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Eliminating regurgitation reduces fibrotic remodeling of functional mitral regurgitation conditioned valves

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Abstract

Functional mitral regurgitation (FMR) is an insidious and poorly understood condition affecting patients with myocardial disease. While current treatments reduce regurgitation, their ability to reverse mitral valve pathology is unclear. We utilized a pseudo-physiological flow loop to study how repair impacted valve composition. Porcine mitral valves were cultured in control geometry (native papillary muscle position and annular area) or high-tension FMR geometry (5 mm apical and 5 mm lateral displacement of papillary muscles, 65% increased annular area) for two weeks. To mimic repair, a reversal condition was created by returning one-week FMR conditioned valves to a non-regurgitant geometry and culturing for one week. Valve composition and material properties were analyzed. After two-week culture, FMR conditioned tissues were stiffer and stronger than control and underwent extensive fibrotic remodeling, with increased prolyl-4-hydroxylase, lysyl oxidase, matrix metalloproteinase-1, and decorin. The reversal condition displayed a heterogeneous, leaflet- and orientation-dependent response. Reversal-conditioned anterior leaflets and circumferential tissue sections continued to have significant fibrotic remodeling compared to control, whereas reversal-conditioned posterior leaflets, chordae tendineae, and radial tissue sections had significantly decreased remodeling compared to FMR-conditioned tissues. These findings suggest current repairs only partially reverse pathology, underscoring the need for innovation in the treatment of FMR.

Key Terms

Functional mitral regurgitation; Mitral valve repair; Organ culture

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Conflicts of Interest

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Introduction

Mitral valve (MV) disease is the most common valvular disorder, with mitral regurgitation affecting 1.7% of the US adult population.²⁸ Functional mitral regurgitation (FMR) is a secondary disorder stemming from left ventricular dysfunction caused by myocardial disease. FMR develops when the left ventricle remodels and dilates, resulting in dilation of the MV annulus and displacement of the papillary muscles (PM) apically and laterally.³³ The results are a decrease in annular contraction and an increase in the tension on the MV leaflets and chordae, causing leaflet tethering.^{18,21} Valves affected by FMR are stiffer, less extensible, and less viscous compared to healthy valves.¹⁵ Together, these factors increase the stresses experienced by the MV and decrease coaptation area between leaflets, thereby leading to mitral regurgitation. FMR impacts the composition of the MV and is commonly associated with fibrotic remodeling.^{15,16}

Because FMR is largely the result of altered valve geometry, multiple surgical treatments aim to reduce regurgitation by correcting the underlying alterations in geometry. Modern treatments fall into three categories: annuloplasty, edge-to-edge repair, and ventricular remodeling.¹¹ Annuloplasty traditionally involves inserting an undersized ring along the annulus of the MV to decrease the anterior-posterior diameter in an attempt to offer better leaflet coaptation. In recent years, percutaneous annuloplasty devices delivered through the coronary sinus have been developed, and many, such as the Monarc¹⁷, Carillon¹, and PTMA²⁵, are in clinical trials with Carillon and Monarc showing promising results in reduction of mitral regurgitation. Edge-to-edge repair is a procedure commonly performed in conjunction with annuloplasty. The commonly-used Alfieri technique requires using echocardiography to find the location of the largest regurgitant jet and then suturing the leaflets together at this location to increase leaflet coaptation.³ The MitraClip is a percutaneous device that fulfills the same role by clipping the MV to create a double-orifice valve.⁹ The MitraClip is available clinically and has become a popular treatment option due to its minimally invasive nature.³⁷ Finally, multiple treatments offer ways to reshape the left ventricle to correct PM positioning and relieve tension from mitral leaflets. Plication of the left ventricular wall using suture to reposition the PM closer to the MV has shown reduction of regurgitation in animal studies.²⁴ The Coapsys device aims to correct left ventricular geometry by placing two pads connected by a chord epicardially and tightening the chord to bring the pads together, thus reshaping the ventricular wall until a reduction in mitral regurgitation is seen on echocardiography.¹² The Coapsys device lowered mitral regurgitation in clinical studies by correcting both annular dilation and PM displacement.²⁶ Recently, the iCoapsys device, a percutaneous version of the Coapsys system, has been developed and has shown early success in reducing regurgitation in animal studies.³¹ The development and assessment of devices and procedures for the treatment of FMR continue to be areas of great interest and extensive research.

The unique heterogeneous composition of the MV is precisely matched to its function. Any changes to valve composition can be detrimental to its ability to open and close properly throughout the cardiac cycle. Most studies assessing the success of MV treatment measure the reduction of mitral regurgitation. While this is the primary parameter of interest, these studies do not evaluate MV composition before and after repair and thus may be missing

critical information that could predict long-term success of treatment. Stephens *et al.* and Connell *et al.* showed in *in vivo* and *in vitro* studies, respectively, that mitral regurgitation causes mitral remodeling and that regurgitation begets further regurgitation due to the loss of function associated with altered composition.^{5,36} Stiffer, less extensible valvular tissues have limited motion compared to healthy tissue and are therefore less able to coapt properly to prevent regurgitation. Therefore, to understand the extent to which mitral repair meant to reduce or eliminate regurgitation truly restores proper valve function, we must study the effect of such repair on tissue composition.

Our lab has designed the Rice University Flow Loop System (RUFLS), a pseudo-physiological flow loop system capable of sterilely culturing living porcine MVs for up to three weeks.¹⁴ RUFLS is able to rapidly induce changes in MVs in response to altered hemodynamics, similar to valves in tachycardia-induced cardiomyopathy animal models.³⁸ We previously established that valves cultured in RUFLS in a high tension geometry resembling that of FMR experienced fibrotic remodeling and became stiffer and stronger after one week.⁵ These changes signified that valve tissues are highly sensitive to altered, physiologically-relevant hemodynamics. We hypothesized that these changes were the result of the valve interstitial cells (VICs) responding to the mechanical environment by actively remodeling their extracellular matrix (ECM). This was supported by a significantly different ECM composition of valves after one week and increased concentrations of ECM-altering enzymes produced by VICs. Furthermore, we hypothesized that VIC response was dynamic and that VICs would modify the profile of ECM-altering enzymes they produced whenever the hemodynamic environment changed. Building on this idea, we hypothesized that removing FMR-conditioned valves from high-tension geometry and returning them to a non-regurgitant environment in RUFLS would reverse the alterations in material properties and composition of the valve. This hypothesis is supported by findings in other cardiac tissues that suggest that removing pathologic mechanical conditions leads to reverse remodeling.^{22,27,34} To test this hypothesis, we created a reversal condition in which intact porcine MVs were cultured for one week in FMR conditions, followed by one week in normal (control-level) regurgitation. This short-term study allows initial analysis on the effects of removing regurgitation on valve composition and material properties, information on which future long-term studies can be based.

Materials and Methods

Organ Culture System

RUFLS design (Supplemental Figure 1) and operation has been previously described.^{5,6,13,14} In brief, MVs were dissected sterilely from adolescent (6 to 9 months) porcine hearts obtained from a commercial abattoir (Animal Technologies, Tyler, TX). The valves were installed into a MV holder that allowed precise control of annular size and PM position. The MV holder was inserted into a “left ventricular” chamber consisting of a fluid compartment separated from an air compartment by a flexible silicone membrane. A piezoelectric pressure regulator (Numatics, Novi, MI) controlled by LabView software (National Instruments, Austin, TX) was used to create a mock cardiac cycle with high-pressure (125 mmHg) systole that forced the culture medium out of the fluid compartment and into the

flow loop through a mechanical aortic valve. Culture medium then traveled to a compliance chamber, where the systolic pressure wave was dampened, followed by a reservoir chamber, which housed medium and allowed gravity to pull it through the MV during the low-pressure diastolic phase of the mock cardiac cycle. The reservoir chamber included an air filter for air exchange. Regurgitant flow and cardiac output were monitored using an ultrasonic meter (Transonic Systems, Ithaca, NY). RUFLS mimicked the cardiac cycle and achieved physiological waveforms and pressures. Approximately 1.2 liters of M199 culture medium (Sigma-Aldrich, St Louis, MO) supplemented with 10% bovine growth serum (ThermoHyclone, Logan, UT) and 2% anti-microbial solution (Mediatech, Manassas, VA) were pumped through the system at a flow rate of 3 liters per minute and a rate of 60 beats per minute. The large volume of culture medium ensured that nutrients remained plentiful for 7 days, at which point medium was changed. The pH of the culture medium was monitored to ensure that its biochemistry was not affected by a buildup of cellular metabolites or insufficient gas exchange. RUFLS was maintained in a cell culture incubator at 37°C and 5% CO₂ for the entire culture period.

Creation of Experimental Culture Conditions

Prior to MV dissection from the porcine heart, the position of the PM relative to the annulus was measured. The PM were assumed to be positioned directly below the commissures, at the midpoint along the anterior-posterior axis. The size of the annulus was measured using a Duran AnCore annuloplasty ring sizing kit (Medtronic, Minneapolis, MN). The acquired annulus and PM measurements constituted the neutral position and were replicated for control models (Figure 1).

The creation of high-tension FMR geometry was achieved by first sewing the valves onto annular rings with area 65% larger than measured annuli, thereby increasing the commissure-to-commissure dimension by 10% and the anterior-posterior dimension by 50%, as previously described.⁵ The valves were then placed in the MV holder and PM were displaced 5 mm apically and 5 mm laterally relative to the measured neutral position to create high-tension tethering associated with FMR. Control and FMR condition valves were cultured for two weeks continuously with a sterile media change after 7 days.

To create the reversal condition, valves were cultured in FMR geometry for one week. After one week of culture, the system was paused and the flow loop was opened and valve removed in a sterile culture hood. The larger FMR-style annular ring was removed, and the valve was sutured onto an annular ring created using the Duran AnCore annuloplasty sizer that was one size smaller than the original annulus size measured in the native heart at the beginning of the experiment. This change reduced the annulus area from its 65% larger FMR level down to a smaller than original area, which was required to prevent mitral regurgitation from recurring. The valve was then placed back into the MV holder, with PMs positioned to bring regurgitation down to control levels (less than 350 mL per minute) (Figure 2). This reduction was accomplished by using previously obtained PM distances from the native heart as a guide and slowly adjusting muscle position until all chordae were slightly taut by visual inspection. The MV holder was then replaced, and the system was turned on. The reversal condition valves were cultured for an additional week in this configuration.

After 2 weeks in culture, the system was disassembled and leaflets were divided into sections for radial and circumferential mechanical testing and histology (Supplemental Figure 2). Anterior clear, anterior rough, posterior circumferential, anterior radial, and posterior radial leaflet sections were utilized for mechanical testing, and anterior and posterior radial leaflet sections were fixed for histology. In addition, anterior basal, anterior marginal, posterior basal, and posterior marginal chordae were collected for mechanical testing.

Histological Staining and Immunohistochemistry

After culture, radial sections of each leaflet were fixed in 10% formalin overnight, dehydrated, embedded in paraffin, and sectioned 5 μ m thick. Herovici staining was used to assess collagen maturity in each condition by differentiating between young collagen, stained blue, and mature collagen, stained red. The methods have been previously described.¹⁹ Immunohistochemistry was performed as previously described to assess the localization of several markers of collagen remodeling, including prolyl-4-hydroxylase (P4H; bs-5090R, Bioss, Woburn, MA; 1:100), lysyl oxidase (LOX; IMG-6442A, Imgenex, San Diego, CA; 1:100), matrix metalloproteinase-1 (MMP-1; ADI-905-472-1, Enzo Life Sciences, Farmingdale, NY, 1:200), and decorin (LF #136, gift from Dr. Larry Fisher, NIH; 1:50).^{10,35} Samples were blinded during staining and remained blinded for all image acquisition and analysis.

Images of anterior leaflet clear zone, anterior leaflet rough zone, and posterior mid-leaflet regions were acquired using white light on a Leica DMLS upright light microscope (Buffalo Grove, IL). Anterior rough zone images were acquired from the distal third of the leaflet, and anterior clear zone images were taken from the proximal half of the leaflet in an area clear from chordal attachments. Posterior leaflet sections were taken from the mid-leaflet region. All sections were selected as representative within their regions within the blinded samples. The full area of the tissue in each image was used for analysis to keep the length of leaflets analyzed consistent throughout samples. Blinded images were analyzed using ImageJ software (NIH, Bethesda, MD). The software was used to split Herovici stained images into hue, saturation, and brightness components to allow quantification of red hue as a percentage of total tissue area, as previously described ($n = 6-7$).³² Blinded immunohistochemistry images were also analyzed with ImageJ software, which quantified the percent of positively stained tissue area compared to matched negative secondary controls ($n = 6-7$).²

Materials Testing

Mechanical analysis was performed as described previously.¹⁴ A pre-determined uniaxial tensile testing protocol was conducted on an EnduraTEC ELF 3220 (Bose, Eden Prairie, MN) on anterior clear, anterior rough, anterior radial, posterior circumferential, and posterior radial leaflet segments (each $n = 6-7$) and anterior basal, anterior marginal, posterior basal, and posterior marginal chordae (each $n = 6-7$). In brief, thickness, width, and gauge length of tissues were measured prior to testing. Testing was conducted in a 37 C phosphate-buffered saline bath. Tissues were preconditioned with ten load-unload triangle waves before

being stretched to failure. Tissues were strained at a rate of 1 mm per second. Any tissue sections that failed prior to the stretch to failure step were excluded from analysis.

Raw load and displacement data was converted to stress-strain curves for analysis using MATLAB (MathWorks, Natick, MA). Gauge length for each tissue section was determined according to previously described methods.⁴ The slope of the second linear region of the bilinear stress-strain curve was used to calculate the elastic modulus. The x-intercept of the line defined by the elastic modulus was calculated as the extensibility. The ultimate stress and strain were defined as the stress and strain values at the maximum stress achieved during the failure testing.

Statistical Analysis

Statistical analysis was performed using R Studio software (Version 1.0.136). Data are expressed as percent difference of the means. Unless otherwise stated, two-way ANOVA with post hoc Tukey Honestly Significant Difference test was performed to determine differences across condition groups (control vs. FMR vs. reversal) and tissue sections (i.e. anterior rough vs. anterior clear). Power analysis was performed to ensure appropriate sample size for all groups ($1-\beta = 0.8$). Results were considered significant at $p < 0.05$.

Results

The results are presented by first comparing FMR-conditioned valves to 2-week control valves in terms of composition and material properties. This is followed by a full description of the reversal-conditioned models compared to both control and FMR-conditioned valves. Cellular viability in RUFLS for up to three weeks has previously been established.^{5,14}

FMR-conditioned valves expressed more widespread collagen remodeling markers

Compared to controls, 2-week FMR-conditioned valve tissues had significantly greater content of MMP-1, an interstitial collagenase involved in collagen degradation in remodeling tissues (+138% anterior clear, +27% anterior rough, +96% posterior, $p < 0.05$; Figure 3). FMR-conditioned valves also had greater expression of decorin, a small leucine-rich proteoglycan involved in collagen fibrillogenesis (+4.6% anterior clear, +13% anterior rough, +31% posterior, $p < 0.01$), and LOX, an enzyme responsible for collagen crosslinking (+26% anterior clear, +19% anterior rough, +23% posterior, $p < 0.05$). FMR leaflets also had increases in P4H, an enzyme involved in the collagen synthesis pathway (+172% anterior clear, +54% anterior rough, +242% posterior, $p < 0.01$).

FMR-conditioned tissues were stiffer, stronger than control tissues

Across all leaflet sections and in both radial and circumferential directions, FMR-conditioned leaflets had a higher elastic modulus (+121% anterior clear, +106% anterior rough, +90% posterior circumferential, +10% anterior radial, +83% posterior radial, $p = 0.002$) and higher ultimate stress (+63% anterior clear, +66% anterior rough, +126% posterior circumferential, +17% anterior radial, +48% posterior radial, $p = 0.0002$) than control tissues (Figure 4). In addition, FMR-conditioned chordae had a greater elastic modulus (+114% anterior basal, +39% posterior basal, 134% anterior marginal, +29%

posterior marginal, $p = 0.005$) and ultimate stress (+52% anterior basal, +57% posterior basal, +106% anterior marginal, +21% posterior marginal, $p = 0.04$) than controls.

Reversal-conditioned valve composition was leaflet dependent

Reversal-conditioned valves had a leaflet-dependent response (Figure 5). The anterior leaflet displayed increased levels of collagen remodeling markers compared to controls, while posterior leaflet marker levels were not statistically different from controls and were decreased compared to FMR-conditioned valves. In the anterior leaflet, compared to controls, reversal-conditioned valves had increased LOX (+27% anterior clear, +21% anterior rough, $p < 0.05$) and MMP-1 (+162% anterior clear, +94% anterior rough, $p < 0.05$). Additionally, the amount of mature collagen measured by Herovici staining was increased in reversal-conditioned anterior leaflets compared to controls (+60% anterior clear, +329% anterior rough, $p < 0.01$).

Reversal-conditioned posterior leaflets, on the other hand, showed no significant difference compared to control posterior leaflets in terms of LOX, MMP-1, P4H, or mature collagen. There was, however, a significant decrease in reversal-conditioned posterior leaflet P4H (−62%, $p < 0.05$) and mature collagen (−71%, $p < 0.05$) compared to FMR-conditioned posterior leaflets. Complete histology data for our three experimental conditions is shown in Supplemental Table 1 and in Supplemental Figures 3–7.

Reversal-conditioned valve material properties were leaflet and orientation dependent

Reversal-conditioned anterior and posterior leaflets displayed dissimilar remodeling patterns affecting material properties (Figure 6). Among anterior leaflet sections, FMR- and reversal-conditioned valves had a higher elastic modulus than controls (FMR: +121% anterior clear, +106% anterior rough, +10% anterior radial, $p = 0.09$, reversal: +181% anterior clear, +113% anterior rough, −18% anterior radial; $p < 0.05$). Reversal-conditioned anterior sections also had a higher ultimate stress than controls (+134% anterior clear, +98% anterior rough, +8% anterior radial, $p < 0.01$). Reversal-conditioned anterior leaflet sections were not statistically different from FMR tissues in terms of elastic modulus ($p = 0.95$) and ultimate stress ($p = 0.49$).

Among posterior leaflet sections, on the other hand, both control and reversal-conditioned valves had significantly lower elastic modulus (control: −47% posterior circumferential, −45% posterior radial, $p < 0.05$; reversal: −35% posterior circumferential, −56% posterior radial, $p < 0.01$) and ultimate stress (control: −56% posterior circumferential, −32% posterior radial, $p < 0.01$; reversal: −19% posterior circumferential, −49% posterior radial, $p < 0.05$) than FMR-conditioned posterior leaflet valves. Reversal-conditioned posterior leaflet sections were not statistically different from control posterior leaflet sections. This pattern held for both elastic modulus ($p = 1.00$) and ultimate stress ($p = 0.69$).

The changes in material properties in reversal-conditioned valves also varied based on leaflet section orientation (Figure 7). In the circumferential direction, both FMR- and reversal-conditioned tissues had higher elastic modulus (FMR: +121% anterior clear, +106% anterior rough, +90% posterior circumferential, $p < 0.05$; reversal: +181% anterior clear, +113% anterior rough, +24% posterior circumferential, $p = 0.053$) and ultimate stress (FMR: +63%

anterior clear, +66% anterior rough, +126% posterior circumferential, $p < 0.05$; reversal: +134% anterior clear, +98% anterior rough, +82% posterior circumferential, $p < 0.01$) than controls. Reversal-conditioned circumferential leaflet sections were not statistically different from FMR-conditioned tissues, both in terms of elastic modulus ($p = 0.99$) and ultimate stress ($p = 0.61$).

Reversal-conditioned radial leaflets had a lower elastic modulus than FMR-conditioned leaflets (−25% anterior radial, −56% posterior radial, $p < 0.05$), and similarly, control radial leaflets had a trend of lower elastic modulus than FMR-conditioned leaflets. Reversal-conditioned radial leaflets also had lower ultimate stress than FMR (−8% anterior radial, −49% posterior radial, $p = 0.26$). Reversal-conditioned leaflet sections in the radial direction were not statistically different from control tissues in elastic modulus ($p = 0.57$) and ultimate stress ($p = 0.92$). Region-specific material properties of leaflets of our three experimental conditions are shown in Supplemental Table 2.

Reversal-conditioned chordae tendineae were less stiff and less strong than FMR-conditioned chordae tendineae

Reversal-conditioned chordae had significantly lower elastic modulus than FMR-conditioned chordae (−14% anterior basal, −19% posterior basal, −53% anterior marginal, −51% posterior marginal, $p < 0.05$ and lower ultimate stress than FMR-conditioned chordae (−31% anterior basal, −49% posterior basal, −44% anterior marginal, −44% posterior marginal, $p < 0.01$) (Figure 8). Control chordae cultured for 2 weeks in the neutral position had lower elastic modulus ($p < 0.01$) and lower ultimate stress ($p < 0.05$) than FMR-conditioned chordae. Reversal-conditioned chordae tendineae were not statistically different from control chordae tendineae. This pattern was seen in elastic modulus ($p = 0.75$) and ultimate stress ($p = 0.98$).

Discussion

We hypothesized that removing FMR-conditioned valves from a high-tension geometry and returning them to a non-regurgitant environment in RUFLS would reverse altered MV material properties and composition. By eliminating regurgitation and returning FMR-conditioned valves to a non-regurgitant geometry we reduced fibrotic remodeling in the posterior leaflet, radially-oriented sections, and chordae tendineae.

FMR-conditioned valves undergo fibrotic remodeling

After two weeks of culture in FMR hemodynamic conditions, porcine MVs demonstrated increased expression of collagen remodeling markers and were stiffer and stronger than controls. This result is consistent with the fibrotic remodeling phenotype found in our previous one-week studies.⁵ The elevated abundance of MMP-1, decorin, P4H, and LOX is consistent with fibrotic remodeling. Our two-week cultures show a progression of changes to material properties associated with fibrotic remodeling beyond what was observed at one week.⁵ The overall leaflet stiffness increased 48% and chordae tendineae stiffness increased 6.3% (non-significant) in one-week FMR-conditioned valves compared to one-week controls, whereas leaflet stiffness increased 76% and chordae stiffness increased 74% across

the two-week FMR-conditioned tissues compared to two-week controls. Additionally, ultimate stress progressed from a 9.3% non-significant increase in leaflets and a 4.2% non-significant increase in chordae at one week to a 57% increase in leaflets and a 58% increase in chordae at two weeks in FMR-conditioned tissues compared to controls. These findings provide support for the continued progression of fibrotic remodeling within FMR, even given the considerable remodeling seen at only one week of culture.

Reversal-conditioned hemodynamics reversed some, but not all, signs of fibrotic remodeling

Our motivation for creating a reversal model was that the most widely used clinical treatments for FMR currently focus on reduction of regurgitation independent of concerns for the resulting forces placed on valve tissues. Whether through annuloplasty⁸, Mitraclip⁹, or chordal transection²⁹, the goal of treatment is to reduce regurgitation and relieve the hemodynamic load from the cardiovascular system. This goal is paramount, as the clinical outcome of the patient is severely impacted by the presence of regurgitation.³³ However, the discovery of an organic component to FMR in the form of fibrotic remodeling highlights the importance of various experimental treatments and devices that not only eliminate regurgitation, but also restore the MV to its original geometry.¹⁵ The rationale of these interventions is that if the valve were placed into its original orientation, the remodeling of the valve would halt and the valve would function properly, leading to a longer lasting repair. Treatments such as plication²³ and the iCoapsys³¹ device aim to return the PMs of the MVs back to their non-displaced positions. The goal of our reversal condition was to mimic repair of this form. By placing the valve annulus in an undersized ring, reminiscent of an undersized annuloplasty, and placing the PMs in a native, non-regurgitant position, we placed the fibrotically remodeled valve in a hemodynamic situation that replicated control hemodynamics as closely as possible. The resulting changes in valve mechanics and composition could then inform whether this style of repair could feasibly impact valve remodeling or if fibrotic remodeling was a phenotype that could not be reversed through mechanical stimulation alone.

Our results showed that FMR condition-induced fibrotic remodeling is a dynamic phenotype that responds to changes in mechanical environment at this early time point. This response was leaflet-dependent, however. Reversal-conditioned anterior leaflets showed continued signs of fibrotic remodeling, building on patterns seen in FMR-conditioned valves, with elevated mature collagen, MMP-1, and LOX compared to controls. Reversal-conditioned posterior leaflets, on the other hand, demonstrated a reverse remodeling process, showing levels of mature collagen, MMP-1, P4H, and LOX lower than the levels found in fibrotic FMR-conditioned leaflets and not statistically different from controls. This observation is consistent with findings in other cardiovascular tissues. Using left ventricular assist devices (LVAD) to unload failing human hearts, for example, resulted in a reduction in fibrosis of LVAD-supported hearts with heterogeneous changes in fibrosis-related molecules.³⁴

In regards to material properties, reversal-conditioned tissues in posterior leaflet sections, radial leaflet sections, and chordae tendineae were not statistically different from control tissues in terms of elastic modulus and ultimate stress. This gives firm evidence that within

tissue sections the mechanical effects of fibrotic remodeling are mitigated from the continued progression observed in our two-week FMR condition. However, reversal-conditioned leaflet sections in the anterior leaflet and in the circumferential direction were significantly stiffer and stronger than controls and were not statistically different from FMR-conditioned tissues. These conflicting results imply that while some mitigation of the fibrotic remodeling has taken place, the changes within reversal-conditioned leaflets are not universal.

One interesting observation is that the specific reversal tissues that most closely resembled controls are those most altered in FMR in terms of geometry or applied force. In FMR, the largest change in tension occurs in the radial direction and results from the lateral and apical displacement of the PMs. This tension is so high that it causes leaflet tenting and leads to regurgitation. This tension is experienced both through radial leaflet segments and chordae tendineae. Moreover, this high tension is particularly pathological for the posterior leaflet, since that leaflet normally experiences low tension and is more subjected to compression, than it is for the anterior leaflet. The anterior leaflet clear region experiences tension in normal healthy valve motion and has a rich collagen region to bear the tensile load under healthy circumstances. Clinically, the type of left ventricular dysfunction that results in FMR minimally dilates this region of the anterior leaflet. We therefore chose to replicate this clinical phenomenon in our FMR condition and did not have significant dilation of the valve in the anterior leaflet clear region. Therefore, the posterior leaflet experienced the majority of the dilation that occurred as a result of our FMR condition. These findings suggest that valve tissues are particularly sensitive to forces in the posterior leaflet, inducing VICs to rapidly remodel their tissue phenotype in response to the changing mechanical environment.

In the leaflets, those regions that underwent reverse remodeling (posterior leaflet and radial sections) are those that have a more GAG-rich ECM, whereas those that did not (anterior leaflet and circumferential sections) are more collagen-rich. Thus, extra collagen is easier to reverse in areas that normally do not normally produce large amounts of collagen. This pre-existing ECM architecture could play a role in the cell phenotypic response, either due to a lack of protection from high stress conditions, or due to an interaction with the cell directly that affects cell phenotypic response to increased tension. The exact mechanism for reverse remodeling seen in this model is a rich avenue for future investigation, as it could reveal mechanisms to induce such behavior in clinical pathologies.

The observation that the MV can recover from the pathologic, fibrotic remodeling of FMR by eliminating regurgitation geometry is a valuable insight, as long as the results of this work are considered in the appropriate context. As previously discussed, the valve models created by RUFLS are limited due to a variety of factors.⁵ The RUFLS system is incapable of providing a physiologic response to regurgitation that would result from the changing hemodynamics that regurgitation places on the cardiovascular system. This includes a lack of cellular response present outside of the valve that is present in living specimens, blood borne factors, and changes that result from the cardiovascular system in response to changing cardiac output. This system is also limited in that it utilizes a flat annulus, while a native MV annulus is saddle shaped. The shape of the annulus has a well-studied impact on the valve function throughout the cardiac cycle.^{20,30} In addition, while porcine MVs are the

closet animal analogue to human MVs, there remain slight anatomical differences that could impact how valves remodel in response to regurgitation and repair.⁷ As with any study utilizing animal tissue, animal-to-animal variability could also impact results. The reversal model also does not simulate any one specific method of FMR correction and instead aims to investigate how valve remodeling is affected by returning valves to control hemodynamics through the repositioning of PMs and the reduction of annular area. Thus, the results are less directly applicable to clinical practice than if a current clinical treatment had been replicated. Finally, the time frame under which this study was conducted is considerably shorter than the natural course of disease leading to valve repair in humans. Most patients would live with FMR for months if not years before repair would be considered, allowing for considerable remodeling of the valve to take place. In addition, once repaired, recovery of the valve would be assessed over the patient's lifetime, in terms of both valve function and the impact on cardiovascular hemodynamics.

Nevertheless, this work, even conducted in this compressed timeframe, provides important observations to the response of valves to hemodynamic conditions. That these changes occur so quickly speaks to the dynamic interplay between valve cells, the ECM they secrete, the mechanics of tissues that change in response to ECM alterations, and the forces the cells experience as a result of changing hemodynamics. That these changes can be reversed, at least at an early time point, shows that fibrotic changes are not permanent and further study is needed to understand if and when repair will no longer allow the valve to recover.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

MV	mitral valve
FMR	functional mitral regurgitation
PM	papillary muscle
GAG	glycosaminoglycan
LOX	lysyl oxidase
MMP-1	matrix metalloproteinase-1
RUFLS	Rice University flow loop system
VIC	valve interstitial cell

ECM	extracellular matrix
LVAD	left ventricular assist device

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Figure 1.

Mitral valve geometry control in RUFLS. (A) The mitral valve is sutured onto an appropriate sized annular ring. (B) Papillary muscles are attached to an eye-hook holder using umbilical tape and suture. (C) The annular ring is held in place at the basal end (left) of the mitral valve holder. A slot-and-screw mechanism is used to position the papillary muscles at the proper apical-basal position. (D) Two nuts on screws sandwich the papillary muscle holder and are used to set the proper medial-lateral position of the papillary muscles. (E, H) Control model is created by placing the valve in neutral position. (F, I) FMR condition created by apical and lateral displacement of papillary muscles from the neutral position (dashed line), as well as (I) 65% greater annular area compared to (H) control annulus. (G, J) Reversal model was created by reducing annulus to one size smaller than original annular size and moving the papillary muscles basally and medially to eliminate regurgitation following one week of culture in the FMR geometry. RUFLS = Rice University Flow Loop System, FMR = functional mitral regurgitation. (A, C, D have been modified from Connell *et al.* 2017⁶ with permission)

Figure 2.

Pressure and flow in RUFLS. (A) Pressure and flow waveforms for control, FMR, and reversal conditions show that the valve leaking seen in the FMR condition is returned to a non-regurgitant, closed form in the reversal condition, similar to that of control. Representative waveforms at approximately 13.5 days of culture are shown. (B) Regurgitation of representative samples over the culture period are shown. Control valves maintain regurgitation levels below 350 mL per minute over the culture period. FMR valves gradually increase regurgitation over the 14-day period. Control and FMR systems are taken down for a sterile media change at 7 days. Reversal valves increase regurgitation for the first 7 days. At this point, they are taken down and repositioned to a non-regurgitant geometry.

They maintain regurgitation levels below 350 mL per minute for the remainder of the culture period. RUFLS = Rice University Flow Loop System, FMR = functional mitral regurgitation

Figure 3.

Fibrotic Remodeling Characteristics Elevated in FMR. Valves cultured in the FMR geometry showed significantly greater P4H, decorin, LOX, MMP-1 expression after a two-week culture compared to valves cultured in the control geometry. These results are indicative of extensive fibrotic remodeling. Representative images of DAB-based immunohistochemistry staining are shown. Scale bar = 250 μ m. ANOVA with post hoc Tukey's HSD test used for significance. * = $p < 0.05$; ** = $p < 0.01$. FMR = functional mitral regurgitation, P4H = prolyl-4-hydroxylase, LOX = lysyl oxidase, MMP-1 = matrix metalloproteinase-1.

Figure 4.

FMR condition tissues are stiffer and stronger than controls. FMR leaflet tissues have a higher elastic modulus and a larger ultimate stress than control leaflet tissues. FMR chordae have a higher elastic modulus and a larger ultimate stress than control chordae. $n = 6-7$.

ANOVA with post hoc Tukey's HSD test used for significance. * = $p < 0.05$; ** = $p < 0.01$;

*** = $p < 0.001$. FMR = functional mitral regurgitation.

Figure 5.

Reversal Model Composition. The anterior and posterior leaflets have different remodeling responses to being relieved from the high-tension environment of FMR and returned to a non-regurgitant state. The reversal-conditioned anterior leaflets show increased levels of ECM remodeling markers compared to control anterior leaflets, while the reversal-conditioned posterior leaflets show decreased levels of ECM remodeling markers compared to FMR-conditioned posterior leaflets. Representative images of DAB-based immunohistochemistry staining are shown. Scale bar = 250 μ m. ANOVA with post hoc Tukey's HSD test used for significance. $^{\wedge} = 0.1 > p > 0.05$; $* = p < 0.05$; $** = p < 0.01$. FMR = functional mitral regurgitation, P4H = prolyl-4-hydroxylase, MMP-1 = matrix metalloproteinase-1, LOX = lysyl oxidase.

Figure 6.

Anterior and posterior leaflet section material properties. FMR- and reversal-conditioned anterior leaflets have higher elastic modulus than control leaflets. Reversal-conditioned anterior leaflet sections have higher ultimate stress than controls. Controls and reversal-conditioned posterior leaflets have higher elastic modulus and ultimate stress than FMR-conditioned tissues. $n = 6-7$. ANOVA with post hoc Tukey's HSD test used for significance. $^{\wedge} = 0.1 > p > 0.05$; $* = p < 0.05$; $** = p < 0.01$. FMR = functional mitral regurgitation.

Figure 7.

Circumferential and radial leaflet section material properties. FMR- and reversal-conditioned circumferential leaflet sections have a higher elastic modulus and ultimate stress than controls. Control and reversal-conditioned radial leaflet sections have a lower elastic modulus than FMR. $n = 6-7$. ANOVA with post hoc Tukey's HSD test used for significance. $^{\wedge} = 0.1 > p > 0.05$; $* = p < 0.05$; $** = p < 0.01$. FMR = functional mitral regurgitation.

Figure 8. Chordae tendineae material properties. Control and reversal-conditioned chordae tendineae have lower elastic modulus and lower ultimate stress than FMR-conditioned chordae tendineae. $n = 6-7$. ANOVA with post hoc Tukey's HSD test used for significance. * = $p < 0.05$; ** = $p < 0.01$. FMR = functional mitral regurgitation.