ORIGINAL ARTICLE

Mechanical Tissue Resuscitation Protects Against Myocardial Ischemia-Reperfusion Injury

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ABSTRACT ***Background and Aim:*** **Reperfusion injury is a complex inflammatory response involving numerous mechanisms and pathways. Mechanical tissue resuscitation is a newly described therapeutic strategy that reduces reperfusion injury. This study further investigates potential mechanisms for the protective effects of mechanical tissue resuscitation while utilizing a bio-absorbable matrix.** ***Methods:*** **Anesthetized swine were subjected to 80 minutes of coronary ischemia and three hours of reperfusion. An absorbable matrix was used to cover the ischemic-reperfused myocardium and apply the mechanical tissue resuscitation (−50 mmHg) throughout reperfusion. Infarct size, myocardial blood flow (microspheres), apoptosis, edema, and hemodynamics were analyzed.** ***Results:*** **Both control and treated groups displayed similar hemodynamics and physiologic parameters. Mechanical tissue resuscitation significantly reduced early infarct size ($16.6 \pm 3.8\%$ vs. $27.3 \pm 2.5\%$ of area at risk, $p < 0.05$). This reduction of infarct size was accompanied by reduced edema formation in both epicardial (27% reduction) and endocardial (58% reduction) samples. Histological examination of both epicardial and endocardial tissues also revealed a reduction in apoptosis (80% and 44% reductions) in MTR-treated hearts.** ***Conclusions:*** **Treatment with mechanical tissue resuscitation during reperfusion reduces both early cell death and the delayed, programmed cell death after ischemia-reperfusion. This cardioprotection is also associated with a significant reduction in interstitial water. Additional cardioprotection may be derived from mechanical tissue resuscitation–induced increased blood flow. Mechanical tissue resuscitation, particularly with a resorbable device, is a straightforward and efficacious mechanical strategy for decreasing cardiomyocyte death following myocardial infarction as an adjunctive therapy to surgical revascularization.** doi: 10.1111/jocs.12247 *(J Card Surg 2014;29:116–123)*

Reperfusion is a complex phenomenon that, while required to salvage ischemic tissue, induces myocardial injury via a multitude of mechanisms and mediators including neutrophils,[1] oxygen radicals,[2] endothelial injury,[3] and complement.[4] Therapeutic approaches for reperfusion injury have primarily focused on pharmacologic inhibition of these well-established mechanisms of injury. Despite the promise shown in animal studies, clinical benefit of such strategies has not been realized.[4,5] More recently, a variety of nonpharmacologic therapeutic approaches have been proposed including postconditioning[6] and local hypothermia.[7]

Negative pressure wound therapy increases cell preservation by reducing inflammation, tissue edema, and improving local blood flow[8,9] within the area that borders a region of irreversible cell death.[10] This tissue ''resuscitation'' may attenuate the ultimate magnitude of cell death within the tissue placed at risk for further injury during reperfusion. We have previously demonstrated that mechanical tissue resuscitation (MTR) is an effective protective strategy for treating a variety of indications, including acute myocardial infarction,[11]

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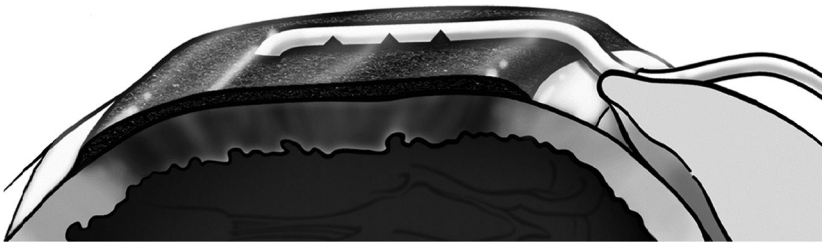

Figure 1. *Illustration of MTR device. MTR was accomplished by placing a PCL matrix directly over the area at risk as shown. The matrix, with vacuum tubing attached, was covered by a POC/collagen film to create a seal. The degree of negative pressure was controlled by a vacuum pump. MTR was applied immediately upon, and throughout, reperfusion.*

burns,[10] and traumatic brain injury.[12] However, in order for this therapeutic approach to be clinically viable, the MTR matrix should be made from materials that are effective as the nondegradable matrices originally described during treatment,[11] while also allowing for biodegradation with little or no tissue reaction. Such a matrix could be either used at the time of open revascularization surgery or placed using a minimally invasive technique following a percutaneous revascularization procedure. In this paper, we confirm and further define the protective effects of MTR on the myocardium while demonstrating the efficacy of bioengineered matrices in the setting of an acute myocardial infarction.

MATERIALS AND METHODS

Preparation of device

The MTR device was constructed using a computer-controlled vacuum pump connected to a silicone tube, an open-cell poly-caprolactone (PCL) foam matrix, and a collagen/poly-octanediol citrate (POC) sealing film. The PCL foam was fabricated using phase separation techniques as previously described.[13] The biodegradable sheeting for securing the matrix to the heart was electrospun using a blend of type I-collagen and poly-1,8-octanediol citrate (POC). After electrospinning, the POC/collagen film was cross-linked in glutaraldehyde vapor, treated with glycine, washed with water, dried, and stored.

Surgical preparation

All animal work was performed in anesthetized swine under approved protocols (Wake Forest Baptist Health Animal Care and Use Committee) and in accordance with the Guide for the Care and Use of Laboratory Animals (1996) published by the National Institutes of Health as previously described with minor changes.[11] Amiodarone and lidocaine were given to control arrhythmias, and heparin was administered as an anticoagulant.

Ischemia-reperfusion protocol

A reproducible, free-wall area at risk on the anterior surface of the left ventricle was made ischemic for 80 minutes by reversible ligation of several diagonal branches of the LAD and reperfused for three hours. Hemodynamic and blood gas analyses were performed at baseline, end of ischemia, and at 30 (R30), 60 (R60), 120 (R120), and 180 (R180) minutes of reperfusion. The control group (n = 6) had no intervention beyond the antiarrhythmic and anticoagulant drugs listed. MTR (n = 6) was accomplished by placement of a PCL matrix directly on the area at risk. The distal end of an evacuation tube was attached to the matrix, with the proximal end connected to a vacuum pump. The matrix and tube were covered with an oversized, elastic, air-impermeable sheet of POC/collagen. The edges of the cover sheet extended onto the epicardium where they were further sealed to the epicardium using fibrin glue (Evicel, Johnson & Johnson, Somerville, NJ, USA). Sub-atmospheric pressure (−50 mmHg) was continuously applied to the myocardium through the open cell matrix via vacuum pump for the duration of reperfusion.

Infarct size

Infarct size was determined as has been previously described.[3,11] The area at risk was identified at the end of 3 hours reperfusion by negative staining with patent blue after re-ligating the coronary ligatures. The left ventricle was cut into 3- to 4-mm-thick sections perpendicular to the long axis of the heart and divided into normally perfused myocardium (blue, nonischemic tissue) and area at risk (unstained). The area at risk was then cut into small pieces and stained with 1% triphenyltetrazolium chloride (TTC, Sigma Chemical, St Louis, MO, USA). The TTC-positive tissue (red, viable tissue) was dissected from the TTC-negative tissue (pale, necrotic tissue). The tissues were then weighed to calculate the area at risk and infarct size using the following formulas:

$$\text{AAR}(\%) = 100 \times \left(\frac{\text{Red} + \text{Pale}}{\text{Blue} + \text{Red} + \text{Pale}}\right)$$

$$\text{Infarct size}(\%) = 100 \times \left(\frac{\text{Pale}}{\text{Pale} + \text{Red}}\right)$$

Regional myocardial blood flow

Regional myocardial blood flow was determined as previously described using neutron-activated microspheres (BioPAL, Inc., Worcester, MA, USA).[11]

Microspheres (15 μm) were injected via the left atrium, while a reference sample of arterial blood was drawn from the femoral artery for 90 seconds.

To understand what role a change in myocardial blood flow may play in the observed myocardial protection, the direct effects of MTR on blood flow were assessed in a separate cohort of animals. Here, blood flow was measured using an ultrasonic flow probe (Transonic Systems, Ithaca, NY, USA) placed around the LAD distal to the first diagonal branch. For these studies, the MTR matrix was placed over the myocardium perfused by the LAD distal to the flow probe and the effect on blood flow of both −50 and −125 mmHg negative pressure was compared. Data were recorded at baseline and at the maximal change in flow during a 60-minute treatment.

Apoptosis and myocardial edema

Epicardial and endocardial samples of tissue from the ischemic, non-necrotic region were prepared for histology using standard paraffin-embedding techniques. All slides were imaged using a digital camera attached to an Axioskope microscope (Carl Zeiss, New York, NY, USA).

Apoptosis was measured using the ApopTag® Peroxidase In Situ Apoptosis Detection Kit (Millipore, Billerica, MA, USA) and counterstained with hematoxylin prior to quantification. All cells within the image field were used to calculate the fraction of TUNEL-positive cells in each field. Data are expressed as the mean fraction of TUNEL-positive cells/field from a sample of 10 fields counted.

Interstitial edema was estimated by quantifying the interstitial space within histological samples. Images were imported into Image J (NIH, Bethesda, MD, USA) and converted into 16-bit black-and-white images. The black/white threshold was adjusted to highlight the interstitial space prior to quantification of percentage of pixels that were either black (tissue) or white (interstitial space). Images that displayed significant tissue artifact or vascular space were excluded from the analysis. The mean of 3–10 fields per sample was used for each data point, and the data were reported as % interstitial space.

Exclusion criteria and statistics

Data from animals were excluded from analysis if: (1) the AAR/LV was $<12\%$ or $>24\%$ ($n=2$); (2) transmural blood flow during the ischemic period was greater than 0.15 mL/min/g tissue ($n=3$); (3) there was inability to maintain seal on the treatment matrix ($n=1$); or (4) an incident of ventricular fibrillation occurred that was nonresponsive to electrical cardioversion (>3 unsuccessful attempts, $n=2$). Group differences in nonrepeated measures variables were compared using a t-test (SigmaStat). When data were non-normally distributed or had unequal variances, the nonparametric Mann-Whitney rank-sum test was performed. For blood flow, arterial blood gas, and hemodynamic variables, two-way repeated measures analysis of variance tests was performed with a post hoc Holm-Sidak test for comparing group differences at individual time points (SigmaStat). All data are presented as mean ± standard error of the mean (SEM).

RESULTS

Animals used in the treated and untreated groups were comparable in age and weight (25.2 ± 0.3 kg) and displayed similar physiological characteristics throughout the experiment (Table 1). There were no significant group differences at any time related to temperature, hematocrit, or blood calcium levels. Arterial pH and carbon dioxide showed small, but statistically significant differences between the groups only at baseline, while the blood oxygen concentration showed group differences only at the last reading of the experiment. These perturbations, which fell within normal ranges, were transient and occurred either prior to initiation of ischemia or at the end of the experiment. Blood pressure and heart rate were monitored throughout the experiment and at no time were group differences found for either mean blood pressure or heart rate (Table 1).

Regional myocardial blood flow

Regional myocardial blood flow was determined in three regions of the myocardium for each group: the normally perfused, nonischemic zone (NI); the ischemic, non-necrotic zone (ISC); and the necrotic zone (NEC). Baseline blood flow was comparable between the two groups in all three regions at baseline (Table 2). Ligation of the coronary arteries caused a significant reduction of blood flow (approaching zero) to the area at risk with no differences between groups. There were no statistically significant group differences in blood flow at any of the reperfusion time points.

Infarct size

Animals were subjected to equivalent areas at risk with ischemic regions of $15.8\pm1.1\%$ and $13.8\pm0.8\%$ of LV in control and MTR groups ($p=0.132$). However, infarct size was significantly reduced in the MTR-treated group (Fig. 2). Application of −50 mmHg subatmospheric pressure reduced infarct size by 39% ($16.6\pm3.8\%$ vs. $27.3\pm2.5\%$ of AAR; $p=0.04$).

Apoptosis

Histological examination of epicardial and endocardial tissue samples from ischemic, non-necrotic regions was used to assess apoptosis. In epicardial samples of untreated animals, $29.3\pm7.7\%$ of the cells examined displayed TUNEL-positive staining indicating induction of apoptosis (Fig. 3). There were significantly fewer TUNEL-positive cells ($5.9\pm2.1\%$, $p<0.01$) in the epicardium of MTR-treated hearts. There was also a reduction in the number of TUNEL-positive cells in endocardial samples of MTR-treated animals ($20.2\pm5.1\%$ vs. $35.9\pm8.6\%$). While this difference did not

TABLE 1
Arterial Blood Gas and Hemodynamic Parameters Throughout the Experiment

	Temp. (°C)	pH	pCO_2 (mmHg)	pO_2 (mmHg)	HCT (%)	Calcium (mmol/L)	HR (min^{-1})	BP (mmHg)
Baseline								
Control	37.1 ± 0.3	7.40 ± 0.04	49 ± 3	525 ± 49	29.0 ± 2.0	2.19 ± 0.20	73 ± 6	76 ± 3
MTR	36.8 ± 0.2	7.49 ± 0.04*	41 ± 4*	649 ± 24	30.1 ± 1.2	1.91 ± 0.13	77 ± 6	72 ± 5
Mid-ischemia								
Control	37.3 ± 0.2	7.43 ± 0.03	43 ± 3	584 ± 39	30.5 ± 1.7	2.25 ± 0.17	68 ± 5	75 ± 6
MTR	37.1 ± 0.2	7.49 ± 0.01	38 ± 0	649 ± 16	33.0 ± 1.6	2.19 ± 0.20	71 ± 5	74 ± 4
End-ischemia								
Control	37.5 ± 0.1	7.39 ± 0.03	47 ± 3	513 ± 33	32.1 ± 1.5	2.29 ± 0.16	69 ± 5	73 ± 5
MTR	37.3 ± 0.1	7.43 ± 0.01	44 ± 1	551 ± 23	31.9 ± 1.5	2.01 ± 0.09	75 ± 8	70 ± 2
R30								
Control	37.6 ± 0.1	7.38 ± 0.02*	45 ± 3	466 ± 59	32.1 ± 1.2	2.26 ± 0.12	94 ± 6	69 ± 5
MTR	37.3 ± 0.2	7.44 ± 0.01	41 ± 2	514 ± 60	32.0 ± 1.7	1.95 ± 0.07	93 ± 12	62 ± 2
R60								
Control	37.8 ± 0.1	7.39 ± 0.01	42 ± 1	432 ± 78	32.1 ± 1.3	2.21 ± 0.08	96 ± 8	66 ± 3
MTR	37.4 ± 0.2	7.42 ± 0.01	42 ± 2	474 ± 45	32.8 ± 2.3	2.06 ± 0.08	83 ± 7	64 ± 3
R120								
Control	37.7 ± 0.2	7.36 ± 0.02	44 ± 2	352 ± 79	30.4 ± 1.4	2.25 ± 0.08	96 ± 6	59 ± 2
MTR	37.4 ± 0.1	7.40 ± 0.01	43 ± 2	462 ± 42	31.4 ± 2.3	2.04 ± 0.08	85 ± 6	62 ± 2
R180								
Control	37.6 ± 0.1	7.35 ± 0.02	42 ± 2	333 ± 90	26.7 ± 1.5	2.08 ± 0.11	103 ± 10	59 ± 3
MTR	37.3 ± 0.2	7.40 ± 0.02	42 ± 3	496 ± 52	27.3 ± 3.4	1.87 ± 0.05	90 ± 6	61 ± 6

MTR, mechanical tissue resuscitation; HCT, hematocrit; HR, heart rate; BP, femoral blood pressure; R30/60/120/180, 30, 60, 120, or 180 minutes following reperfusion.
*$p < 0.05$ vs. control within a specific time.

TABLE 2
Regional Myocardial Blood Flow Determined Using Microspheres

Time	Group	NI	ISC	NEC
Baseline	Control	0.71 ± 0.10	0.81 ± 0.16	1.00 ± 0.20
	MTR	0.98 ± 0.16	1.18 ± 0.18	1.11 ± 0.12
Ischemia	Control	0.59 ± 0.09	0.05 ± 0.01**	0.02 ± 0.01**
	MTR	0.57 ± 0.04	0.06 ± 0.01**	0.02 ± 0.01**
R30	Control	0.59 ± 0.05	2.00 ± 0.26**	1.94 ± 0.40**
	MTR	0.56 ± 0.06**	2.12 ± 0.30**	1.96 ± 0.28**
R180	Control	1.05 ± 0.25**	1.49 ± 0.40**	1.51 ± 0.42
	MTR	0.70 ± 0.15	1.16 ± 0.30	0.99 ± 0.10

NI, nonischemic region; ISC, ischemic, non-necrotic tissue; NEC, necrotic tissue; MTR, mechanical tissue resuscitation; R30/180, 30 or 180 minutes following reperfusion.
*$p < 0.05$ vs. control within a region.
**$p < 0.05$ vs. baseline within a group.

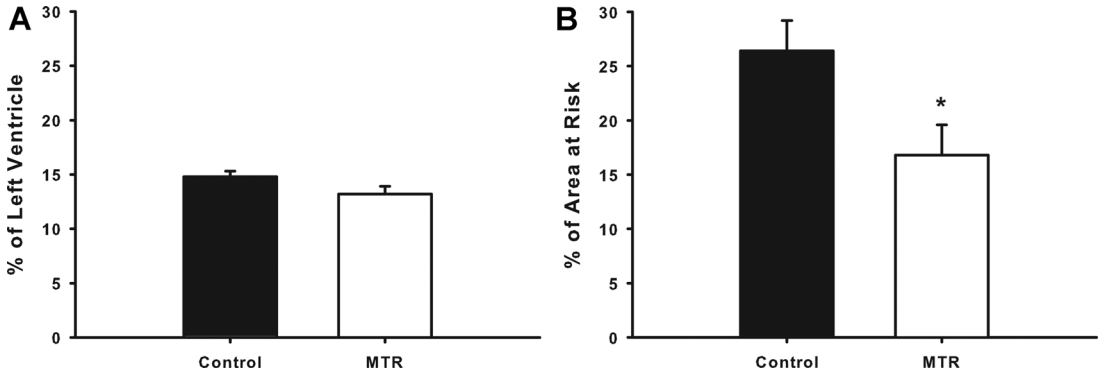

Figure 2. *Infarct size following myocardial ischemia and reperfusion. (A) Both groups had equivalent areas subjected to 80 minutes of ischemia and three hours of reperfusion. (B) MTR treatment during the reperfusion phase significantly reduces the amount of tissue death. Infarct size is expressed as necrotic area normalized for area at risk. *$p < 0.05$ vs. untreated controls.*

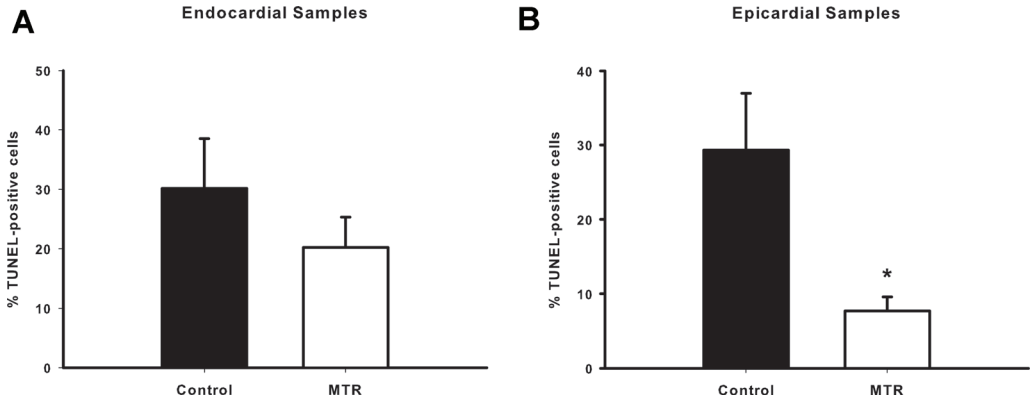

Figure 3. *Induction of apoptosis following myocardial ischemia and reperfusion. Apoptosis was assessed in both endocardial (A) and epicardial (B) samples of ischemic, non-necrotic tissue following 80 minutes of ischemia and reperfusion. Apoptosis was quantified from histological samples using a TUNEL-staining kit. Apoptosis is reported as the percentage of cells staining positive for nick-end labeling. $^{*}p < 0.05$ vs. untreated controls within a region.*

reach statistical significance, it represents a greater than 30% reduction in apoptosis. Together, these data suggest that MTR reduces myocardial apoptosis following myocardial ischemia-reperfusion, with a more robust effect occurring closer to the source of negative pressure ($5.9 \pm 2.1\%$ vs. $20.2 \pm 5.1\%$, $p < 0.05$).

Interstitial edema

Nonischemic myocardial tissue displayed the densely packed myocytes of normal myocardial tissue with an average interstitial spacing of $1.7 \pm 0.3\%$. The inflammatory response to ischemia-reperfusion resulted in the increase in interstitial spacing of myocytes of both endocardial ($14.3 \pm 2.4\%$) and epicardial (10.7%) samples. MTR-treatment (Fig. 4) reduced the interstitial spacing of endocardial myocytes by approximately 50% ($14.3 \pm 2.4\%$ vs. $6.0 \pm 0.5\%$, $p = 0.004$). A similar, but less robust response was observed in the epicardial samples ($10.7 \pm 2.1\%$ vs. $7.8 \pm 1.1\%$, $p = 0.267$). Together, these data demonstrate that MTR reduces ischemia-reperfusion–induced myocardial edema.

Blood flow and MTR

In naïve hearts ($n = 4$ for each pressure), blood flow and hemodynamic changes were measured prior to and following application of MTR to the anterior surface of the left ventricle at either −50 or −125 mmHg. At the lower pressure, no significant change from baseline blood flow was observed. However, there was a $23.3 \pm 7.1\%$ increase in blood flow ($p < 0.05$ vs. baseline) in hearts treated with −125 mmHg pressure MTR. This change in blood flow occurred without any significant increase to either heart rate or blood pressure in either of the treatment groups (Fig. 5).

CONCLUSIONS

In this study, mechanical tissue resuscitation was applied throughout reperfusion by treating the ischemic-reperfused myocardium directly with controlled

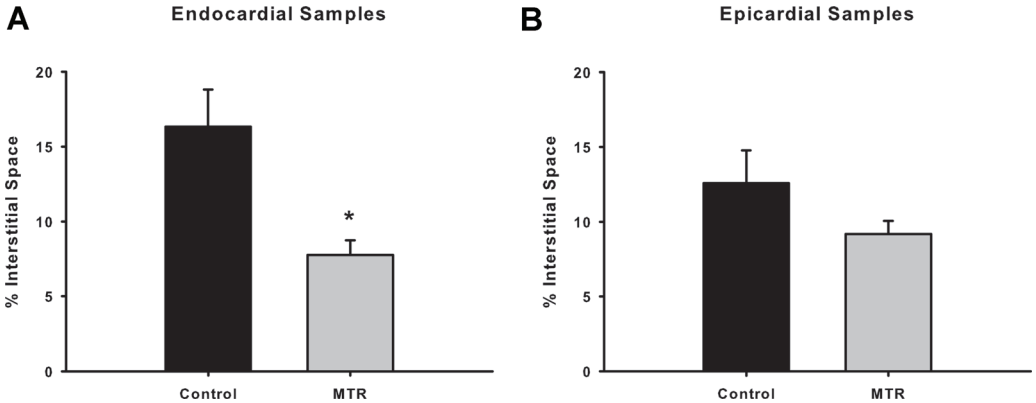

Figure 4. *Myocardial edema following myocardial ischemia and reperfusion. Myocardial edema assessed in both endocardial (A) and epicardial (B) samples of ischemic, non-necrotic tissue following 80 minutes of ischemia and reperfusion. Edema was measured from histological samples by quantifying the amount of interstitial space between myocytes within multiple high-powered fields using image analysis software. Edema is reported as the percentage of cells staining positive for nick-end labeling. $^{*}p < 0.05$ vs. untreated controls within a region.*

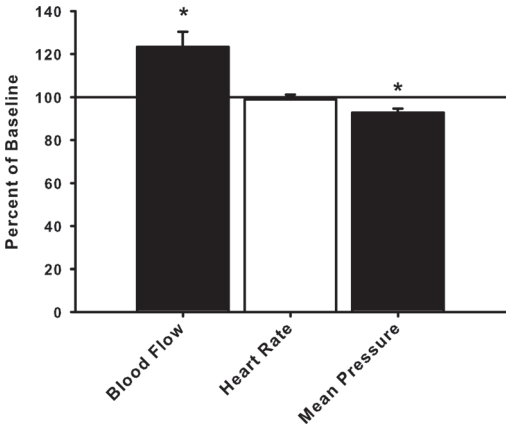

Figure 5. *Effect of MTR on myocardial blood flow in naïve hearts. A separate cohort (n = 4/group) of animals was instrumented with a transit-time flow probe on the LAD. After baseline conditions were achieved, MTR (either −50 or −125 mmHg) was applied to the myocardium distal to the flow probe and changes to myocardial blood flow were measured. While blood flow increased with MTR at the higher pressure, there were no significant increases to either heart rate or blood pressure. *$p < 0.05$ vs. baseline.*

sub-atmospheric pressure. This therapeutic approach was achieved by directly applying bioabsorbable materials developed in the laboratory with no discernible acute adverse hemodynamic, functional, or physiological effects to the epicardial surface of the heart. Consistent with our previous work,[11] application of MTR resulted in significant tissue preservation following myocardial ischemia-reperfusion. Specifically, MTR ameliorated both the acute death of myocytes and the initiation of apoptosis in cells that survive the early inflammatory response to reperfusion. Furthermore, MTR reduced infarct size in treated animals by 39% compared to untreated controls.[11] This reduction in infarct size was similar in magnitude to our previously reported data (55% reduction at −50 mmHg, not statistically different than present study). Along with infarct size reduction, MTR also reduced the magnitude of TUNEL-positive cells by almost 75% in the epicardium and 30% in the endocardium, potentially reducing the later wave of cell death that normally occurs within the ischemic-reperfused myocardium. The increased response within epicardial tissue is likely due to activation of second messenger signaling due to the exertion of negative pressure on the surface of the heart, which may be less robust further from the source of subatmospheric pressure.

In addition to the alterations in apoptosis, we hypothesize that several other mechanisms may account for MTR-mediated cardioprotection including the reduction of postischemic edema, changes in local blood flow, and the removal of inflammatory cytokines from the ischemic-reperfused myocardium. There is a well-established relationship between interstitial water accumulation and a decrease in normal ventricular performance[14,15] as well as the remodeling response to myocardial injury.[16] In our study, interstitial spacing of the myocytes was used as a surrogate marker for tissue edema as reported by others.[17,18] Similar to published results reported for untreated animals,[17] normal myocardium from the nonischemic region exhibited tightly packed muscle fibers with little spacing while the ischemic-reperfused region of the heart displayed significant edema formation with increased interstitial spacing of muscle fibers. MTR-treated myocardium exhibited reduced interstitial spacing in histological samples of the area at risk. In contrast to the apoptosis data, the effect of MTR was greater within the endocardium than in the epicardium. This phenomenon may be due to a bulk shift in interstitial water from the endocardium toward the epicardium as a result of the constant force of negative pressure originating at the epicardial surface. It is unclear whether longer treatment of the ischemia-reperfused myocardium during reperfusion would lead to additional removal of interstitial water from both the endocardial and epicardial regions. It is also possible that the movement of extracellular water may carry with it the soluble inflammatory mediators associated with ischemia-reperfusion injury as has been demonstrated in both humans and animals treated with negative pressure therapy.[19] Thus, reduction in myocardial edema and accelerated resolution of edema may play a key role in treating acute myocardial infarction.

A third potential mechanism for the observed protection is increased MTR-induced local blood flow. No significant differences in blood flow (using microspheres) were observed in the ischemic-reperfused myocardium of MTR-treated and untreated animals. However, blood flow was determined at just two time points during MTR treatment using a limited, discrete sampling of transmural tissue, which may confound the data considering the regional effect of MTR on apoptosis and edema. To directly assess the effects of MTR on blood flow, we used an ultrasonic flow probe placed proximally on the LAD coupled with MTR treatment of the myocardium perfused by the distal LAD in a separate cohort of animals. By integrating the blood flow signal across the entire treated region, we were able to increase the blood flow signal and thereby improve the power to detect changes in total blood flow. Even so, no significant blood flow changes were observed when MTR was applied at −50 mmHg. However, a significant increase in blood flow was observed (23%) when MTR was applied at −125 mmHg. This increased blood flow was similar in magnitude to that previously reported.[20] While blood flow increased, there were no significant increases in blood pressure or heart rate, the primary determinants of myocardial oxygen demand. With the tight relationship between myocardial oxygen demand and blood flow, the fact that these parameters did not change suggests that MTR exerts some direct action on either the myocardium or the coronary vasculature resulting in vasodilation and increased blood flow. While other investigators[21] have reported increases in epicardial blood flow within seconds of applying low (−50 mmHg), but not high (−125 mmHg), magnitudes of negative pressure, our data show the opposite phenomenon. They also observed an increase in epicardial blood flow in normal, ischemic, and ischemic-reperfused epicardium[22,23] and

showed that the effect of negative pressure on blood flow was a function of distance from the source of negative pressure.[24] While there are differences in the details of both methodology and results, both groups have demonstrated an effect of negative pressure on blood flow.

One goal of the present study was to identify an absorbable matrix that could be used to create an MTR patch that would not have to be removed from patients. The data from this study provide proof of concept evidence that the absorbable materials used display appropriate biophysical properties for use in MTR. The PCL foam we used in the present study compares favorably with the nonabsorbable PVA foam used previously.[11] Both types of matrices were able to transduce the negative pressure from the vacuum pump to the epicardial surface, resulting in benefit to the treated area. In addition, the PCL foam has been shown to maintain negative pressure for at least 72 hours with no cytotoxicity.[13] Although in vitro degradation studies predict that ~80% of the PCL foam will degrade within 33 weeks[13] whereas non-modified POC elastomers have been manufactured that degrade nearly completely within 12 hours in vitro,[25] elastomeric modifications, such as co-spinning with collagen, can significantly alter the rate of degradation. While the timing of degradation is not ideal, the results of this study suggest that appropriate materials can be manufactured to maintain the ability to treat with MTR for approximately 72 hours while being absorbed quickly beyond that time. This would allow for maximal treatment of myocardial ischemia-reperfusion injury without residual material remaining within the pericardial space longer than necessary.

In summary, this study demonstrates that MTR, applied specifically at the injured area of myocardium, can reduce myocardial injury following ischemia-reperfusion and decrease the ultimate size of the infarct. In addition, MTR reduces both acute edema formation and the initiation of apoptosis within the area at risk. Because this therapy was applied during reperfusion only, a number of clinical populations could derive benefit from such an intervention. For example, patients undergoing open surgical revascularization procedures could employ this technique as a straightforward adjunctive therapy that would not interfere with bypass grafts. Moreover, the MTR device could be adapted for delivery thoracoscopically for patients reperfused with minimally invasive techniques. Since we showed that MTR can be applied using bioabsorbable materials, the potential to place this device in patients without the requirement to perform a second intervention for removal also seems reasonable. Overall, since MTR is a mechanical treatment for ischemia-reperfusion injury that acts through multiple mechanisms, it could potentially be more effective than many of the single-target pharmacologic interventions attempted previously. This therapeutic modality also has the potential to be used with both open- and closed-chest reperfusion strategies resulting in widespread utility within the clinic.

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