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Design of a Miniature Tissue Culture System to Culture Mouse Heart Valves

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Abstract

Valvular heart disease is a leading cause of morbidity and mortality in adults but little is known about the underlying etiology. A better understanding of the genetic and hemodynamic mechanisms involved in growth and remodeling of heart valves during physiological and pathological conditions is needed for a better understanding of valvular heart disease. Here, we report the design of a miniature tissue culture system (MTCS) that allows the culture of mitral valves from perinatal to adult mice. The design of the MTCS is novel in that fine positioning and cannulation can be conducted with hearts of different sizes (perinatal to adult). Perfusion of the heart and hence, culture of the mitral valve in its natural position, occurs in a hydraulically sealed culture bath environment. Using the MTCS, we successfully cultured the mitral valve of adult mouse hearts for 3 days. Histological analysis indicated that the cultured valves remained viable and their extracellular matrix organization was similar to age-matched native valves. Gene expression could also be modified in cultured valves by perfusion with medium containing beta-galactosidase-expressing adenovirus. Thus, the MTCS is a new tool to study the genetic and hemodynamic mechanisms underlying the three-dimensional organization of the heart valves, which could provide insights in the pathology of valvular heart disease and be used in animal models for the development of tissue-engineered heart valves.

Keywords

Mitral valve; Culture; Mouse; Valve disease; Development

INTRODUCTION

Valvular heart disease is a leading cause of morbidity and mortality in adults^{16,18} and is characterized by the disruption of the structural integrity of the leaflets. The highly organized structure of the valve is established by spatiotemporal modulation of gene expression during development and hemodynamic forces acting locally on the leaflets.⁵ The use of genetically modified animal models and *in vitro* explant assays allowed the identification of key-genes and the dissection of several signaling pathways during early valve development. In addition, the importance of hemodynamics on early valve development has been demonstrated.¹⁰ Little

is known, however, on the molecular and hemodynamic mechanisms underlying postnatal maturation of the valves and in the etiology of valvular diseases. In patients with mitral valve prolapse or Marfan syndrome, both linked to genetic mutations,^{7,13} alterations in the mitral valve structure are clinically expressed after birth, suggesting that changes in hemodynamics occurring after birth and during subsequent life might play an important role in the etiology of these mitral valve diseases.

To study the respective impacts of genetic factors and hemodynamics on the postnatal dynamic structure of the mitral valve in mouse requires a system to culture mouse mitral valve from postnatal to adult mice, with intact connections to the heart wall (via anchoring to the annulus fibrosus and papillary muscles), and in the presence of a flow that can be altered experimentally. Current systems do not adequately address these requirements. The well-established culture of mitral valve precursors has proven extremely valuable in unraveling the mechanisms regulating early valve development in avian and mouse models.^{1,2,4-6,12,15} However, in this culture system the structural organization of the valve is not maintained and the effect of hemodynamics cannot be studied. Dissection of the AV ring of postnatal heart to establish a similar culture cannot be considered since it would result in valves missing their most distal part, i.e., their attachment to the papillary muscles of the heart. Perfusions of adult rodent hearts in Langendorff preparations^{14,20} are intended for studies on the myocardium and are not suitable to perfuse the mitral valve. Moreover, available bioreactors used to culture tissue-engineered valves,⁸ or to mechanically test bioprosthetic valves,¹⁹ are designed for aortic valves from porcine or sheep origin.

The purpose of our study was to develop a novel system that allows valves to be cultured in their natural position in the heart in the presence of a circulating culture medium. This objective cannot be achieved with traditional culture methods or systems. Here, we describe the design of a new system, the miniature tissue culture system (MTCS), to culture the mitral valve from perinatal to adult mouse hearts in the presence of various flow speeds. In the MTCS, the mitral valve maintains its natural position in the heart, i.e., with intact points of attachment to the annulus fibrosus and to the papillary muscles, and perfusion with culture medium is controlled by a peristaltic pump. Therefore, the MTCS is a powerful tool to decipher the genetic and hemodynamic contributions in maturation and remodeling of heart valves during physiological and pathological conditions.

MATERIALS AND METHODS

Design of the MTCS

The MTCS was designed using Pro/Engineer Computer Aided Design software (Parametric Technology Corporation, Needham, MA, USA). To culture the mitral valve with intact connections to the annulus fibrosus and supporting apparatus, mouse hearts were cannulated through the pulmonary vein and apex. The cannulation system of the MTCS was designed to have several degrees of freedom in a hydraulically sealed bath environment in order to adjust to the anatomy of the heart and to accommodate hearts of different sizes. The MTCS is composed of a perfusion chamber, which is mounted on a stand and connected to a hydraulic system.

Perfusion Chamber—The perfusion chamber was fabricated from biocompatible polycarbonate with clearances needed to cannulate a range of mouse heart sizes (length of heart at embryonic day (*E*) 18.5 is ~2 mm; at 2 months of age is ~5–6 mm) (Fig. 1). The perfusion chamber consists of a bath area with sections that allow the integration of subcomponents: the rotating stage, ball cannula, cylinder cannula, legs, and is closed by a lid.

The rotating stage, which allows linear motion in Z-direction (Fig. 1), was fabricated from 1" polycarbonate round stock and a ¼"-20 nylon screw, which threads into the chamber. The rotating stage's screw converts the rotating stage's rotary motion into a linear motion, and also locks the rotating stage in place when not turned. Biocompatible grease (Dow Corning High Vacuum Grease, VWR Catalog # 59344-055) is used to seal the chamber. Clearance is provided for the rotating stage with the polycarbonate legs.

The ball cannula (Fig. 1) can rotate in the XY and YZ planes, and therefore be positioned with multiple degrees of freedom (15 degrees from center) within a socket. A clearance hole was drilled into a commercially available ¼" polycarbonate ball which was counterbored, allowing the assembly of a standard luer stub needle (26 gauge) with epoxy. The socket was drilled into the chamber to provide a close tolerance fit, while the ball cannula was snapped into place and could be locked into position with an M3 screw tightened from the top of the chamber. Finally, the socket was sealed with Dow Corning High Vacuum Grease.

The cylinder cannula, which allows linear motion of a needle in the Y-direction, XY-plane (Fig. 1), was fabricated by drilling a clearance hole into a commercially available ¼" polycarbonate round stock cut into size. The cylinder has a clearance hole and was counterbored allowing a standard luer stub needle (23 gauge) to be assembled with epoxy. The cylinder was drilled and tapped, perpendicular to the clearance hole, for an M3 nylon screw used as a guide screw during linear actuation. A shaft was drilled into the chamber providing a running clearance fit for the cylinder cannula. The cylinder presses up against a commercially available ¼" stainless steel spring (McMaster Carr catalog number 1986K2) with a spring constant of 1.57 lbs/in. (274.95 N/m). The chamber was drilled and tapped for an M3×.5 mm lead screw, which moves the cylinder cannula assembly with linear motion by pressing against the guide screw attached to the cylinder. The lead screw both converts the rotary motion of the screw into linear motion of the cylinder cannula, and coupled with the compression spring locks the cylinder cannula in place.

Polycarbonate legs were cut into size from a ¼" round stock and threaded on one end with a die (¼"-20), allowing assembly into their respective locations in the chamber base that are drilled and tapped for ¼"-20 threads and drilled and tapped for an M3 screw on the other end to allow attachment to the stand. These polycarbonate legs allow the horizontal positioning of the perfusion chamber on the platform of a dissecting microscope and the vertical positioning in the stand. This design allows the legs to be removed for easy insertion of the chamber and subcomponents into sterilization bags.

The lid and gasket were designed to seal the bath and provide access to the bath fluid through ports, while being transparent to allow visual inspection of the heart. The lid was fabricated from transparent polycarbonate material with clearance holes for M3×0.5 screws that attach the lid to the chamber. A hydraulic seal is maintained with a gasket fabricated from commonly used gum rubber material (McMaster Carr catalog number 8525T53).

Stand—The perfusion chamber is mounted on a polycarbonate stand by inserting the legs into the clearance holes present in the stand, and screwing them in place with M3 screws. The stand was designed to hold four perfusion chambers upright for simultaneous culture of valves in control and experimental conditions, a peristaltic pump, and bubble traps (Fig. 2).

Hydraulic System—The hydraulic circuit begins with a reservoir (modified 50 mL Falcon tube) containing the culture medium, which connects to a miniature 4-channel peristaltic pump (Instech P625/900.143) that passes the medium through a bubble trap into the perfusion chamber, and then back to the reservoir. The reservoir volume for the 3-day culture experiment was 30 mL in a 50-mL falcon tube. For the adenovirus experiments, a smaller reservoir was

used (1 mL of solution in a modified 2 mL cryogenic vial, Nalgene) to minimize the perfusion volume. Connections were made with platinum-cured silicone tubing (Nalgene) and commercially available tube fittings. The culture medium is oxygenized by bubbling with 95% O₂/5% CO₂ gas.

The flow speed was maintained between 150 and 300 μ L/min. The corresponding shear stress (dynes/cm²) was calculated using the following equation⁵: $\tau = 4\mu Q/\pi r^3$ where μ is the viscosity (dynes*s/cm²), Q the volumetric flow rate (cm³/s), and r the radius (cm), in this case half of the diameter of the orifice of the mitral valve at the level of the annulus fibrosus or free edge. The shear stress was ranging from 0.5 dynes/cm² at the level of anchoring to the fibrous annulus to 38 dynes/cm² at the level of the free edge of the valve. The circulating culture medium allowed renewal of supplies of oxygen and nutrients to the valve tissue.³ The assembled MTCS was designed to fit in a standard 5% CO₂ incubator at 37 °C.

Sterilization and Sterility Tests

The MTCS and all instruments were sterilized in the UMDNJ sterilization facility by low-temperature ethylene oxide (ETO) gas. The culture medium appearance (e.g., clarity) was regularly checked during MTCS operation for signs of contamination. At the end of the culture experiments, the culture media was inspected for fungi and bacteria under an inverted microscope.

Cannulation of Mouse Hearts in the MTCS

All procedures were approved by the Institutional Animal Care and Use Committee of UMDNJ. Hearts from FVB mice were harvested and washed in PBS at room temperature. Using a dissecting microscope the tip of the apex was removed and the pulmonary vein was exposed for cannulation with the cylinder and ball cannula, respectively. Hearts were then transferred in the perfusion chamber filled with BGJb medium (Gibco, Carlsbad, CA, USA) containing antibiotic–antimycotic (Invitrogen, Carlsbad, CA, USA). First, the height of the rotating stage was adjusted, followed by positioning of the ball cannula through the pulmonary vein into the left atrium just above the entry to the left ventricle and its ligation proximal to the left atrium entry. Leakage was prevented through ligation of the left atrial appendage, and the aorta and pulmonary artery. The cylinder cannula was then positioned with linear motion through the apical opening in the left ventricle. The medium was removed and the cylinder cannula was sealed with a biocompatible cyanoacrylate (Loctite, 4014). The whole heart was ligated to the cannulation system. Culture medium was circulated through the mitral valve into the left ventricular chamber with no right side or coronary circulation (Fig. 3). Hearts were checked for leakage by injecting medium through them with the bath still empty. The perfusion chamber was then filled with medium, closed with the gasket and lid, positioned on the stand, and connected to the hydraulic system. The MTCS was placed in a 5% CO₂ incubator and hearts were cultured by perfusing BGJb medium (Gibco, Carlsbad, CA, USA) containing antibiotic–antimycotic (Invitrogen, Carlsbad, CA, USA) through the left side of the heart. There was no circulation introduced outside of the heart. The level of the medium in the perfusion chamber was marked and monitored during the culture to check for leakage in the cannulated heart.

Adenovirus-Mediated Gene Delivery

Medium containing beta-galactosidase-expressing adenovirus (gift from Dr. M. Abdellatif; 5×10^8 pfu/mL) was perfused for 24 h through the left side of the heart. The heart was processed for whole-mount beta-galactosidase staining,⁹ embedded in paraffin, and sectioned (10 μ m). Nuclear fast red was used as counterstaining.

Histology

At the end of the culture, hearts were washed in PBS, fixed overnight in 4% paraformaldehyde/PBS, embedded in paraffin, and sectioned (6 μ m). Cell death was monitored by TUNEL assay using the *In Situ* Cell Death Detection Kit (Roche Applied Science, Indianapolis, IN, USA) and by immunofluorescence using an antibody directed against cleaved caspase-3 ((Asp175) (5A1), Cell Signaling Technology Inc., Danvers, MA, USA). The Tyramide Signal Amplification Biotin System Kit (Perkin-Elmer, Waltham, MA, USA) was used to increase the signal, followed by visualization using Alexa-conjugated streptavidin (Molecular Probes, Carlsbad, CA, USA). To examine the extracellular matrix organization in the valve, immunofluorescence was performed using primary antibodies directed against collagen I (Southern Biotechnology Associates, Birmingham, AL, USA) and versican (AB1033; Chemicon, Millipore, Billerica, MA, USA), followed by Alexa488 (green) or Alexa594 (red) conjugated secondary antibodies (Molecular Probes, Carlsbad, CA, USA).

Statistics

All data are presented as means $\pm$ SE. Statistical significance was determined by calculating a probability value (p) with Student's t -test. Values of $p < 0.05$ were considered to be significant.

RESULTS

MTCS Operation

To test if the MTCS could accommodate hearts of different sizes, hearts from E18.5 to 4-month-old mice were cannulated (Fig. 4). The rotating stage, the ball cannula, and cylinder cannula provided sufficient range of motion to cannulate both the pulmonary vein and apex in all cases. No leakage was observed in the MTCS or through the perfused heart under operating pressures and the pump was able to perfuse the mitral valve. The volume of the closed circuit, excluding the amount in the reservoir, was 1 mL, compatible with the use of minimum amounts of growth factors or adenoviruses during experiments. Microscopy analyses showed no signs of bacteria or fungi growing in the MTCS at the end of the culture, indicating successful sterilization of all the components of the MTCS.

Histological Analysis of Adult Mitral Valve Cultured for 3 Days

Hearts from 8-week-old adult mice ($n = 4$) were cannulated in the MTCS in order to culture the mitral valve for 3 days. This 3-day culture period was determined to be optimal to evaluate the efficacy of the MTCS and culture procedure while studying in parallel the effect of culture on the valve. This culture period also corresponds to protocols previously described for Langerdorff-perfused hearts (1 day)²⁰ and for *ex vivo* study of aortic heart valves (4 days).¹⁹

At the end of the 3-day culture, hearts were processed for histological analysis and compared to age-matched hearts that were not cultured (control). The cultured and control hearts were harvested at the same time from a group of aged-matched mice. Control hearts were immediately fixed, whereas the cultured hearts were cannulated in the MTCS and cultured for 3 days. Apoptosis was assessed by immunostaining for the cleaved form of caspase 3 and by TUNEL staining. No statistical difference was found between the percentage of apoptotic cells in the valves of cultured hearts (cCasp3: $0.61 \pm 0.17\%$, total counted nuclei was 8678, $n = 4$; TUNEL: $0.39 \pm 0.17\%$, total counted nuclei was 9704, $n = 4$;) and control (non-cultured) age-matched hearts (cCasp3: $0.02 \pm 0.02\%$, total counted nuclei was 7197, $n = 3$; TUNEL: $0.23 \pm 0.24\%$, total counted nuclei was 6442, $n = 3$). In the cultured valves, the apoptotic cells that were found were single or clustered at the atrial side of the leaflets (Fig. 5a). This suggests that the presence of these apoptotic cells is not due to lack of oxygen or nutrients, in which case widespread apoptosis would have been expected, but is a sign of adjustment of the valve to

different hemodynamic conditions. In contrast, when whole postnatal hearts were cultured in a Petri dish, i.e., without the MTCS perfusion system described in this study, massive cell death occurred ($49.72 \pm 1.52\%$, total counted nuclei was 1372, $n = 3$), which is statistically different ($p < 0.05$) from the percentage of apoptotic cells in the valves of cultured and non-cultured age-matched hearts.

In order to evaluate the whole valvular structure of both native control and cultured valves, 6- μm thick serial sections were generated through the entire valvular region. One out of every ten sections was observed under the microscope and no noticeable morphological difference could be detected between native and cultured valves. Figure 6 shows representative sections at the level of the nodular thickenings. The structure of the valves was also analyzed by examining the expression of the extracellular matrix components collagen I and versican, which are predominantly found at the ventricular and atrial side of the leaflets, respectively. ¹¹ Collagen I and versican expression patterns were similar in the cultured (Fig. 6a) and native (Fig. 6b) mitral valves. Therefore, valves cultured in the MTCS for 3 days stay alive and maintain their characteristic pattern of extracellular matrix expression.

Adenovirus-Mediated Gene Transfer

We tested if adenovirus-mediated gene transfer could be achieved in cultured mitral valve. For this experiment, hearts were harvested from a group of mice (5 days old), which were independent of those used for the 3-day culture experiment. The hearts were perfused in the MTCS for 24 h with medium containing beta-galactosidase-expressing adenovirus and processed for histological analysis. LacZ staining was detected in the endothelial cells aligning the atrial surface of the valve, which are the cells directly in contact with the inflowing medium, and in underlying mesenchymal cells (Fig. 7).

DISCUSSION

We describe the design of a unique system, the MTCS, that allows the culture of the mitral valve from perinatal to adult mice in the presence of a continuous flow, which can be experimentally modified, in a closed circuit. We showed that the mitral valve maintained its characteristic pattern of extracellular matrix over a 3-day culture period and that gene expression could be altered efficiently by adenoviruses added to the perfusion medium.

The design of the MTCS met several requirements. First, the valve had to be cultured with intact connections to the annulus fibrosus and supporting apparatus. For this, whole mouse hearts were cannulated through the pulmonary vein and the apex of the left ventricle, allowing perfusion of the mitral valve while sitting in its natural position in the heart. This simulates the natural filling of the left ventricle through the mitral valve, which cannot be achieved by introducing flow through the aortic valve in existing Langendorff setups. Second, the perfusion system had to accommodate hearts of different sizes, from ~2 mm for E18.5 mouse heart to at least 6 mm for an adult mouse heart. For this, the rotating stage allowed Z translation in the YZ-plane and thus positioning of hearts in front of the cannulas (Fig. 1). The cylinder cannula allowed linear motion of a needle in the Y-direction, XY-plane, adjusting its position to the size of the heart. The ball cannula allows the entry of the needles into the pulmonary vein with different angular degrees. Third, the perfusion system should allow manipulation of the tissue under a dissecting microscope and culture in an incubator. The use of the polycarbonate legs allowed the horizontal positioning of the perfusion chamber on the platform of a dissecting microscope and vertical positioning on a stand placed in the incubator. Four, the system had to be hydraulically sealed. This was achieved by close tolerance fits of the ball cannula and the screw of the rotating stage, a rubber stopper at the cylinder cannula, and the lid and gasket. Five, the system had to be sterile. A sterile environment was provided by ETO gas sterilization of the different components of the perfusion chamber and instruments, dissection of the hearts,

and cannulation under a hood with laminar flow and with the addition of antibiotic–antimycotic solution to the medium. Six, the system should permit the use of small volume of perfusion to allow the use of growth factors or adenoviruses in the medium. The volume of the closed hydraulic circuit, excluding the reservoir, was 1 mL. Finally, the stand was designed to hold four perfusion chambers, which allows the simultaneous culture of hearts in control and experimental conditions.

Side-by-side histological analysis of cultured and native mitral valves showed similar collagen and versican distribution (Fig. 6). This distribution follows a ventricular–atrial axis, with the stress-resistant collagen I located at the ventricular side providing strength to the valve, and versican localized at the atrial side providing cushioning.¹¹ In addition, gene expression could be manipulated using adenovirus-mediated gene transfer (Fig. 7), which holds great promise for the use of the MTCS to study the role of genetic factors in valvular structure.

Some limitations are inherent to the design of the MTCS. The placement of the outflow cannula is through the apex of the heart, which is not the natural outlet for the left ventricle. Notice, however, that in this configuration, the flow through the mitral valve mimics the flow through the mitral valve during the filling of the left ventricle. Another limitation is that we did not maintain a beating myocardium, and therefore the cultured valves do not close (they remain open). As a consequence, the mechanical forces acting on the cultured valve are different than in the native valve, which might be reflected in the presence of a few apoptotic cells at the atrial side of the cultured valves (Fig. 5), although no significant difference was found in the percentage of apoptotic cells in the valves of cultured and non-cultured age-matched hearts. In addition, although the cultured mitral valve remained viable for 3 days in the MTCS, the surrounding myocardium died after 3 days of culture due to lack of coronary circulation.

Taken all together, the objective of culturing viable heart valves was achieved by developing a novel system that allows valves to be cultured in their natural position in the heart in the presence of a circulating culture medium. There are no limiting factors to the fixturing and perfusion equipment that would prevent investigators from culturing for periods greater than 3 days. Therefore, the MTCS can be used in the future to explore the effects of culture time and flow rate on valve growth and development.

The MTCS has, however, several other potential applications. The lack of contraction of the myocardium gives the opportunity to study the role of non-pulsatile flow in valve remodeling, because the valve does not change position and is, therefore, subjected to a continuous unidirectional flow. In addition, the effects of pulsatile flow on the mitral valve could be studied by using a pulsatile pump. A reversing flow could be generated by configuring the peristaltic pump with an electrical circuit to change the direction of the flow cyclically, but could cause several technical difficulties, such as tearing of valvular tissue by the reversing flow. Furthermore, the effect of flow could also be analyzed on the mitral valves from genetically modified mice. The aortic valve, instead of the mitral valve, could be cultured by cannulating the apex (inflow) and aorta (outflow). The system could be reconfigured to serve as a culturing system for whole heart studies (modified Langendorff's setup) through the cannulation of the aorta with the cylinder cannula (inflow) and the generation of an outflow by replacing the ball cannula with a stopper and a luer stub needle. Moreover, flow could be introduced outside of the heart; through the lid ports (B) described in Fig. 2. Other miniature tissues could also be used in the MTCS, such as small rodent tissues including arteries, veins, kidneys, stomach, and intestines.

In conclusion, we designed a new culture system, the MTCS, to culture the mitral valve from perinatal to adult mice in the presence of a continuous flow, which can be experimentally modified. In future applications, the MTCS could contribute to a better understanding of the

genetic and hemodynamic mechanisms involved in maturation and remodeling of heart valves during physiological and pathological conditions. This would provide insights in the pathology of valve diseases and contribute to development of tissue-engineered heart valves. Replacing a diseased valve with a tissue-engineered valve would have a significant clinical impact not only on adults, but especially on the pediatric population.^{16–18}

ABBREVIATIONS

MTCS	Miniature tissue culture system
E	Embryonic day
ETO	Ethylene oxide
IVS	Interventricular septum
MiVa	Mitral valve
LV	Left ventricle

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FIGURE 1.

The MTCS perfusion chamber and its subcomponents. (a) Photograph of the assembled perfusion chamber and rotating stage, ball cannula, and cylinder cannula. Scale bar: 20 mm. (b) Top (XY-plane) and (c) side cross-section (YZ-plane) drawings of the perfusion chamber. Hearts of different sizes can be positioned for cannulation by using the rotating stage, which allows Z translation (arrow a) in the YZ-plane of the perfusion chamber. The ball cannula can be positioned (arrow b) with multiple degrees of freedom (15 degrees from center) within the socket drilled into the perfusion chamber and the cylinder cannula provides linear motion through a spring-loaded shaft drilled into the perfusion chamber to allow the translation of a needle in the Y-direction (arrow c).

FIGURE 2.

The MTCS in operation. Photograph of four MTCS chambers attached to a stand. The perfusion chambers (A) are filled with culture medium and sealed with the gasket/transparent polycarbonate lid assembly, which has fluid ports (B) to provide access to the bath. The MTCS chambers are mounted onto a polycarbonate stand (C) allowing perfusion of the hearts in their natural upright position. The hydraulic circuit begins with a reservoir which connects to a peristaltic pump (D) that pumps the medium through a bubble trap (E) before entering in the MTCS chambers (A).

FIGURE 3.

Schematic representation of the heart ligation and perfusion in the MTCS. Front and back views are shown of the ligated heart. The ball cannula (BC) is positioned through the pulmonary vein into the left atrium (LA) just above the entry to the left ventricle (LV) and ligated proximal to the left atrial entry. The cylinder cannula (CC) is positioned through the apical opening in the left ventricle (LV) and sealed with a biocompatible cyanoacrylate. Leakage was prevented through ligation of the left atrial appendage, and the aorta and pulmonary artery. RA, right atrium; RV, right ventricle.

FIGURE 4.

Mouse hearts of different developmental stages can be cannulated in the MTCS. The hearts of (a) embryonic (E18.5), (b) 12-day-old, and (c) adult (8 weeks old) mice were positioned in the perfusion chamber with the rotating stage, and cannulated through the pulmonary vein and apex with the ball cannula and cylinder cannula, respectively. Scale bar = 1 mm.

FIGURE 5.

Cultured mitral valves remain viable. Representative sections of 8-week-old mouse hearts that were cultured for 3 days in the MTCS (a) or fixed immediately at harvest (b), and processed for immunostaining for cleaved caspase 3 expression. Arrows indicate positive staining. IVS, interventricular septum; MiVa, mitral valve; LV, left ventricle. Scale bar = 100 μ m.

FIGURE 6.

Cultured mitral valves maintain collagen I and versican organization. Representative sections of 8-week-old mouse hearts cultured for 3 days (a) or fixed immediately at harvest (b) and processed for immunostaining for collagen I (green) and versican (red) expression. IVS, interventricular septum; MiVa, mitral valve; LV, left ventricle. Scale bar = 100 μ m.

FIGURE 7.

Adenovirus-mediated gene transfer in cultured mitral valves. Representative sections of 5-day-old mouse hearts that were cultured for 24 h in the presence of adenovirus harboring a CMV-beta-galactosidase construct and processed for beta-galactosidase staining. Serial sections were counterstained with nuclear fast red. MiVa, mitral valve; LV, left ventricle. Scale bar = 100 μ m.