

Functional Comparison of Transmyocardial Revascularization by Mechanical and Laser Means

Keith A. Horvath, MD, Noam Belkind, BS, Irene Wu, BS, Rodney Greene, BS, John Doukas, PhD, Jon W. Lomasney, MD, David D. McPherson, MD, and David A. Fullerton, MD

Division of Cardiothoracic Surgery, Northwestern University Medical School, Chicago, Illinois, and Selective Genetics, Inc, San Diego, California

Background. As a result of the clinical benefit observed in angina patients treated by transmyocardial revascularization (TMR) with a laser, interest in mechanical TMR has been renewed. Although the injury induced by mechanical TMR is similar to laser TMR, the resultant impact on myocardial contractility is unknown. The purpose of this study was to determine whether mechanical TMR improves ventricular function as compared with laser TMR in chronically ischemic myocardium.

Methods. After establishing an area of chronic myocardial ischemia, 25 domestic pigs were randomized to treatment by: excimer laser (group I), a hot needle (50°C) (group II), a normothermic needle (group III), an ultrasonic needle (40 KHz) (group IV), or no treatment (group V). All devices create a transmural channel of the same diameter; 22 ± 1 transmural channels were created in each animal. Regional myocardial contractility was assessed by measuring ventricular wall thickening at rest and with dobutamine stress echocardiography. Six weeks after revascularization, the animals were restudied at rest and with stress. Postsacrifice and histologic analysis of angiogenesis and TMR effects was then assessed.

Results. Laser TMR provided significant recovery of ischemic myocardial function. This improvement in contractility after laser TMR was a 75% increase over the baseline function of the ischemic zone ($p < 0.01$). Mechanical TMR provided no significant improvement in function posttreatment. In fact, TMR achieved with an ultrasonic needle demonstrated a 40% worsening of the contractility versus the pretreatment baseline ($p < 0.05$). Histologic analysis demonstrated a significant increase in new blood vessels in the ischemic zone after laser TMR, which was not demonstrated for any of the other groups ($p < 0.05$). Additionally, evaluation of the mechanical TMR channels demonstrated significant scarring, which correlated with the functional results.

Conclusions. Using devices to create an injury analogous to the laser, mechanical TMR failed to improve the function of chronically ischemic myocardium. Only laser TMR significantly improved myocardial function.

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Recent randomized and nonrandomized, single and multiinstitutional controlled trials [1–9] have led to the approval and clinical acceptance of transmyocardial laser revascularization (TMR) as a means of providing angina relief for patients whose severe disease is not amenable to conventional methods of revascularization, such as coronary artery bypass grafting (CABG) and percutaneous transluminal coronary angioplasty with stenting (PTCA). Before CABG, PTCA, and laser TMR, mechanical methods to create transmural channels and thereby to revascularize the myocardium were reported [10–15]. With the clinical success of laser TMR, there has been renewed interest in mechanical TMR. In laboratory studies, mechanical TMR has been shown to induce an injury to the myocardium, similar to laser TMR [14–17]. In response to this injury, there has been an increase in

angiogenesis. Whether this increased angiogenesis is meaningful and can lead to an improvement in myocardial function has not been demonstrated. We have previously shown that laser TMR results in functional improvement in a large animal model of chronic myocardial ischemia [18]. The purpose of this study was to determine whether mechanical TMR improves ventricular function as compared with laser TMR in chronically ischemic myocardium.

Material and Methods

Animal Model

Animals received humane care as approved by the Center for Experimental Animal Research at Northwestern University and in compliance with the “Guide for the Care and Use of Laboratory Animals” published by the

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Address reprint requests to Dr Horvath, Division of Cardiothoracic Surgery, Northwestern University Medical School, 201 E Huron St, Galter 10-105, Chicago, IL 60611; e-mail: khorvath@nmh.org.

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To accurately mimic the clinical scenario, we employed a standard animal model of chronic myocardial ischemia. For these experiments, the animals underwent three operations over a 12-week period. In the first operation, the ameroid occluder, which slowly constricts the circumflex artery, was placed. Six weeks later, during the second operation, the animals were randomized into five groups. The groups were established according to treatment of the ischemic territory. Group I, treatment with excimer laser; group II, treatment with a hot needle (50°C); group III, treatment with a normothermic needle; group IV, treatment with an ultrasonic needle (40 kHz); and group V, no treatment (control). At the final operation, an additional 6 weeks later, the animals were restudied and sacrificed.

Twenty-seven Yorkshire pigs weighing 12 to 17 kg were anesthetized with Telazol (10 mg/kg), Xylazine (0.25 mg/kg), and Atropine (2 mg) intramuscularly, followed by sodium thiamylal (2.5%, 10 mg/kg) intravenously. After intubation, maintenance anesthesia was maintained with isoflurane (Abbott Laboratories, Chicago, IL). Before exposure of the heart, lidocaine (1 mg/kg) was administered intravenously. The same anesthetic regimen was used for each of the three different surgical procedures that were performed.

Operative Technique

At the initial operation, with sterile technique, the heart was exposed through a small left thoracotomy, and the pericardium was opened. The proximal left circumflex artery was dissected free, and an ameroid constrictor (Research Instruments Manufacturing, Corvallis, OR) with an internal diameter of 2.5 mm was placed in the same location for each animal, specifically around the origin of the left circumflex artery. The pericardium and chest were then closed. The animals were allowed to recover, and were ambulatory before leaving the operating room suite. They were monitored daily by a veterinarian and his staff as well as the surgical team. Adequate food and water were provided and intake as well as weights were measured daily. Antibiotics were administered intramuscularly for 3 days postoperatively. Pain medications were also given intramuscularly until the animals were ambulating without difficulty and exhibiting normal activity levels. At the second operation, through a larger left thoracotomy, the pericardium was reopened and the heart reexposed. Blood pressure was monitored through an arterial catheter placed in the left internal mammary artery. Electrocardiographic monitoring was also used. Rest and dobutamine stress epicardial echocardiographies (7.5 MHz, Model 128; Acuson Inc, Mountain View, CA) were performed to confirm the presence of chronic ischemia in the circumflex distribution and to rule out infarction. In this animal model, dobutamine stress echocardiography provided an assessment of the viability of the myocardium and a method of determining the extent of the ischemia. Dobutamine was administered intravenously starting at 5 $\mu\text{g}/\text{kg}/\text{min}$ and

titrated to a maximum infusion rate of 50 $\mu\text{g}/\text{kg}/\text{min}$ to achieve at least a 50% increase in the resting heart rate. Animals were then randomized into one of the five aforementioned groups. The circumflex territory (ischemic zone) was then treated with TMR. Transmural channels (22 ± 1) were created in a distribution of one channel/ cm^2 in each of the TMR-treated animals.

In control animals, the same procedure was performed except the ischemic zone was not treated. The thoracotomies were then closed and the animals were allowed to recover. The aforementioned postoperative care was then reinstated. At the time of sacrifice, 6 weeks later, the animals had a repeat thoracotomy. At that time, they underwent repeat rest and dobutamine stress echocardiography. The animals were then sacrificed and the hearts were harvested for histologic analysis.

TMR Devices

According to group assignment, transmural channels were created by one of four different methods. Group I had laser TMR. The excimer laser employed (Xenon Chloride 308 nm; Acculase Inc, Carlsbad, CA) had a pulse rate of 240 Hz and an operative energy of 10 mJ/pulse. To minimize the mechanical effects of the quartz laser fiber traversing the myocardium, the fiber was advanced at a rate of 1.5 cm/s. This rate allows the fiber to traverse the myocardium behind the wave of laser ablation. Group II was treated with a hot needle (Hearten Medical, Tustin, CA), which is a thermoelectric device that achieved a temperature of $50^\circ \pm 1^\circ\text{C}$ before manual advancement through the myocardium. Group III involved treatment with a "normothermic needle" consisting of the quartz laser fiber that was used in group I, and that fiber was mechanically advanced through the myocardium without firing the laser. Group IV was treated with an ultrasonic needle (Hearten Medical, Tustin, CA), which was identical to the thermoelectric needle except that the channel was created with ultrasonic (40 KHz) instead of thermal energy. Group V had no treatment of the ischemic area. All devices were of the same 1-mm diameter.

Echocardiographic Analysis

The echocardiographic images were recorded on to a half-inch videotape. End-diastolic and end-systolic images were then digitized off-line from the videotape with a dedicated software package (Prism Lite for Windows, Version 5.14; Tomtec Imaging Systems, Broomfield, CO). The digitized images were spatially calibrated, and the endocardial and epicardial contours were traced. The software then automatically calculated the wall motion along the hundred evenly distributed lines of site around the contour. By standard segmental contraction analysis, the mean wall motion score for each segment was obtained (48 segments for each short-axis image). Segmental contraction was defined as the change in wall thickness between systole and diastole as measured in centimeters. Echocardiographic analysis was performed by an independent observer blinded to the treatment that the animals received. Segmental contraction was compared in all segments at all times using each animal as its

Table 1. Contractility in the Ischemic Zone

	Baseline	Posttreatment
Laser	0.38 ± 0.07 ^a	0.67 ± 0.12 ^b
Hot needle	0.43 ± 0.06 ^a	0.52 ± 0.03 ^c
Control	0.36 ± 0.05 ^a	0.39 ± 0.03 ^c
Normothermic needle	0.46 ± 0.06 ^a	0.35 ± 0.04 ^c
Ultrasonic needle	0.47 ± 0.06 ^a	0.29 ± 0.04 ^b

Myocardial function at baseline and after treatment in the ischemic zone for all groups (cm of wall thickening, mean ± standard error).

^a $p = NS$, by analysis of variance. ^b $p < 0.05$, baseline versus posttreatment by paired t test. ^c $p = NS$, baseline versus posttreatment by paired t test.

own control. As an additional control, the data from the untreated animals were compared with those of the laser and mechanical TMR-treated animals.

Histologic Analysis

After formalin fixation, the sections were imbedded in paraffin and stained with hematoxylin and eosin, Mallory's trichrome, and factor VIII via immunohistochemistry. Sections were also stained so as to detect smooth muscle cell-specific α -actin using the primary antibody 1A4 (Dako Corp, Carpinteria, CA), followed by biotinylated horse anti-mouse IgG, streptavidin-horseradish peroxidase, and 3,3'-diaminobenzide (Vector Laboratories, Burlingame, CA); hematoxylin was used as a counterstain. Blood vessels were identified by positive factor VIII staining and counted at 200 \times magnification. The counts in the ischemic zone were not done at the site of the TMR channels. For evaluation of fibrosis and collagen deposition as a result of laser and mechanical TMR, sections were taken through the actual TMR sites. With a Mallory's trichrome stain, collagen deposition was then assessed on a scale of 0 to 5. No evidence of collagen deposition was scored as a zero, and replacement of the myocardium with dense scar encompassing the entire 200 \times field was scored as a 5. Additionally, after anti- α -actin staining, TMR sites were photographed as nonoverlapping microscopic fields, and an image analysis software package (Image-Pro Plus; Media Cybernetics, Silver Spring, MD) was used to quantify the area (mm²) of positively stained smooth muscle cells. The highest six values for each section were grouped, and are presented as the mean ± standard error. The histologic sections were reviewed by two cardiac pathologists masked to the treatment group.

Statistical Analysis

Continuous data are presented as mean ± standard error. Changes in contractility were assessed using a paired t test for each treatment group. One-way analysis of variance was used to compare differences in angiogenesis, myocardial fibrosis, and change in contractility between treatment groups; between-group comparisons were made using the Tukey method of adjustment for multiple pairwise comparisons. All statistical tests were

two-tailed, and p less than 0.05 was regarded as statistically significant.

Results

Two deaths occurred in group IV (ultrasonic needle) during the second operation. In each case, the ultrasonic needle stimulated an intractable ventricular arrhythmia from which the animal could not be cardioverted. These two animals were excluded from the analysis. Twenty-five animals survived and were randomized into the five groups. There were no significant resting hemodynamic or electrocardiac differences between the animals at the second or third operations. Additionally, all animals underwent the same degree of dobutamine stress at each operation. Mean arterial pressures demonstrated a modest increase with stress, and there was no significant difference between the resting and stress blood pressure measurements for operation 2 versus operation 3.

Echocardiographic measurements of the segmental contraction in the ischemic zone at baseline (operation 2) and posttreatment (operation 3) for the various groups are depicted in Table 1. The segmental contraction after placement of the ameroid constrictor (operation 2) demonstrated hypokinesia of the ischemic zone of the myocardium subtended by the occlusion. There was no change in segmental contraction after ameroid placement in the nonischemic zone. There was no significant difference in the baseline resting function for all animals ($p = NS$), and these measurements are consistent with historical controls [18]. The posttreatment resting function of the ischemic zone showed a wide variation in segmental contraction according to treatment group (Table 1). Expressed as a change in contractility from baseline, the functional results after TMR treatment are depicted in Figure 1. These results show a significant improvement in function for animals treated with laser TMR ($p < 0.01$) and a worsening or no change

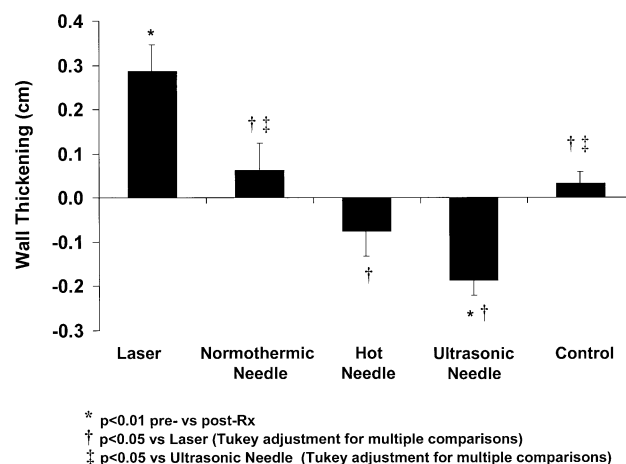


Fig 1. Functional results of treatment of myocardium by various transmyocardial revascularization devices or no device (control). Shown is the change in contractility from baseline to posttreatment as measured echocardiographically.

Table 2. Blood Vessels in the Ischemic Zone

	Blood Vessels
Laser	31 ± 2 ^a
Hot needle	19 ± 2
Normothermic needle	17 ± 1
Ultrasonic needle	14 ± 2
Control	18 ± 2

Blood vessels per 200× hpf in the ischemic zone as detected by factor VIII immunohistochemistry.

^a *p* < 0.05, laser versus all other groups (Tukey adjustment for multiple comparisons).

in function with mechanical TMR (*p* < 0.05 for mechanical vs laser TMR, *p* < 0.05 for ultrasonic needle vs control).

Histologic analysis involved an assessment of angiogenesis based on the number of blood vessels per high-powered field (hpf) as detected by factor VIII immunohistochemistry. This assessment was performed in sections of ischemic tissue that did not include a TMR site. Results of this angiogenic response in the ischemic zone are listed in Tables 2-4. Only laser-treated TMR tissue demonstrated a significant increase in the number of blood vessels/hpf away from the actual TMR site (Table 2). The results from the anti- α -actin staining of arteriole area (mm²) in the ischemic zone for the various groups are recorded in Table 3. Again, only the laser-treated tissue demonstrated a significant difference in the anti- α -actin staining, which is a measure of more mature blood vessels that contain smooth muscle (*p* < 0.05 for laser TMR vs all other groups). Histologic investigation of the TMR sites themselves specifically staining for collagen and fibrosis demonstrated a significant increase in scarring seen after treatment by mechanical TMR but not after laser TMR (*p* < 0.05 for laser TMR vs all other groups) (Table 4). Representative sections of the TMR sites from the ischemic zones of each of the groups are presented in Figure 2. These provide a pictorial demonstration of the results of the Mallory trichrome stain in the first frame, the anti- α -actin stain in the second frame, and the factor VIII staining in the third frame of the series of photomicrographs for each group.

Table 3. Arteriolar Area in the Ischemic Zone

	Arteriolar Area
Laser	2.4 ± 0.4 ^a
Hot needle	0.74 ± 0.1
Normothermic needle	0.63 ± 0.2
Ultrasonic needle	0.60 ± 0.1
Control	0.80 ± 0.2

Arteriolar area in the ischemic zone as measured in mm² after anti- α -actin immunohistochemistry.

^a *p* < 0.05, laser versus all other groups (Tukey adjustment for multiple comparisons).

Table 4. Collagen in the Ischemic Zone

	Collagen
Laser	0.6 ± 0.2 ^a
Normothermic needle	1.4 ± 0.2
Hot needle	2.8 ± 0.4
Ultrasonic needle	3.8 ± 0.2

Collagen in the ischemic zone as detected by Mallory's trichrome staining.

^a *p* < 0.05, laser versus hot needle and ultrasonic needle (Tukey adjustment for multiple comparisons).

Comment

While the exact mechanism whereby laser TMR achieves its clinical effect is yet to be determined, it has generally been accepted that angiogenesis plays a role, if not the primary role, in achieving the clinical benefit. It is also accepted that angiogenesis will occur as part of the healing response to tissue injury. Experimentally, mechanical TMR has been shown to elicit a similar angiogenic response histologically as is seen with laser TMR [15-17, 19]. These studies have shown increase in vascularity in and along the TMR channels. Not surprisingly, there is evidence of increased angiogenic growth factors along the channels as well. The question arises that if mechanical TMR can generate the same angiogenic response, then why is a laser necessary?

The necessity may lie in the method of delivery of energy to the tissue. Simple mechanical or kinetic energy may not have as far-reaching stimulation of angiogenesis as is provided by a laser. Additionally, the injury that occurs must not be so great as to do more harm than good. If the injury destroys tissue such that the resulting scar has new blood vessels but cannot contract, it will be of little value to the patient. Hence, the angiogenesis that arises must be meaningful. In addition to providing new blood vessels that provide an increase in perfusion, functional recovery of the ischemic myocardium should be attainable.

We have employed several different mechanical devices in these experiments to mimic the laser-induced injury. The hot needle and ultrasonic needle used delivered energy that in vitro was similar to the energy from the laser. The normothermic needle or laser fiber was also used, and while not the same as other mechanical devices that have been tested, was the exact same "needle" that was used for the delivery of the laser energy. Therefore, any differences observed between the normothermic needle and the laser-treated animals should be due to the laser energy alone. Previous works [14-17] have used a variety of solid needles, hollow needles, and mechanical drills. Not all of these experiments were done in ischemic models, and none of this work demonstrated a recovery of function after mechanical TMR. We have demonstrated a significant improvement in recovery of function seen with laser TMR that was not seen with mechanical TMR. This recovery of function has been previously demonstrated both experimentally and clinically [18, 20, 21] after laser TMR. The results from the

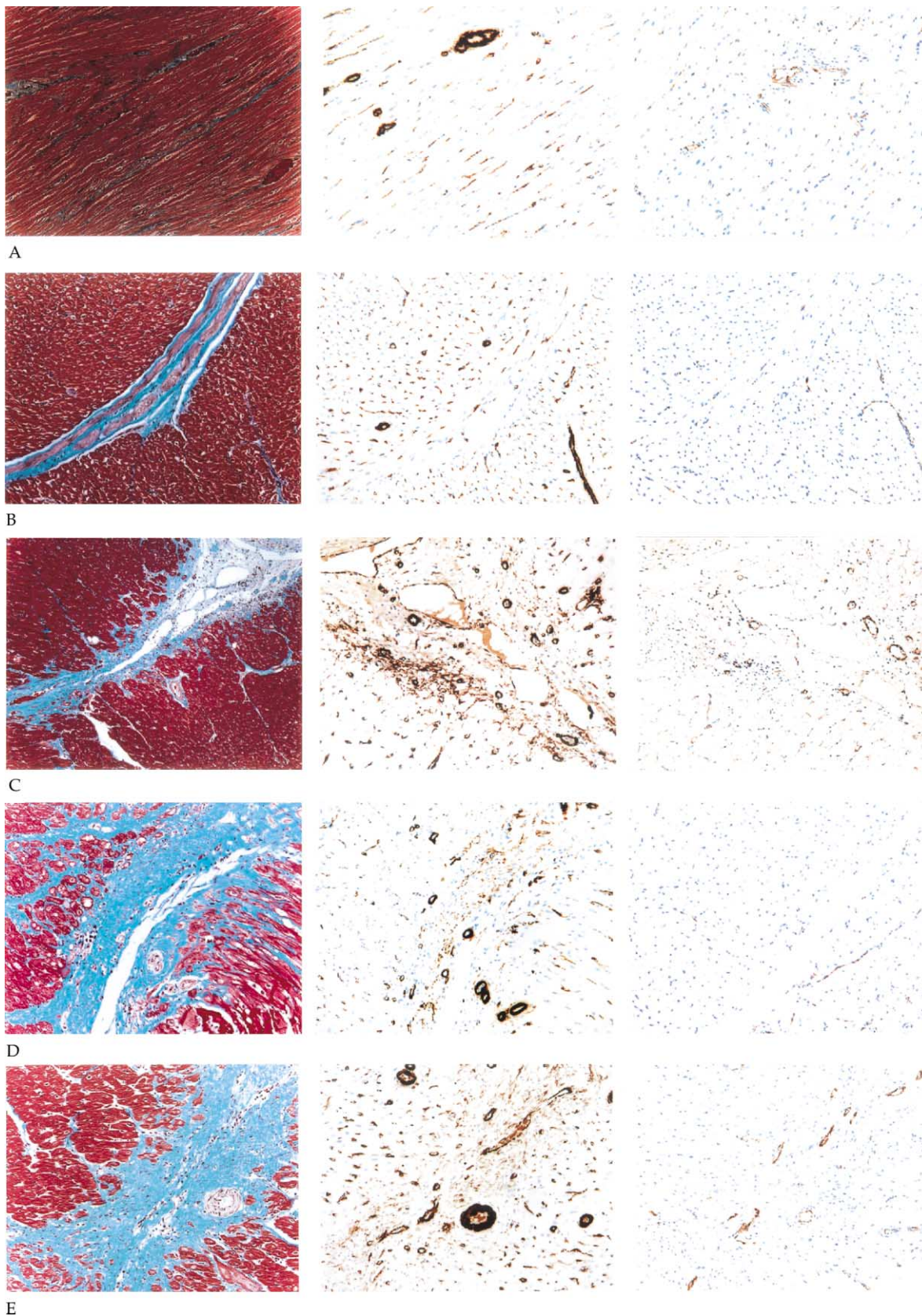


Fig 2. Representative photomicrographs of the ischemic zone from each group (200× magnification). Each series has a section stained for collagen (first frame, Mallory's trichrome), endothelial cells (second frame, factor VIII), and arterioles (third frame, anti- α -actin). (A) Untreated control; (B) normothermic needle; (C) excimer laser; (D) hot needle; (E) ultrasonic needle.

present work demonstrate that this recovery of function may be due to a minimization of scar formation after laser TMR with a maximization of angiogenesis. This increase in angiogenesis was noted only in the laser-treated ischemic zone as detected by factor VIII and anti- α -actin staining. Focusing on the TMR channel itself, the mechanical devices created more fibrosis and collagen deposition than was seen with the laser. This scar formation will obviously affect the contractility of the heart, which is what we observed.

Previous experimental work using an excimer laser has been reported [22]. This previous report demonstrated no significant improvement in perfusion or function after excimer TMR. Although these conclusions are obviously different than those reached in the present study, the studies are significantly different as well. As described, our study was performed to address the issue of whether laser energy is needed or whether a similar response could be obtained with mechanical means. The previous work was a comparative analysis of different lasers [22]. Although an excimer laser was used in both studies, the animal model employed was not the same (a hydraulic occluder that decreases blood flow as opposed to an ameroid constrictor, which creates total occlusion in the artery). The period of follow-up was markedly different (6 months vs 6 weeks). Most importantly, the excimer lasers were not the same. The operating parameters for the lasers were significantly different. For example, the repetition rate was 30 Hz for the previous study and 240 Hz for ours. As demonstrated by our report, mechanical TMR is not very effective, and the excimer laser previously studied was not designed to advance through the myocardium behind a wave of laser energy, and in fact was advanced manually like our mechanical devices through the myocardium. As a result, although an excimer laser was used in both studies, the inability to demonstrate an improvement in function using the aforementioned laser is not surprising based on our findings.

In summary, using devices to create an injury analogous to the laser, mechanical TMR failed to improve the function of chronically ischemic myocardium. Only laser TMR significantly improved myocardial function as a result of meaningful angiogenesis.

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