Biologic Glue Increases Capillary Ingrowth After Cardiomyoplasty in an Ischemic Cardiomyopathy Model

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The authors investigated the multi-step mechanism of healing after cardiomyoplasty, focusing on the process of angiogenesis. The authors contend that enhancement of angiogenesis and prevention of ischemia-reperfusion injuries immediately after muscle mobilization will be effective in improving cardiomyoplasty results. After cardiomyoplasty, autologous biologic glue (ABG) was administered between the latissimus dorsi muscle (LDM) and myocardium. By 2 months, a new pseudo interlayer was present that bridged the gap between the LDM and myocardium. Neovascularization was visible in the form of numerous small capillaries. Marked degeneration of the LDM was noted, possibly caused by muscle ischemia-reperfusion damage after mobilization.

Pockets were created of ischemic and nonischemic LDM to test for angiogenesis. One was left free of ABG (control); one received ABG only; one received ABG with aprotinin; and one received ABG with pyrolostatin. The greatest angiogenesis was seen with ABG and pyrolostatin. Some of the capillaries were large and had erythrocytes inside. Biopsy samples showed 9.4 ± 1.9% of the sample was occupied by blood vessels (compared with 3.6 ± 0.7% in control muscle).

These preliminary studies prove the feasibility of the authors' concept and provide evidence that angiogenesis can accelerate the healing process and provide an organic bridge between the LDM and myocardium after cardiomyoplasty, **ASAIO Journal 1996;42:**M480-M487.

For many years the surgical treatment of end-stage cardiac failure has been limited to the use of temporary mechanical circulatory support devices (as a bridge to transplant) and cardiac transplantation. The discovery that skeletal muscle, if stimulated using a special training protocol, could acquire fatigue resistance has piqued interest in its possible use in cardiac assistance.  

Four approaches have been investigated regarding the use of skeletal muscle in cardiac assist: cardiomyoplasty, in which skeletal muscle is wrapped around the heart; aortomyoplasty, in which skeletal muscle is wrapped around the descending aorta; a skeletal muscle ventricle connected to the circulation as a synchronous or counterpulsation assist; and a mechanical ventricular assist device using skeletal muscle power. However, only dynamic cardiomyoplasty currently is used for surgical treatment of patients with congestive heart failure. Potential benefits of cardiomyoplasty are attributable to the wrap itself, muscle flap stimulation, additional blood supply, or a combination of these factors. Despite remarkable symptomatic improvement, the primary mechanism of beneficial effects remains a matter of controversy, and the full potential of cardiomyoplasty has not been realized.

The first step in cardiomyoplasty, as well as all other forms of skeletal muscle assist, is latissimus dorsi muscle (LDM) mobilization, which leaves the muscle ischemic. Poor histologic structure of the LDM after cardiomyoplasty may result when the ischemic muscle is wrapped around the heart and paced. Under these circumstances, cardiomyoplasty may not provide significant support of cardiac function. Revascularization begins only after 6–8 months, which may be too late to benefit some patients. It is necessary to accelerate this process while simultaneously enhancing capillary ingrowth from the LDM to the myocardium.

It is our contention that enhancement of neovascularization (angiogenesis) and prevention of ischemia-reperfusion injuries immediately after muscle mobilization will be effective in improving cardiomyoplasty results and useful for other types of skeletal muscle powered assistance. We hypothesized that autologous biologic glue (ABG) could be used as a drug depot for reduction of local ischemia-reperfusion lesions and as an interlayer between the stimulated LDM and the mobile myocardium to improve adhesion formation and cardiomyoplasty results.

Methods

The animal studies reported in these investigations conform to the **Guiding Principles Regarding the Care and Use of Animals** of the American Physiological Society and to all federal laws and regulations regarding animal use.

Operative Technique

Animal Preparation. Twelve adult sheep were operated on as described here. Twenty-four hours before surgery, amoxicillin (15 mg/kg IM) was administered to all animals and continued for an additional 5 days after surgery to guard against infection. Sterile technique was followed at all times to reduce the potential for infection. All surgical procedures and biopsies were conducted while the animals were under general anesthesia. Anesthesia was induced with diazepam (5 mg/kg IV) and thiopental sodium (20–25 mg/kg IV). The animals were intubated, placed on a Drager (North American Drager, Telford, PA) ventilator, and maintained on halothane gas anesthesia (1–2% with 40% O₂). Oxygen saturation lev-
els and heart rates were monitored via a pulse oximeter placed on the animal's tongue.

**Dynamic Cardiomyoplasty.** With the animal in the lateral position, a 25 cm cutaneous incision from the left axilla to the costovertebral angle was made at the level of the lateral border of the scapula to the intersection between the iliac crest and paraver tebral muscles. The LDM was dissected free from the iliac crest, vertebral, and inferior scapular angle, and 9th to 12th rib attachments. Dissection was performed carefully, mainly with scissors, to minimize injury to the vascular supply. Collateral blood vessels arising from intercostal arteries were ligated. The muscle flap was then free of its distal attachments with the neurovascular pedicle carefully preserved.

Two intramuscular leads (Medtronic, Inc., Minneapolis, MN) were placed parallel to each other and perpendicular to the thoracodorsal nerve branches according to the Medtronic protocol. The cathode electrode was placed first in the vicinity of the main nerve trunk branches into the muscle. Care was taken that the electrode surface was not exposed on the surface of the muscle and that the distal tip of the electrode was within the muscle. The fixation disk was sutured to the muscle epimysium. This adjustment limited current loss and protected the adjacent tissue from stimulation. The anode was placed approximately 5 cm distally into the muscle, parallel to the cathode. About 4 cm segment of the anterior portion of the second rib was resected, and the LDM flap was transposed into the anterior mediastinal space. The window in the rib was closed carefully.

Cardiomyoplasty was performed through a left anterolateral thoracotomy, with the pericardium widely open. The LDM flap was not sutured to the myocardium; rather, all sutures were placed into the pericardium. The first two sutures were placed close to the left branches of the pulmonary artery and the inferior vena cava. (The LDM was placed without heart elevation.) The apex of the left ventricle was elevated 2–3 cm, and the mid part of the LDM flap was placed under the heart. The heart was immediately returned to its natural position and the electrocardiogram monitored. If there were no severe cardiac dysrhythmias, the next suture was placed as deep as possible at the level of the right ventricle. In four sheep (series 2), ABG was administered between the LDM and the myocardium. In another four sheep (series 1), the space between the LDM and the myocardium was left free of ABG and served as a control. A Medtronic stimulator was implanted subcutaneously in a separate pocket, and the muscle leads were tunneled to the stimulator. All incisions were closed, and the animal was allowed to recover. The Medtronic's protocol for electrical stimulation training was started 2 weeks after cardiomyoplasty with a cardioresynchronization rate of 1:2, an amplitude of 5 V, and a frequency of 10 Hz. Simulation was begun with single impulses for 2 weeks, followed by double impulses for 2 weeks, triple impulses for 2 weeks; finally, 2 months after cardiomyoplasty, trains of impulses (30 Hz; a burst of 6 impulses, and a pulse width of 210 µsec) were synchronized 2 to 1 with the cardiac cycle.

**Skeletal Muscle Pockets.** After the sheep were placed under general anesthesia, they were put in a lateral position, and a longitudinal skin incision was made from the left axilla toward the costovertebral angle. A 6 × 16 cm flap of subcutaneous adipose tissue was dissected free, leaving the lateral part connected. The anterior border of the LDM was completely mobilized. Several vessels originating from intercostal arteries that penetrated the muscle were ligated. Vessels entering the LDM from the spinal posterior, the profound posterior, and the superficial anterior areas were not disturbed. Two distinct sections of LDM were identified: (a) the posterior portion with its undisturbed vascular supply and the anterior portion in an ischemic state. These sections were separated from each other, exposing the anterior flap to even greater ischemia, as compared with LDM typical after normal subtotal mobilization. We were left with three distinct tissue sections: LDM in situ (nonischemic), ischemic LDM, and an adipose tissue flap. The adipose tissue flap was placed on top of the in situ LDM, and the ischemic LDM section was placed on top of the adipose tissue. The three tissue layers were sutured together, forming four double pockets from the “sandwich” of the in situ LDM adipose tissue-ischemic LDM layers. Each pocket measured 3 × 5 cm and consisted of either in situ LDM as nonischemic LDM and adipose tissue. Different compositions of ABG were tested in each of these pockets. One double pocket was left free of ABG and served as a control; the second contained ABG alone, without any other additives; the third contained ABG with aprotinin (an inhibitor of serine proteinases, 1,000 U/ml); and the fourth contained ABG with pyrolostatin (a free radical scavenger, 10 µM). The same type of ABG was inserted into both the in situ and ischemic LDM halves of the double pocket. Biopsy samples were taken from the ischemic and in situ LDM before glue was applied and 3 hr after LDM mobilization. The wound was closed and the animal allowed to recover.

**Formation of Fibrin Interface.**

**Preparation of Autologous Biologic Glue.** Autologous cryoprecipitate was prepared from each animal's citrated blood. Each protein preparation was produced under sterile conditions using a standard procedure (American Association of Blood Banks Technical Manual, 10th Edition, 1990). Blood was centrifuged, and the decanted plasma was frozen. After the plasma was frozen for 48 hr at −80°C, it was thawed at 4°C for 4 hr and centrifuged again. The yellow-white precipitate was collected and stored at −18°C. The resultant cryoprecipitate containing concentrated fibrinogen, Factor XIII, fibrinectin, and vitronectin was reconstituted in 40 ml of phosphate buffer solution (PBS) at a concentration of 10 mg/ml of coagulable protein. We used a thrombin preparation (Johnson & Johnson Patient Care Inc., New Brunswick, NJ) approved by the United States Food and Drug Administration to prepare our fibrin meshwork. This enzymatically active compound was dissolved in PBS (approximately 40 ml) to yield an enzyme concentration of 250 U/ml.

**Application of Autologous Biologic Glue.** When the fibrinogen and thrombin are mixed together, the resultant preparation immediately creates a meshwork of fibrin fibers, known as a fibrin clot. Thus, to apply the ABG to the tissue surface, two separate syringes were filled with thrombin and cryoprecipitate, respectively. An equal amount of each compound was applied to the surface at the same time. Total glue volume ranged from 15–20 ml in each pocket (series 1) to 70–80 ml between the LDM and myocardium (series 3).
Aprotinin (Pentapharm Ltd., Basel, Switzerland) was added to the fibrinogen solution extempore at a concentration of 1,000 U/ml. Pyrrolostatin (Kamiya Biomedical Co., Thousand Oaks, CA) was solubilized in dimethylsulfoxide and added to the thrombin solution extempore at a resultant concentration of 10 μM.

Histologic Studies

Biopsies. Biopsy specimens for light microscopic study and immunohistochemistry were taken from both the ischemic and non-ischemic LDM before pocket creation and at 3 hr after LDM mobilization (day 0), and on days 14, 28, and 56. Samples (3 × 4 mm) were placed in 10% formalin and taken to the hospital's pathology department for embedding and sectioning.

Light Microscopic Study. Samples were taken from all pockets for histologic examination. Transverse sections were made for conventional histologic (hematoxylin and eosin) staining and for subsequent evaluation. Multiple slides were made of each biopsy sample. Histologic data were submitted for interpretation to two independent observers. Particular attention was paid to evidence of muscle regeneration, thickness and composition of the reparative response, and density of neovascularization.

Conventional Indirect Immunoperoxidase Staining (Immunohistochemistry). To assess angiogenesis, conventional indirect immunoperoxidase staining was used after fixation, and proteolytic digestion of formalin-fixed tissue was followed by incubation with a polyclonal rabbit antibody to human von Willebrand factor (VWF, Dakopatts A/S, Denmark). In preliminary experiments we established that this antibody cross-reacted with ovine vWF. This analysis was particularly important because it yielded information about overall angiogenesis and vascularity in the ABG interface and the adjacent skeletal muscle. We evaluated the degree of vascularity by counting the number of vessels per unit area. Photographic enlargements (8 × 10 inches) were made from the light micrographs. Two photocopies of each photo were made and weighed (original weight or OW) after cutting off the waste edges. Large capillaries were removed from the copies with scissors; smaller capillaries were burned off with electric cautery. After all capillaries were excised, the copies were again weighed (cut weight or CW). The percent of capillaries was calculated using the following formula: [(OW − CW)/OW] × 100. The mean value of each photo was calculated from two different copies.

Data Analysis

Histologic data were submitted for interpretation by an independent, blinded observer in the hospital's pathology department. Values are reported as mean plus or minus the standard deviation of the mean, unless stated otherwise. All data were compared using the Student Newman-Keuls' t-test. A p value of less than 0.05 was considered statistically significant.

Results

Dynamic Cardiomyoplasty

Without Autologous Biologic Glue (Series 1). Visual examination of the heart-muscle complex showed that there were gaps between the LDM and the myocardium that prevented organic contact between the tissues, even 12 weeks after cardiomyoplasty. In several places, weak adhesions were detected, which were subsequently analyzed with a microscope. The LDM was atrophic and looked ischemic. The peripheral portion, which had more ischemic damage after mobilization, had changed more than the proximal part and contained many areas of adipose tissue.

Light microscopic analysis showed areas of total necrosis and degeneration in the LDM. The adhesions connecting the LDM and the myocardium contained fibrotic and granulation tissue (Figure 1A). Blood vessels occasionally were noted in the LDM but were absent in the interlayer between the LDM and the myocardium.

With Autologous Biologic Glue (Series 2). When ABG was
applied between the LDM and the myocardium, a new pseudo interlayer was present at 2 months that physically bridged the gap between the myocardium and the LDM. In addition, blood vessels of various diameters were detected in the interlayer during biopsy, and there was considerable bleeding. The flap of LDM appeared nonischemic, with a thickness approximating that of levels before the wrap. Adipose degeneration was significantly reduced.

Light microscopic study showed (Figure 1B) the presence of well vascularized areas in the interlayer and between muscle fibers. There also were nonsignificant zones of necrosis and calcification. Degenerative changes and fibrotic areas were greater in the peripheral part of the LDM but were less than in animals in series 1.

Immunohistochemistry revealed more specific data for evaluating angiogenesis in the interface. At high magnification, neovascularization of the fibrin glue layer is clearly visible in the form of numerous small capillaries lined by endothelial cells. At least some of the newly formed capillaries are functional, as concluded from the presence of erythrocytes (Figure 1C).

Because marked degeneration of the LDM flap was noted in both series, we inferred that this was attributable to ischemia-reperfusion damage after muscle mobilization. To investigate this damage and study its possible prevention, we created the pocket model (series 3). This model also allowed us to investigate the influences of different agents in decreasing or eliminating the damaging effects.

Skeletal Muscle Pockets

Because the LDM pockets were subcutaneous, it was easy to obtain biopsy samples and to visually evaluate muscle condition, the intensity of bleeding during the biopsy, and the durability of the contact between the ischemic and nonischemic parts of the LDM. Data from the ischemic part of the LDM and the interlayer are presented here.

Light Microscopic Study

Control Pocket. Biopsies taken through day 56 produced moderate blood oozing. There were no strong connections between the ischemic LDM flap and the adipose tissue. In biopsies taken 3 hr after muscle mobilization, there was a margination of leukocytes and some interstitial hemorrhaging. This was typical for all pockets at this point after the operation (Figure 2). This phenomenon of margination of leukocytes was present until day 14. In biopsy specimens obtained from days 14 through 56, various stages of necrosis were discernible. The muscle looked damaged and edematous, and some muscle fibers had a wrinkled appearance through day 56. The peak of these changes was noted on day 28 (Figure 3A–C).

Autologous Biologic Glue Only Pocket. There was considerable bleeding from the pocket with only ABG. Strong adhesions had grown through the adipose tissue connecting the ischemic and nonischemic layers of LDM by day 14. Leukocyte margination on day 14 was considerably less than at 3 hr after mobilization. Occasional fiber degeneration also was noted on day 14. Fibrosis and calcified necrosis were less than in control pockets. These changes also were noted in some samples on day 56 (Figure 4A–C).

Figure 2. Light micrograph of an ischemic LDM biopsy taken from the control pocket (without ABG) at 3 hr after LDM mobilization; inset is 2X magnification.

Autologous Biologic Glue With Aprotinin or Pyrolostatin. The same intense bleeding and strong adhesions were present in pockets containing aprotinin and pyrolostatin as were noted in the pocket with only ABG on days 14, 28, and 56. Granulation tissue invaded from the ischemic part of the LDM into the interface (Figure 5A–C). There also were degenerative changes at the interface between the muscle and fat tissue. In some samples, muscle calcification was present, and there were considerable regressive changes. We found no significant morphologic differences between pockets treated with aprotinin and those treated with pyrolostatin.

Immunohistochemistry

Control Pocket. In the control pocket without ABG there were few vessels between the muscle fibers, and partially degenerated areas were present in the ischemic LDM (Figure 6A, B). At day 14, capillaries occupied 3.0 ± 0.9% of the area in the ischemic tissue. At day 28, the fractional area occupied by capillaries was approximately the same (2.9 ± 0.9%) and was only slightly greater at day 56 (3.6 ± 0.7%) (Table 1). There were no statistical differences among any of the days tested. Most capillaries at day 56 were larger than 50 μm in diameter.

Autologous Biologic Glue Only Pocket. When ABG was used, numerous small capillary structures were present (Figure 6C). The percent area occupied by capillaries at day 14 was 4.1 ± 0.4%; at day 28, 5.0 ± 0.2% (p < 0.05 vs day 56);
and at day 56, 5.5 ± 0.2% (Table 1). Most capillaries at day 56 were larger than 50 μm in diameter.

**Autologous Biologic Glue and Aprotinin Pocket.** Intense neovascularization, represented by an increased number of capillaries, was reflected by the presence of large vascular structures (Figure 6D). At day 14, the percent area occupied by capillaries was 5.2 ± 2.1%. This percent increased to 7.8 ± 1.4% at day 28 and to 8.5 ± 1.1% at day 56 (Table 1). No statistical differences were found among the days tested. At day 56, most of the capillaries were larger than 50 μm in diameter.

**Autologous Biologic Glue and Pyrrolostatin Pocket.** The greatest degree of angiogenesis was seen in the pockets with ABG and pyrrolostatin (Figure 6F). By day 14, capillaries occupied 7.9 ± 1.9% of muscle area. This percent area decreased to 7.0 ± 1.4% at day 28 but increased to 9.4 ± 1.9% on day 56 (Table 1). However, no statistical differences were found among the days tested.

**Discussion**

Apart from its effect on systolic augmentation, cardiomyoplasty with a stimulated muscle wrap may have an important girdling effect on the left ventricle, which prevents dilatation and deterioration of left ventricular function.15,16 In preliminary clinical investigations, we saw this effect in patients after dynamic cardiomyoplasty17; however, in our experimental investigations, we noted no durable contact between the LDM and myocardium even 2 months after surgery.18 This lack of physical contact between the tissue layers may be the reason that myocardial revascularization does
not take place quickly enough for patients with ischemic dilative cardiomyopathy, who are most in need of its effects.

To overcome this lack of contact we created a biologic glue-derived interface between the myocardium and the LDM wrapped around the heart. We have implemented the use of a biologic glue interlayer, based on a fibrinogen-thrombin composition, for acceleration of the process of angiogenesis after cardiomyoplasty. We hypothesized that the use of an ABG would serve as a provisional adhesive between the tissues. The glue also may allow the LDM to better conform to the geometric shape of the heart and allow for accelerated therapeutic angiogenesis. We showed in this study that with the administration of ABG, we can create strong adhesions between the myocardium and the LDM and provide the provisional matrix for angiogenesis. Eight weeks after cardiomyoplasty using ABG, blood vessels of various diameters, including developed arteries and veins, were detected in the fibrin layer. We also saw numerous small capillaries lined by endothelial cells, some with erythrocytes inside.

Experimental and clinical data support the usefulness of angiogenic factors in expediting angiogenesis. Surgical implantation of a modified fibrin glue (containing endothelial cell growth factors) between the aorta and the myocardium induced site-directed angiogenesis, resulting in the growth of new blood vessels to the heart, as determined by angiographic and histologic studies. Other investigators have shown that in vivo administration of growth factors enhanced

Figure 6. Immunohistochemical micrographs of ischemic LDM biopsy samples taken on day 56 (A) in the control pocket when peroxidase was used without the primary antibody (as a background control); (B) in the control pocket without ABG (with the primary antibody); (C) in the pocket with ABG only; (D) in the pocket with ABG and aprotinin; and (E) in the pocket with ABG and pyrolostatin.
Table 1. Percent Area of Muscle Occupied by Capillaries

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<th>Day 14 (%)</th>
<th>Day 28 (%)</th>
<th>Day 56 (%)</th>
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<tbody>
<tr>
<td>Control</td>
<td>3.0 ± 0.9</td>
<td>2.9 ± 0.9</td>
<td>3.6 ± 0.7</td>
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<tr>
<td>ABG only</td>
<td>4.1 ± 0.4</td>
<td>3.0 ± 0.2*</td>
<td>3.5 ± 0.2*</td>
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<tr>
<td>ABG + aprotinin</td>
<td>5.2 ± 2.1</td>
<td>7.6 ± 1.4**</td>
<td>8.5 ± 1.1**</td>
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<tr>
<td>ABG + pyrrolasein</td>
<td>7.9 ± 1.9*</td>
<td>7.0 ± 1.4**</td>
<td>9.4 ± 1.9**</td>
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ABG, autologous biologic glue. Values are mean ± SD.
* p < 0.05 versus Control.
** p < 0.05 versus day 56.
† p < 0.05 versus ABG.

endothelial cells seem to be the first cell type to be damaged during ischemia-reperfusion injury. In addition, polymorphonuclear leukocytes bind to endothelium altered by ischemia and extravasate, often causing damage to both the vessel wall and surrounding muscle tissue. Endothelium-leukocyte interactions may explain many of the irreversible reactions associated with the early phases of ischemia-reperfusion injury. Thus, the development of successful therapeutic strategies to reduce reperfusion injury in the LDM is crucial to future vascularization of the myocardium.

If ischemia is unavoidable, the best way to keep ischemic injury to a minimum is to reoxygenate the tissue as soon as possible. Fibrin glue is able to effectively stop bleeding by forming a layer around the traumatized LDM. Because of the importance of endothelial cells as a source of angiogenesis they must be protected. It is necessary to find components that will prevent their injury. We decided to investigate the use of two different compounds to guard against this pathologic process. Aprotinin, a natural inhibitor of serine proteinases, may represent the optimal inhibitor for prevention of proteolytic degradation. Because of its low toxicity, aprotinin has been used for decades as a therapeutic agent to treat diseases caused by dermal slits of the proteinase system. The topical application of aprotinin in the pericardial cavity significantly reduced postoperative blood loss after cardiac surgery. During muscle mobilization, iron is released, which is cytotoxic to vascular endothelium and acts as a catalyst for reactive oxygen metabolites. Pyrrolasein, a scavenger of free radicals, may be useful for prevention of the vascular endothelium's damage. We postulated that aprotinin and pyrrolasein contained in ABG, our drug delivery depot, would decrease LDM ischemia immediately after cardiomyoplasty and increase the LDM's angiogenic potential. Both aprotinin and pyrrolasein seemed to prevent the mobilized muscle from severe ischemia-reperfusion damage and to stimulate greater angiogenesis.

It is our contention that prevention of ischemia-reperfusion injuries immediately after muscle mobilization and enhancement of angiogenesis after cardiomyoplasty would effectively improve the clinical outcome of cardiomyoplasty by protecting existing vascular networks. The development of successful therapeutic strategies to reduce reperfusion injury in the LDM is crucial to future vascularization of the myocardium. In mobilized muscles, the intrinsic degeneration of myofibrils that precedes regeneration is accompanied by a parallel degeneration of blood vessels. Thus, vascular remodeling of mobilized LDM, primary to healing and transformation into a fatigue resistant state, has direct clinical relevance for the full potential of cardiomyoplasty.

References
In Vitro Testing of Endothelial Cell Monolayers Under Dynamic Conditions Inside a Beating Ventricular Prosthesis

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Thromboembolic complications remain a major problem associated with the long-term clinical use of cardiac prostheses. A promising approach toward resolving this predicament is lining the blood contacting surfaces with a functional monolayer of endothelial cells (EC). In developing an endothelialized cardiac prosthesis, the authors in the past focused on establishing a confluent EC monolayer on the luminal surface of ventricular blood sacs. In this study, the au-