Polymer Injection Therapy to Reverse Remodel the Papillary Muscles

Efficacy in Reducing Mitral Regurgitation in a Chronic Ischemic Model

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Background—Ischemic mitral regurgitation (MR) results from displacement of the papillary muscles caused by ischemic ventricular distortion. Progressive left ventricular (LV) remodeling has challenged therapy. Our hypothesis is that repositioning of the papillary muscles can be achieved by injection of polyvinyl-alcohol (PVA) hydrogel polymer into the myocardium in chronic MR despite advanced LV remodeling.

Methods and Results—Ten sheep underwent ligation of the circumflex branches to produce chronic ischemic MR over 8 weeks. PVA was injected into the myocardium underlying the infarcted papillary muscle. Two-dimensional and 3D echocardiograms and hemodynamic data were obtained before infarct (baseline), before PVA (chronic MR), and after PVA. PVA injection significantly decreased MR from moderate to severe to trace (MR vena contracta, 5.8±1.2 to 1.3±1.3 mm; chronic MR to post-PVA stage; P=0.0003). This was associated with a decrease in infarcted papillary muscle–to–mitral annulus tethering distance (30.3±5.7 to 25.9±4.6 mm, P=0.02), tenting volume (1.8±0.7 to 1.4±0.5 mL, P=0.01), and leaflet closure area (8.8±1.3 cm² to 7.6±1.3 cm², P=0.004) from chronic MR to post-PVA stages.

PVA was not associated with significant decreases in LV ejection fraction (41±3% versus 40±3%, P=NS), end-systolic elastance, τ (82±36 ms to 72±26, P=NS), or LV stiffness coefficient (0.05±0.04 to 0.03±0.01).

Conclusions—PVA hydrogel injections improve coaptation and reduce remodeling in chronic MR without impairing LV systolic and diastolic function. This new approach offers a potential alternative for relieving tethering and ischemic MR by correcting papillary muscle position. (Circ Cardiovasc Interv. 2010;3:499-505.)

Key Words: mitral regurgitation • left ventricular remodeling • coronary artery disease

Ischemic mitral regurgitation (MR) is an important sequela of coronary artery disease that significantly increases late mortality.1,2 Extensive evidence has shown that IMR results from remodeling of the ischemic left ventricle (LV), leading to displacement of the papillary muscle (PM), annular dilation, and therefore tethering of the mitral leaflets.3–5

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Therapy for ischemic MR remains challenging and controversial.6–11 There is general agreement that the mitral leaflets are tethered between their ventricular (PM) and annular attachments, both of which may be abnormally displaced.14,15

Mitrail ring annuloplasty, performed at the time of bypass surgery, is the current therapy for ischemic MR and reduces mitral annular area by reducing the anterior-posterior diameter. However, mitral ring annuloplasty does not directly address the fundamental problem of ischemic LV distortion and dilation, which is progressive, so that initial annular compensation for ventricular dilatation may be overwhelmed.10,11

Therapies that directly reverse the ischemic distortion of the LV wall and leaflet tethering have included surgical plication of the infarcted LV, surgical relocation of the PM, placement of external patches or discs over the infarct, and leaflet augmentation, all of which remain relatively invasive.16–20 An alternative approach that is potentially less disruptive to the LV wall uses biomaterials injected directly into the myocardium, acting as tissue-bulking agents to reposition the PMs. Biomaterial injections could have the effect of displacing the PM toward the mitral annulus, potentially buttressing the weakened muscle wall to limit outward bulging. This could be achieved in the beating heart under imaging guidance and without cardiopulmonary bypass.

One type of biomaterial that has physical and mechanical properties as a tissue-bulking agent is polyvinyl alcohol...
based hydrogel (PVA hydrogel). The base polymer, PVA, is biocompatible, biologically inert, and in current use in human applications. It can be formulated so that it is injectable but forms a stable gel at body temperature.

We have previously tested the injection of a PVA hydrogel in an acute model of ischemic MR. Injection of PVA in the acute model of ischemic MR decreased MR, providing proof of concept for PVA hydrogel injection therapy in the unremodeled ventricle; however, acute ischemic MR is not commonly encountered in the clinical setting. Therefore, it is important to establish whether injecting PVA hydrogel remains effective in a model of chronic ischemic MR with remodeling of the infarcted wall, which represents the most clinically relevant and challenging situation.

Our hypothesis is that injection of PVA hydrogel into the myocardium is effective in reducing MR in a chronic ischemic model.

Methods
As detailed by Llaneras et al., anesthesia was induced in Dorsett hybrid sheep with sodium thiopental (12.5 mg/kg IV), and the trachea was intubated and ventilated at 15 mL/kg with a mixture of 2% isoflurane and oxygen. All animals received glycopyrrolate (0.4 mg IV) and vancomycin (0.5 g IV) 1 hour before incision. The heart was exposed through a sterile left thoracotomy. After the pericardium was opened, baseline imaging was performed and the second and third circumflex obtuse marginal branches were ligated to infarct the inferoposterior wall. The chest was then closed, and the animals recovered.

After 8 weeks, each animal had a second thoracotomy under general anesthesia. A micromanometer-tipped Millar catheter was placed in the LV to measure heart rate, pressure, dP/dt, relaxation constant (τ), and stiffness coefficient. Echocardiographic imaging was performed at the following stages: baseline, chronic MR, and PVA hydrogel injection (post-PVA) (Figure 1).

This study was reviewed and approved by our institutional Animal Care Committee.

Polymer Injection
An 11% PVA hydrogel aqueous solution with 36% polyethylene glycol (used as a gellant) (Cambridge Polymer Group, Inc, Boston, MA) was preformulated and stored in 10-mL syringes at room temperature (20°C to 30°C) as a solid gel. The formulation of the PVA was modified from the acute studies to decrease viscosity, making it easier to inject into scarred myocardium yet retain the same gelling profile. These syringes were packaged in heat-vacuum packed bags and terminally sterilized using 25-kGy gamma radiation (Steris Isomedix, Northboro, Mass). The materials were tested for cytotoxicity and endotoxicity using conventional in vitro tests (LAL and Agarose overlay, respectively). This PVA formulation was designed to gel at or near body temperature (<45°C). The PVA hydrogel syringes were heated to >90°C in a water bath to achieve liquid state and then allowed to cool. The cooled PVA formulation remains injectable for a reasonable period (5 to 8 minutes).

The temperature of the polymer solution is monitored as it exits the needle with a thermometer attached to the needle exit point to ensure that injection occurs at 39°C to 40°C. Once injected into the myocardium, the PVA substantially solidifies over a 5- to 10-minute period. The PVA hydrogel was injected into the myocardium underlying the PMs using an 18-gauge short-bevel needle through a long-tipped catheter. The location of the injections was guided by direct visualization of anatomic landmarks as well as by real-time echocardiography. Additional injections were performed as needed, guided by echo assessment of MR reduction. If there was no MR reduction after an initial injection, injections were repeated adjacent to the initial site to broaden the area of tissue bulking until MR was reversed.

Data Collection and Analysis
LV pressure was recorded along with an ECG lead on a multichannel physiological recorder (Sonometrics Inc, London, Ontario, Canada). Two-dimensional, Doppler, and 3D echo data were collected using an X3 matrix array transducer and iE33 Ultrasound machine (Philips Medical Systems, Andover, Mass). Epicardial imaging was performed through an agarose standoff to minimize near-field effects. For 3D reconstruction, the probe was positioned along the apex to align the LV apex through the center of the mitral valve. By adjusting the mitral valve to the center of the screen, sector settings were optimized for image and color resolution. Three-dimensional data sets were acquired using the full-volume mode over 4 to 7 sequential heart beats with ECG gating and suspended respiration. All images were stored on a DVD disk and transferred for offline analysis using QLAB Advanced Quantification Software, version 6.0 (Philips Medical Systems). Three-dimensional data sets were exported from QLAB in a Cartesian-volumetric format and analyzed using customized software. MR was quantified by measuring the vena contracta in a long-axis view perpendicular to the coaptation
LV and Mitral Valve Measures

LV end-diastolic and end-systolic volumes were obtained by 3D echo, using endocardial borders from 6 planes at equal angular intervals and a surfacing algorithm. Mitral geometry was reconstructed from rotated images at mid-systole, when the leaflets most closely approach the annulus. Mitral annular area was measured at mid-systole by manually tracing the annular points that were defined as the annular hinge points where the leaflets inserted. The PMs were traced to identify their tips by reviewing several adjacent images. The tethering length over which the mitral leaflets and chordae are stretched between the PMs and the relatively fixed fibrous portion of the annulus were measured from posteroanterior PM tip to the medial trigone of the aortic valve (medial junction of aortic and mitral annuli). Leaflet closure surface area was defined as surface area separating the LV and LA and calculated at mid-systole by tracing leaflet surfaces using 6 imaging planes and applying a surface fitting algorithm. The tenting volume of the leaflets is the volume between the leaflets and the least squares of the mitral annulus at mid-systole. LV volumes and contractile performance were assessed using 4 sonomicrometer crystals (Sonometrics) placed over the LV epicardium at the base and apex (long axis) and the anterior and posterior walls (short axis). Pressure-volume loops were constructed from continuous tracings of LV volume, calculated using a standard algorithm, and Millar micromanometer pressure. Sonomicrometry and pressure data were measured using CardioSOFT Pro 3.4 (Sonometrics). The slope (elastance) of the end-systolic pressure-volume relationship as a measure of contractile state was taken as a measure of LV contractility.33 End systole was defined as the maximum ratio of LV pressure to LV volume; end-diastole was defined by the trough in the LV pressure tracing after atrial contraction. The end-diastolic pressure-volume relationship data from LV caval occlusion were fitted to an exponential equation: LV end-diastolic pressure = D*exp(Kp*LV[end-diastolic volume]), where D is a curve-fitting variable and Kp is the stiffness coefficient, which is a measure of the compliance of the ventricle. The relaxation time constant (τ) was calculated as the time for LV pressure to fall from peak negative dP/dt to half its value.

Histological Studies

In a subset of animals, after death, hearts were excised for histological examination of tissue architecture after injection. Injected myocardium was fixed in 10% formalin and histopathologic examination performed on 5-μm sections stained with hematoxylin and eosin and Masson trichrome stain for microscopic examination.

Statistical Analysis

The efficacy of PVA hydrogel injection was tested by repeated-measures ANOVA (baseline, chronic MR, and post-PVA hydrogel). Significant differences were examined by Student t test. The Wilk-Shapiro test was used to test for normal distribution for Student t tests. A probability value of 0.05 was considered significant. SPSS 16.0 (SPSS, Inc) was used to perform statistical analyses.

Results

In 14 sheep, an infarction was created by ligation of left circumflex branches. Two animals died after ligation but before PVA hydrogel injection and 2 did not develop MR. The remaining 10 animals developed moderate to severe MR and underwent PVA hydrogel injection. PVA injection decreased MR from moderate or greater to trace (vena contracta: 5.8±1.2 mm versus 1.8±1.3 mm, chronic MR versus post PVA; P=0.0003). There was a significant decrease in LV ejection fraction after infarction compared with baseline (56±8% to 41±3%; P<0.0001). However, there was no change in LV ejection fraction (chronic MR 41±3% versus 40±3%, post PVA p=ns; Figure 2) and there were no new wall motion abnormalities after PVA injection compared with the chronic MR stage. Figure 3 shows reduction in MR from moderate to trace in an animal after injection of a total of 4 mL of PVA given in 2 aliquots of 2 mL. Three-dimensional echocardiography is used to guide injection with visualization of PVA gel within the myocardium post injection (Figure 4).

Three-dimensional echocardiography examination of mitral valve geometry showed that tethering length (distance from mitral valve to infarcted PM) increased from 24.6±4.6 mm at baseline to 30.3±5.7 mm at chronic MR stage (P=0.001) and decreased to 25.9 mm after PVA injection (chronic MR versus post-PVA; P=0.02). Similarly, leaflet closing area and tenting volume increased significantly from baseline to chronic MR stage. These mitral geometric parameters returned to baseline levels with significant differences between chronic MR and post PVA stages: tenting volume (1.8±0.7 to 1.4±0.5 mL, chronic MR to post-PVA; P=0.01) and leaflet closure area (8.8±1.3 to 7.6±1.3 cm², chronic MR to post-PVA; P=0.004) (Figure 5). Mitral annular area did not change significantly throughout the stages.

Heart rate, LV pressure, and maximal dP/dt were unchanged between chronic MR stages and PVA injection. There were also no significant changes in elastance (Emax), τ (relaxation constant), or the LV diastolic stiffness coefficient after PVA injection (Table), indicating no detrimental effect on LV systolic or diastolic function with localized PVA injection into the myocardium.

A total of 3.3±1.0 mL of PVA was injected. In 6 of 10 sheep, between 2 to 3 injections (each containing 2 to 3 mL of PVA gel) were performed to achieve MR reduction. Gross
anatomic examination showed the PVA hydrogel encapsulated into the myocardium, and histology showed that PVA had gelled in a discrete collection adjacent to infarcted myocardium and dense scar (Figure 6).

Discussion
This study demonstrated the efficacy of PVA hydrogel injection in reducing MR in a chronic ischemic model of MR. This appears to occur without detriment to LV systolic or diastolic function.

Polymers have been used in an increasing number of biological applications including plastic and reconstructive, vascular, and urologic uses. PVA is a chain of carbons with alternating hydrogen and oxygen. It is made by hydrolysis of polyvinyl acetate and is highly hydrophilic, biologically inert, and biocompatible. PVA hydrogel is formed by PVA chains connected by hydroxyl groups, using polyethylene glycol as a gellent to initiate gelling. PVA is currently used as embolization spheres in vascular applications, as cartilage replacement and nerve sheath guides and as rheology modifiers in soft contact lenses for dry eye treatment. These current PVA applications use the solid form of PVA, which is not a suitable formulation for application in the beating heart. Therefore, an injectable formulation of PVA hydrogel was developed and tested for use as a tissue bulker in infarcted myocardium. PVA can be formulated to be an injectable liquid when heated to 90°C but begins to gel at controlled rates at body temperatures.

PVA injection did not result in a decrease in measures of global LV systolic or diastolic function. Because the PVA injections are focused on a relatively small area of the heart, the reduction in MR appears to result from reverse localized remodeling. PVA hydrogel injection may reposition the PMs both by causing direct tissue displacement and in principle by altering the myocardial mechanics so that the underlying myocardium bulges less. The localized nature and relatively small volume of PVA injections would not be expected to have a significant impact on LV function or volumes. This was reflected in the lack of change in standard measures of LV function after PVA injection compared with the chronic MR stage. However, with extensive PVA injections as might be necessary in large infarction and extensive remodeling, alterations in diastolic LV function might occur and must be further assessed.

PVA injections did not result in disruption of the surrounding myocardial fibers. Both MRI studies performed in the acute ischemic MR studies and histological examination showed that PVA injections produced a well-defined cross-linked gel surrounded by necrotic myocardium and fibrous
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The PVA hydrogel did not disrupt or “ooze” into the surrounding myocardial architecture; rather, the PVA hydrogel formed a stable, well-encapsulated gel within 5 minutes after injection at body temperatures. The cross-linking of PVA occurs through hydrogen bonding, which exceeds the bonding forces likely to be present with adjacent tissue planes. Initial pilot studies on acute ischemic MR demonstrated proof of concept for PVA injection therapy. However, it was critical to demonstrate efficacy in a chronic ischemic model of MR that is the clinically most relevant setting for therapy, reflecting patients presenting with chronic remodeled infarction and ischemic MR. Additionally, it was important to establish the ability of PVA hydrogel to form a stable tissue bulking gel not only in acute myocardial infarction but also in chronic infarction, in which there is an admixture of necrotic myocytes and fibrous scar. The advantage of a polymer-based approach is that polymer chemistry has advanced to the point where physical and material characteristics can be modified to adapt to specific clinical situations. For example, injection characteristics may be different in chronic infarction, in which there is more fibrous tissue than in acute infarction. The formulation of the PVA was modified from the acute studies to decrease viscosity, making it easier to inject into scar yet retain the same gelling profile. Furthermore, the viscosity and rheology of the PVA hydrogel can be further adjusted so that longer but narrower delivery systems used in minimally invasive techniques could potentially be used.

PVA injection may also function as an “internal stiffener or patch,” acting to limit further remodeling by constraining the myocardium. Injection of collagen into infarcted rat myocardium resulted in a reduction in post myocardial infarction LV remodeling, supporting the concept that localized tissue bulking can limit adverse LV remodeling. In addition, finite element modeling of material injection into the myocardium has predicted beneficial effects on ventricular mechanics.

Clinical Implications
Polymer injection has potential advantages for treating ischemic MR as it directly modifies ischemic ventricular distor-
tion. Previous studies have demonstrated efficacy of therapies that directly address ventricular distortion or leaflet tethering, such as external patching over the infarct, augmentation of the posterior leaflet by insertion of a pericardial patch, or surgical relocation of the PM, but those approaches remain relatively invasive. PVA hydrogel injection has the potential to be less invasive applied in the beating heart and hence may be more widely applied. It can also be tailored to the individual patient, adjusting for the variable degree of tethering among patients, with potential benefit to both PMs and at annular and subannular levels.

Limitations
This study examined acute PVA injections into chronic ischemic MR and the efficacy of chronic PVA injections in maintaining MR reduction remains to be determined in future studies.

PVA hydrogel injections may be limited in globally dilated and dysfunctional left ventricles because this may require more extensive reverse remodeling than possible with localized tissue bulking therapy. However, it could be possible to make serial sequential injections over both PMs to achieve symmetrical reverse remodeling of PM displacement.

Surgical and echocardiographic observations demonstrate variations in the anatomy underlying ischemic MR, including symmetrical versus asymmetrical leaflet coaptation and mismatched coaptation of posterior and commissural scallops related to variations in chordal and segmental leaflet anatomy. Just as in other forms of mitral valve repair, various

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Figure 5. Changes in mitral valve geometry at baseline, post–chronic MR, and post-PVA stages. *P<0.05 relative to baseline and chronic MR; **P<0.05 relative to chronic MR and after PVA. Scale for leaflet area, mitral annular area, and tenting volume are displayed on the left-hand side of the graph; scale for the tethering length is displayed on the right hand-side of the graph.

Figure 6. A, Gross appearance of PVA hydrogel (arrows) injected into chronic infarction. B, Histology showed PVA (blue) had gelled in a discrete collection adjacent to infarcted myocardium and dense scar (arrows).
approaches may therefore be needed to achieve repair in the largest number of patients; this study describes one approach that targets ventricular remodeling in the region that controls mitral valve tethering. If tethering is seen by imaging not to be the predominant mechanism of MR in a given patient, other approaches can be used instead, and PVA injection is not an exclusive solution. If annular dilatation should predominate and polymer injection should leave sufficient residual MR, it can certainly be combined with annular reduction, either by ring annuloplasty or conceivably by perianular polymer injection, which remains to be tested.

Nevertheless, despite anatomic variations, positive results should be obtained by releasing valve tension from the ventricular end of the “tug-of-war” in between its ventricular and annular attachments.

The model used incorporates the key aspects of the clinical situation (infarct location, PM displacement, leaflet tethering and MR) in a way that allows testing proof of principle.

Conclusion
PVA hydrogel injections improve coaptation and reduce remodeling chronic MR without impairing LV systolic and diastolic function. This new approach offers a potential alternative for relieving ischemic MR in the beating heart by correcting PM position, thus relieving tethering that causes ischemic MR.

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Disclosures
Dr Braithwaite and Jason Berlin and are employees of Cambridge Polymer Inc and Dr Muratoglu has an equity interest in Cambridge Polymer Inc.

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**CLINICAL PERSPECTIVE**

Ischemic mitral regurgitation (IMR) is an important consequence of coronary artery disease that increases morbidity and late mortality. The fundamental mechanism underlying IMR is distortion of the ischemic ventricular wall, which results in restricted mitral leaflet closure from displacement of the papillary muscle. Mitral ring annuloplasty, commonly placed at the time of coronary bypass surgery for IMR, although effective, does not directly address the ventricular distortion and papillary muscle displacement. The use of biologically inert polymer biomaterials are used as bulking agents for a number of clinical uses in urological, orthopedic, plastic surgical, and vascular disciplines. This chronic experimental study explores a novel approach to treating ischemic IMR in which a polymer specifically designed to cross-link once injected into the myocardium, forming a stable gel. This results in myocardial tissue bulking and repositioning of the infarcted myocardial wall with relief of left ventricular distortion and deformation, thereby restoring normal mitral leaflet closure. This new approach offers a potential alternative for relieving tethering and IMR by correcting papillary muscle position and can be performed in the beating heart.
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