Agler D, Shiota T, Cosgrove D. Annular geometry and motion in human ischemic *mitral* regurgitation: novel assessment with three-dimensional echocardiography and computer reconstruction.*The Annals of thoracic surgery. 2004*; 78(6):2063-8.

75. Gorman JH, 3rd, Ryan LP, Gorman RC. Pathophysiology of ischemic mitral insufficiency:does repair make a difference? *Heart failure reviews.* 2006;11(3):219-229.

76. Lamas GA, Mitchell GF, Flaker GC, Smith SC, Jr., Gersh BJ, Basta L, Moye L, Braunwald E, Pfeffer MA. Clinical significance of mitral regurgitation after acute myocardial infarction. Survival and Ventricular Enlargement Investigators. *Circulation.* 1997;96(3):827-833.

77. Ciarka A, Braun J, Delgado V, Versteegh M, Boersma E, Klautz R, Dion R, Bax J, Van de Veire N. Predictors of mitral regurgitation recurrence in patients with heart failure undergoing mitral valve annuloplasty.*The American journal of cardiology. 2010*; 106(3):395-401.

78.Otsuji Y, Kumanohoso T, Yoshifuku S, Matsukida K, Koriyama C, Chihaya K, Minagoe S, Levine R, Robert A; Tei C. Isolated annular dilation does not usually cause important functional mitral regurgitation: comparison between patients with lone atrial fibrillation and those with idiopathic or ischemic cardiomyopathy. *Journal of the American College of Cardiology,* 2002; 39(10):1651-1656.

79. Gorman J, Jackson B, Gorman R, Kelley S, Gikakis N, Edmunds L. Papillary muscle discoordination rather than increased annular area facilitates mitral regurgitation after acute posterior myocardial infarction. *Circulation*, 1997;96(supp):II-124-II_127.

80. Tibayan F, Rodriguez F, Langer F, Zasio M, Bailey L, Liang D, Daughters G, Ingels N, Miller D. Does septal-lateral annular cinching work for chronic ischemic mitral regurgitation?*J Thorac Cardiovasc Surg*. 2004;127(3):654-63.

81. Otsuji Y, Levine R, Takeeuchi M, Sakata R, Tei C. Mechanism of ischemic mitral regurgitation. J Cardiol 2008:51:145-156.

82. Sabbah H, Rosman H, Kono T, Alam M, Khaja F, Goldstein S. On the mechanism of Functional Mitral Regurgitation. *Am Heart J,* 1993;72:1074-1076.

83. Katoh T, Ikeda N, Nishi K, Gohra H, Hamano K, Noda H, Fujimura Y, Esato K. A newly designed adapter for testing an ex vivo mitral valve apparatus. *Artificial Organs*. 1999; (10):920-3.

84. Espino D, Hukins D, Shepherd D, Watson M, Buchan K. Determination of the pressure required to cause mitral valve failure. *Med Eng Phys*. 2006;28(1):36-41.