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五條理志，許俊鋭	(シリーズ)再生医学のいま-基礎研究から臨床への展開に向けて-44-細胞移植：心臓病における臨床応用への潮流	治療	92 (12)	2793 ～ 2799	2010
五條理志，豊田雅士，梅澤明弘	組織工学と再生医療	人工臓器	39 (3)	202 ～ 207	2010
五條理志，上大介	(特集)臨床工学技士が知っておきたい人工臓器と再生医療の展望：心臓における再生医療	Clinical Engineering	22 (1)	9～14	2011

五條理志，許俊鋭	心不全治療の新展開-外科医との共働による新たな治療戦略 心不全に対する再生医療　幹細胞移植・細胞シート	内科	108	107 ～ 111	2011
五條理志	【血管・心筋再生はどこまできたか】治す　重症心不全に対する再生治療　心臓移植・人工心臓患者に心臓再生医療をどう活かすか	Heart View	15	854 ～ 859	2011

III. 研究成果の刊行物・別刷

Artificial
Organs

Artificial Organs
34(4):280–288, Wiley Periodicals, Inc.

Serum-independent Cardiomyogenic Transdifferentiation in Human Endometrium-derived Mesenchymal Cells

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Abstract: Media with high concentrations of serum are commonly used to induce cardiomyogenic transdifferentiation in mesenchymal stem cells; however, serum contains numerous unknown growth factors and interferes with definition of specific cardiomyogenic transdifferentiation factors secreted from feeder cells. In the present study, we determined whether the transdifferentiation of human mesenchymal cells can be observed in a FBS-free medium. The efficiency of transdifferentiation was observed in 10% FBS-containing standard medium (10%FBS) and in FBS-free medium containing insulin and thyroxin (FBS-free). In the present study, we used human uterine endometrium-derived mesenchymal cells (EMC100, EMC214) and menstrual blood-derived mesenchymal cells (MMCs). After cardiomyogenic transdifferentiation, the efficiency and physiological properties of cardiomyogenesis (fractional shortening of the cell [%FS] and action potential [AP]) were evaluated. The efficiency of transdifferentiation in EMC100 and in MMCs increased 36%* and 163%* (*$P < 0.05$), respectively. The %FS in EMCs increased to 103%*. AP-duration more than 250 ms with a marked plateau was only observed in FBS-free (3/19), and not in 10% FBS (0/41). The cardiomyogenic transdifferentiation of human mesenchymal cells can be observed in the FBS-free medium. Phenotypes of generated cardiomyocytes were significantly more physiological in FBS-free than in 10% FBS. **Key Words:** Cardiomyogenesis—Human mesenchymal stem cells—Serum free—Assay system—Cardiomyogenic factors.

Many types of stem cells, for example, embryonic stem cells (1,2), marrow-derived mesenchymal stem cells (MSCs) (3–5), circulating endothelial progenitor cells (6), and cardiac residential precursor cells (7,8), etc., have been shown to transdifferentiate into cardiomyocytes in vitro; therefore, such cells are good candidates for cardiac stem cell therapy. However, only the marrow-derived MSCs (9), hematopoietic stem cells (10–12), and skeletal myoblasts (13) have been used for clinical patients at the present time, since these cells can be obtained in an autologous manner and there is no ethical problem. The effects of this stem cell therapy are still limited, and among these stem cells, only the marrow-derived MSCs are known to cause cardiomyogenic transdifferentiation in vitro. The cardiomyogenic transdifferentiation efficiency of the MSCs was limited in vitro; therefore, we tentatively conclude that the observable effect of the MSC transplantation in clinical patients is due either to grafted MSC-induced neovascularization (3,14) or the paracrine effect (15) on the residual host myocardium. To further improve the efficacy of cardiac stem cell-based therapy, cardiomyogenesis from engrafted stem cells is essential.

The precise mechanisms for cardiomyogenesis from the human MSCs are still unclear. Nonspecific

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demethylation of DNA by 5-azacitidine initiated cardiomyogenic transdifferentiation of murine marrow-derived MSCs (16); however, in human MSCs, it failed to cause cardiomyogenesis (17). For human MSCs, environmental factors are believed to play a pivotal role in cardiomyogenesis, since co-cultivation with murine cardiomyocytes (feeders) is essential in vitro. Nevertheless, specific cardiomyogenic differentiation factors are difficult to determine, because environmental factors are complex and numerous. Previously, we have reported that the human uterine endometrial gland-derived mesenchymal cells (EMCs) or menstrual blood-derived mesenchymal cells (MMCs) have an extremely high cardiomyogenic transdifferentiation efficiency (76–97% and 27–32%, respectively) (18) compared to the marrow-derived MSCs (0.3%). This also suggests that our feeder secretes significant cardiomyogenic transdifferentiation factors for human mesenchymal cells.

Serum-containing media are commonly used for the culture procedures, and the serum may be one of the major environmental factors for cardiomyogenesis. The serum contains numerous growth factors, some of which are unknown. Therefore, it is very difficult to determine feeder-derived specific cardiomyogenic transdifferentiation factors by use of serum-containing media. In the present study, we showed cardiomyogenic transdifferentiation in our in vitro cardiomyogenic transdifferentiation assay system with a chemically defined FBS-free medium. Finally, we established a FBS-free in vitro cardiomyogenic transdifferentiation system to detect feeder-derived humoral factors for cardiomyogenesis of human mesenchymal cells.

MATERIALS AND METHODS

Cardiomyogenic induction

Fetal cardiomyocytes were obtained from the hearts of day 17 mouse fetuses, as described previously (17). Cultured cardiomyocytes were plated at $5 \times 10^4/cm^2$ on the culture dish as feeders for the co-culture system. In the control experiment, the obtained cardiomyocytes were suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, then plated on the 1% gelatin-coated dishes. In the test experiment, we used a FBS-free medium, which was designated as ACCITT medium (19): M199 medium containing 5 mM creatine, 2 mM L-carnitine, 5 mM taurine, 0.1 nM thyroxin (T3), 0.1 μM insulin, 2.5 mM pyruvate, and 2 mg/mL heat-treated bovine serum albumin (Fraction-V). In order to negate the possible contamination of serum, cells were washed with PBS three times after collection. In the FBS-free medium, cells attached to the gelatin-coated dishes were rare; therefore, a laminin-coated dish was used for the experiment. Human EMCs and MMCs were obtained as described previously (18). In short, individual human endometrial glands were isolated under a microscope and then seeded. After the retroviral transfection of HPV16E6, E7, and hTERT, endometrial cell strains of EMC100 and EMC214 were generated by the limiting-dilution methods. MMCs were established by primary culture of human menstrual blood. EGFP-labeled (17) EMCs and MMCs were plated on the feeders at $3 \times 10^3/cm^2$.

Immunocytochemistry

The samples were stained with mouse monoclonal anticardiac troponin-I antibody (# 4T21 Lot 98/10-T21-C2, HyTest, Turku, Finland) diluted 1:300, or monoclonal anti-α actinin antibody (Sigma, St. Louis, MO, USA) and anti-connexin 43 antibody diluted 1:300. For nuclei staining, 4′-6-diamidino-2-phenylindole (DAPI) (Molecular Probes, Eugene, OR, USA) was used. Tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse IgG (Sigma), TRITC-conjugated goat antirabbit IgG (Sigma), and Cy5-conjugated goat antimouse IgG (Chemicon, Temecula, CA, USA) were used as secondary antibodies (20). A laser confocal microscope (FV1000, Olympus, Tokyo, Japan) was used for immunocytochemical analysis (18,20,21).

Calculation of induction rate

EMCs and MMCs were dissociated 1 week after cardiomyogenic induction by 1% trypsin and 0.25 mM EDTA for 5 min at 37°C. These cells were then dissociated by 0.5% collagenase type-II (Worthington Biochemical, Lakewood, NJ, USA) and 10 mM 2,3-butanedione monoxime (BDM) (Sigma) for 20–40 min at 37°C. After centrifugation (1000 rpm, 5 min), the EMCs and MMCs were seeded on the poly-L-lysine-coated dishes for 2–3 h at room temperature. The dishes were fixed and immunocytochemically stained by the anti-troponin-I antibody (16 h, 4°C), then observed by the laser confocal microscope. The cardiomyogenic induction rate was calculated as the fraction of human cardiac troponin-I positive cells in the EGFP-positive cells. The rate was calculated as the average of five separate experiments. The calculations were done by analyzing photomicrographs recorded under the same conditions (18,20,21).

Functional analysis of transdifferentiated cardiomyocytes

Seven days after the cardiomyogenic induction, action potential (AP) was measured by use of standard glass microelectrodes, as described previously (17,18,20,21). Immediately after the recording, Alexa 568 was injected into the cell via recording microelectrodes to confirm that the recording AP was obtained from EGFP-positive human cells. Since thyroxin may affect the AP morphology, the medium was exchanged for a thyroxin-free medium 2 days before the AP recording. The extent of dye transfer was monitored under a fluorescent microscope, and digital images were recorded with a digital camera.

The fluorescent image of spontaneously beating EMC-derived cardiomyocytes was monitored using a CCD camera and was stored as digital video. The images of EMCs at day 7, 14, and 21 of co-culture in 10% FBS and FBS-free medium were recorded; then the cell contraction was analyzed using an image edge detection program made by Igor Pro 4 (Wavemetrics, Inc., Lake Oswego, OR, USA).

RNA extraction and reverse transcriptase (RT)-PCR

RT-PCR was done as described previously (17,18,20,21). Primers for the following genes were used: cardiac transcription factors—Csx/Nkx-2.5; cardiac structural proteins—cardiac troponin-I (cTnI) and cardiac troponin-T (cTnT). The internal control was 18s rRNA. PCR primers were prepared such that they would amplify the human but not the mouse genes.

Statistical analysis

Data were expressed as the mean ± SE. The difference between the two groups was determined with Student's *t*-test. Statistical significance was set at $P < 0.05$.

RESULTS

EGFP-labeled EMC100s, EMC214s, and MMCs cultured in either 10% FBS or FBS free started beating 3 days after the cardiomyogenic induction.

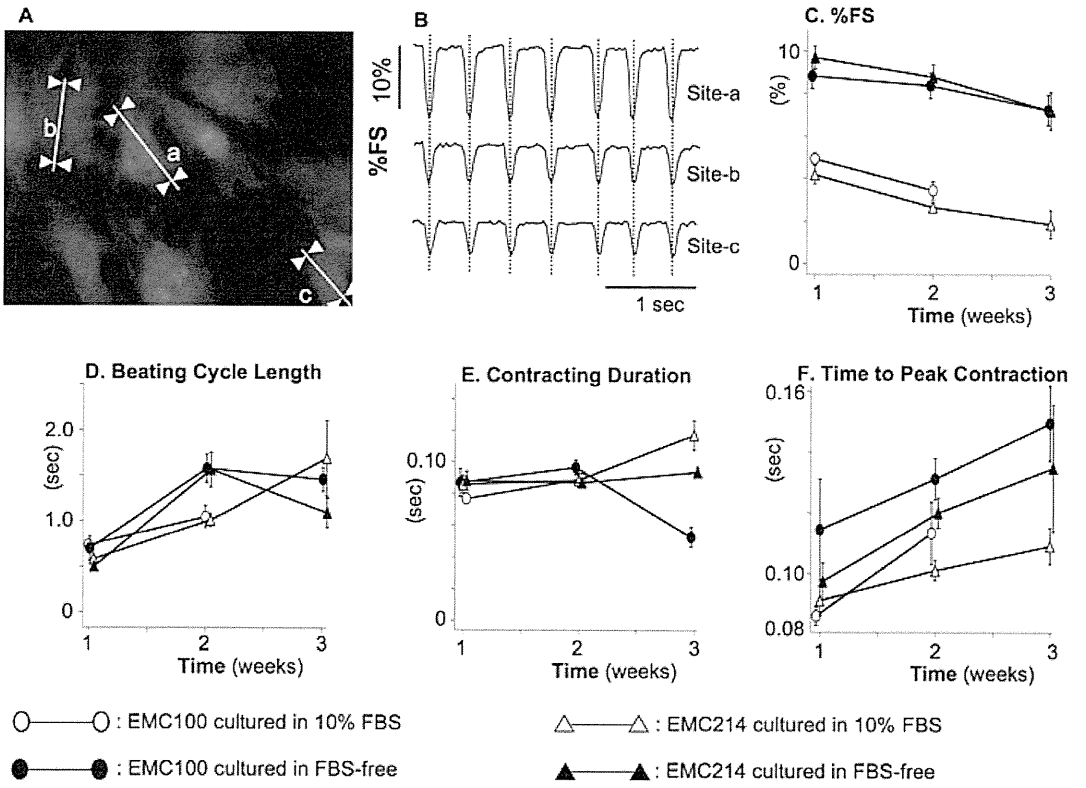


FIG. 1. Contraction parameter of EMC100 and EMC214-derived cardiomyocytes. (A) A representative fluorescent microscopic image of EMC-derived cardiomyocytes and detected fractional shortening (%FS) along the white line obtained from sites a, b, c are shown in (B). (C–F) Calculated contraction parameters are averaged as a function of time after cardiomyogenic induction. %FS cultured in FBS-free ACCITT are significantly greater than %FS cultured in DMEM with 10% FBS.

The EGFP-labeled human cells beat strongly in a synchronized manner on day 5 and continued to contract at least until day 21.

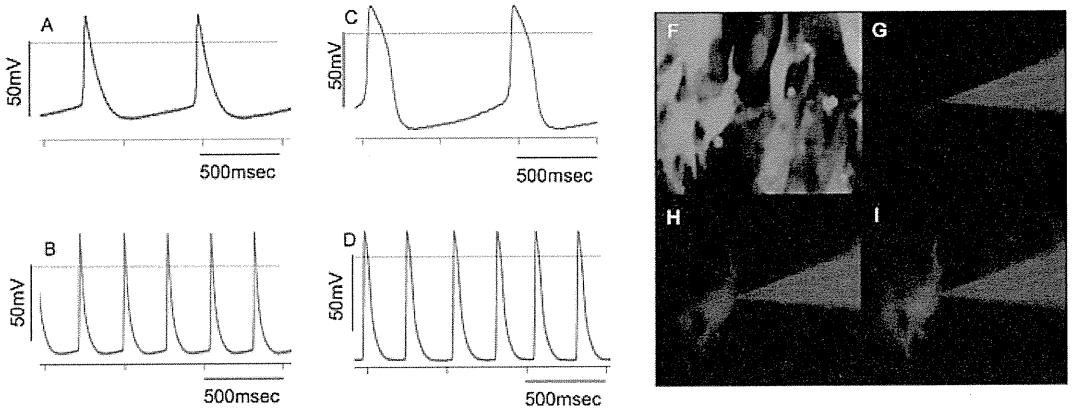
Functional analysis of differentiated EMCs

We analyzed the parameters of cell contraction using an image edge detection program. Edges were selected along the long axis of the EGFP-positive cells (Fig. 1A). The recorded contraction pattern showed synchronous beating of the cells (Fig. 1B). %FS of EMCs cultured in FBS-free medium was larger than that of EMCs cultured in 10% FBS medium. This difference was statistically significant and lasted until day 21 of co-culture. Other parameters—beating cycle length, contracting duration, and time to peak contraction—were not statistically significant, except contraction duration of EMC214s on day 21 of co-culture, which was observed to be significantly longer in FBS-free medium (Fig. 1C–F).

APs from 60 spontaneously beating EGFP-positive EMC214s were obtained. There was no difference in the observed AP morphological category, i.e., sinus node-like and working cardiomyocyte-like AP, between the 10% FBS and FBS-free medium (Fig. 2A–D). However, the maximum diastolic potential was significantly hyperpolarized, and the amplitude was greater in the FBS-free medium than in 10% FBS medium (Fig. 2E). AP duration more than 250 ms was only observed in FBS-free medium (0/41 vs. 3/19). The Alexa 568 dye spread into the GFP-positive adjacent EMCs, while not diffusing into the GFP-negative murine cardiomyocytes, suggesting there was no heterologous cell-to-cell gap connection, but that there was a homologus connection among EMCs in vitro (Fig. 2F–I).

FBS-free culture increased the cardiomyogenic transdifferentiation rate of EMCs and MMCs

The fraction of human cardiac troponin-I positive cells among the EGFP-positive cells was defined as the cardiomyogenic transdifferentiation rate (Fig. 3A–C). While EMCs and MMCs without co-culture did not show any troponin-I expression, EMCs and MMCs became positive for human cardiac troponin-I antibody as a result of the co-culture. The efficiency of cardiomyogenic transdifferentiation of EMC100s and MMCs was significantly increased (36 and 163%, respectively) as the result of cultivation



E

	Amplitude (mV)	MDP (mV)	APD90 (msec)	Vmax (V/sec)	n
10% FBS	66.5 ± 1.4	-53.1 ± 0.9	159.9 ± 4.9	9.46 ± 0.02	41
FBS-free	74.5 ± 2.6*	-57.3 ± 1.1*	179.6 ± 19.5	10.71 ± 1.31	19

DMP : Maximum Diastolic Potential
APD : Action Potential Duration
Mean ± SE

FIG. 2. Electrophysiological parameter of EMC214-derived cardiomyocytes. (A–D) A representative action potential (AP) shape of EMC214-derived cardiomyocytes. AP cultured by DMEM with 10% FBS showed triangular action potential shapes in AP with marked pacemaker-like potential (A) and without pacemaker-like potential (B). On the other hand, AP cultured with FBS-free ACCITT medium showed a prominent plateau in AP with pacemaker-like potential (C) and without it (D). (F) EGFP-labeled EMCs (green) were injected with Alexa 568 solution (red). (G–I) through a microelectrode to confirm that the recorded signal was obtained from EMCs. G, H, and I are sequential images as a function of time after dye transfer (0, 2, and 5 min after the dye transfer, respectively). Measured action potential parameters are averaged and shown in panel E.

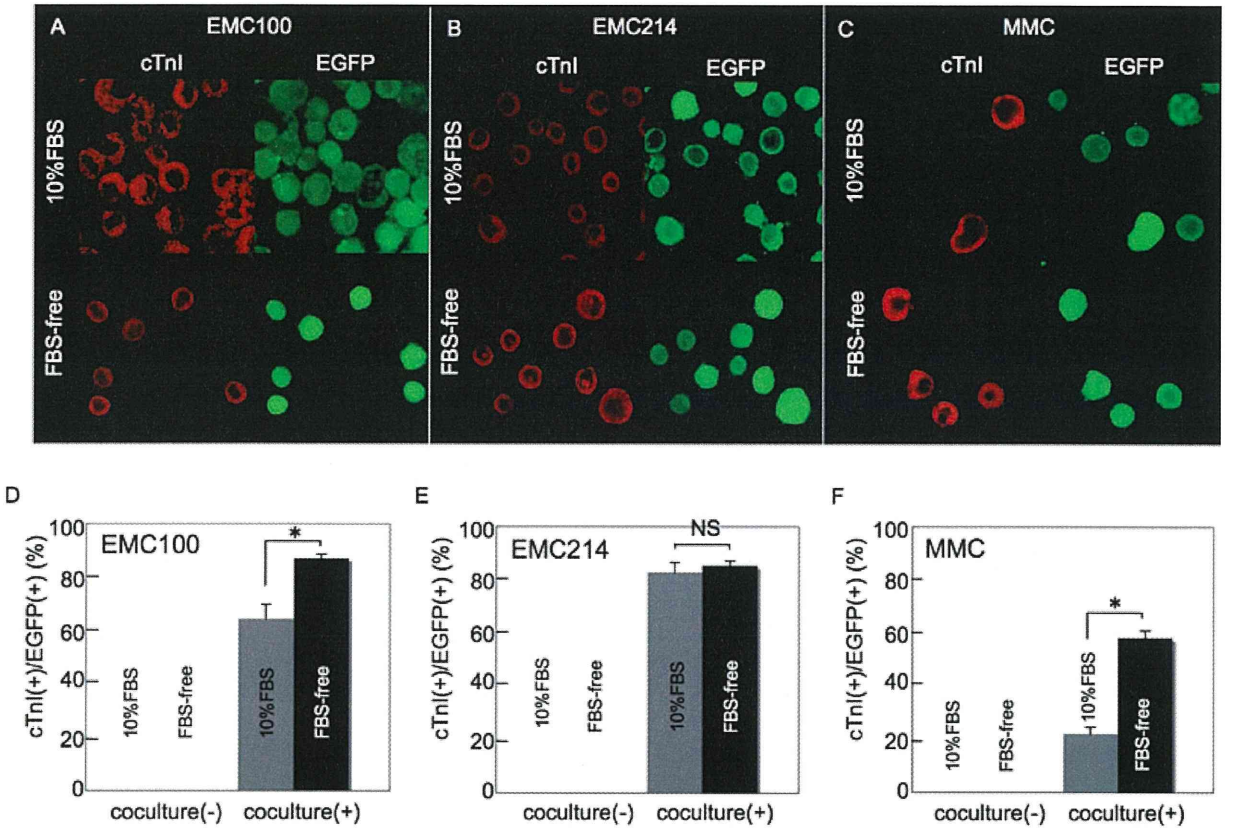


FIG. 3. Facilitating cardiomyogenic transdifferentiation efficiency of EMCs and MMCs by use of FBS-free ACCITT medium. (A–C) Representative immunocytochemical images are shown. Isolated EGFP-positive (green) EMC100s (A), EMC214s (B), and MMCs (C) with co-cultivation with murine cardiomyocytes by 10%FBS containing DMEM (DMEM) or FBS-free ACCITT medium (ACCITT) were stained with anticardiac troponin-I antibody (cTnI; red). (D–F) Cardiomyogenic transdifferentiation efficiency is defined as a proportion of cTnI-positive cells in EGFP-positive cells and averaged data are shown. ACCITT significantly increased the transdifferentiation efficiency of EMC100s and MMCs.

with FBS-free medium (Fig. 3D,F). Since the induction rate was saturated in the EMC214s (more than 80%), FBS-free medium tended to increase the cardiomyogenic induction rate of EMC214s; however, it is not statistically significant (Fig. 3E).

Immunocytochemistry

Immunocytochemical staining was done at day 7 of cardiomyogenic induction. EGFP-positive EMCs, when cultured in either medium, are positive for cardiac troponin-I (Fig. 4), α-actinin, and connexin 43 (Fig. 5). Cardiac troponin-I and α-actinin showed clear striation patterns, which indicated that differentiated EMCs were physiologically functioning. In the FBS-free medium, EMC-derived rod-shaped cardiomyocytes could be observed frequently (Fig. 4I–L). Connexin 43 was stained at the margin of α-actinin positive EMC-derived cardiomyocytes, suggesting tight electrical communication between the EMCs.

RT-PCR

EMCs constitutively expressed some cardiomyocyte-specific genes (18). In the present study, we confirmed that the FBS-free medium does not affect the mRNA expression of Nkx2.5, cardiac troponin I, cardiac troponin T, and cardiac actin at the default state (Fig. 6). These cardiomyocyte-specific genes were constitutively expressed before and after the cardiomyogenic induction.

DISCUSSION AND CONCLUSIONS

Serum-independent cardiomyogenic transdifferentiation of EMCs and MMCs

One of the most important findings in the present study is that serum in the culture medium is not an essential factor for cardiomyogenic transdifferentiation in human mesenchymal cells.

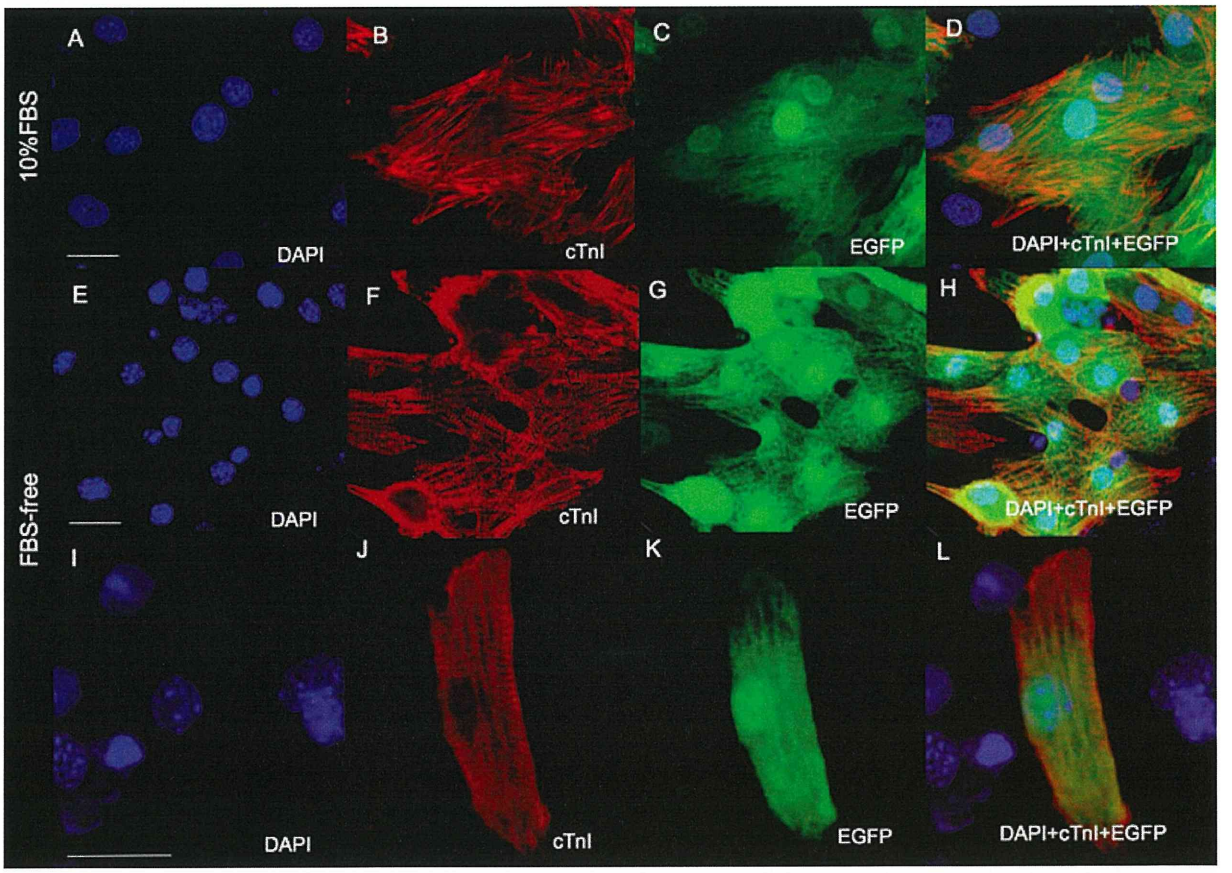


FIG. 4. Confocal microscopic view of EMC214-derived cardiomyocyte immunostained with anticardiac troponin-I antibody. Immunocytochemistry of transdifferentiated EMC214s with anticardiac troponin-I (cTnI) antibody. The cells were stained with DAPI (blue) and anti cTnI antibody (red). EGFP-positive (green) human EMC214s expressed cTnI. Note the clear striation staining pattern of EMC214s. Scale bar denotes 20 μm. Both EMC214s cultured with 10%FBS containing DMEM or FBS-free ACCITT medium expressed cTnI. EMC214s cultured with ACCITT medium sometimes showed a rod shape. Scale bar denotes 20 μm.

Several stem cell types are used for clinical patients. Of these stem cells, mesenchymal cells are reported to show cardiomyogenesis in vitro (16, 17), while others fail to show evidence of cardiomyogenesis in the experimental condition (22–24). There are preclinical candidates for stem cell sources that have cardiomyogenic ability (e.g., embryonic stem cells, cardiac presursor cells, etc.) (1,2,5,7,8,25); however, they may not be used for clinical patients, since clinical application has several problems. Thus, the analysis of key mechanisms for cardiomyogenic transdifferentiation in the human mesenchymal cell is important in order to expand the efficacy of current cardiac stem cell therapy. For cardiomyogenic transdifferentiation in the human mesenchymal cell, environmental factors, e.g., co-cultivation with cardiomyocytes obtained from other species (feeder cardiomyocytes), were reported as essential in the previous study (17). Feeder cardiomyocytes may secrete a humoral factor for cardiomyogenic transdifferentiation in human mesenchymal cells in vitro. Definition of these cardiomyogenic transdifferentiation factors in vitro are important, because it may improve the cardiomyogenic transdifferentiation efficiency of engrafted mesenchymal cells in vivo, and consequently, improve the efficacy of current cardiac stem cell therapy. The conventional culture medium, however, contains serum, which has numerous humoral factors that preclude the reliable detection of feeder-derived cardiomyogenic transdifferentiation factors. Thus, our serum-independent cardiomyogenic assay system may provide an effective model for identifying feeder-derived cardiomyogenic transdifferentiation factors in vitro. Furthermore, it is also important to state that serum in the culture medium does not play an essential role in cardiomyogenic transdifferentiation of human mesenchymal stem cells in vitro.

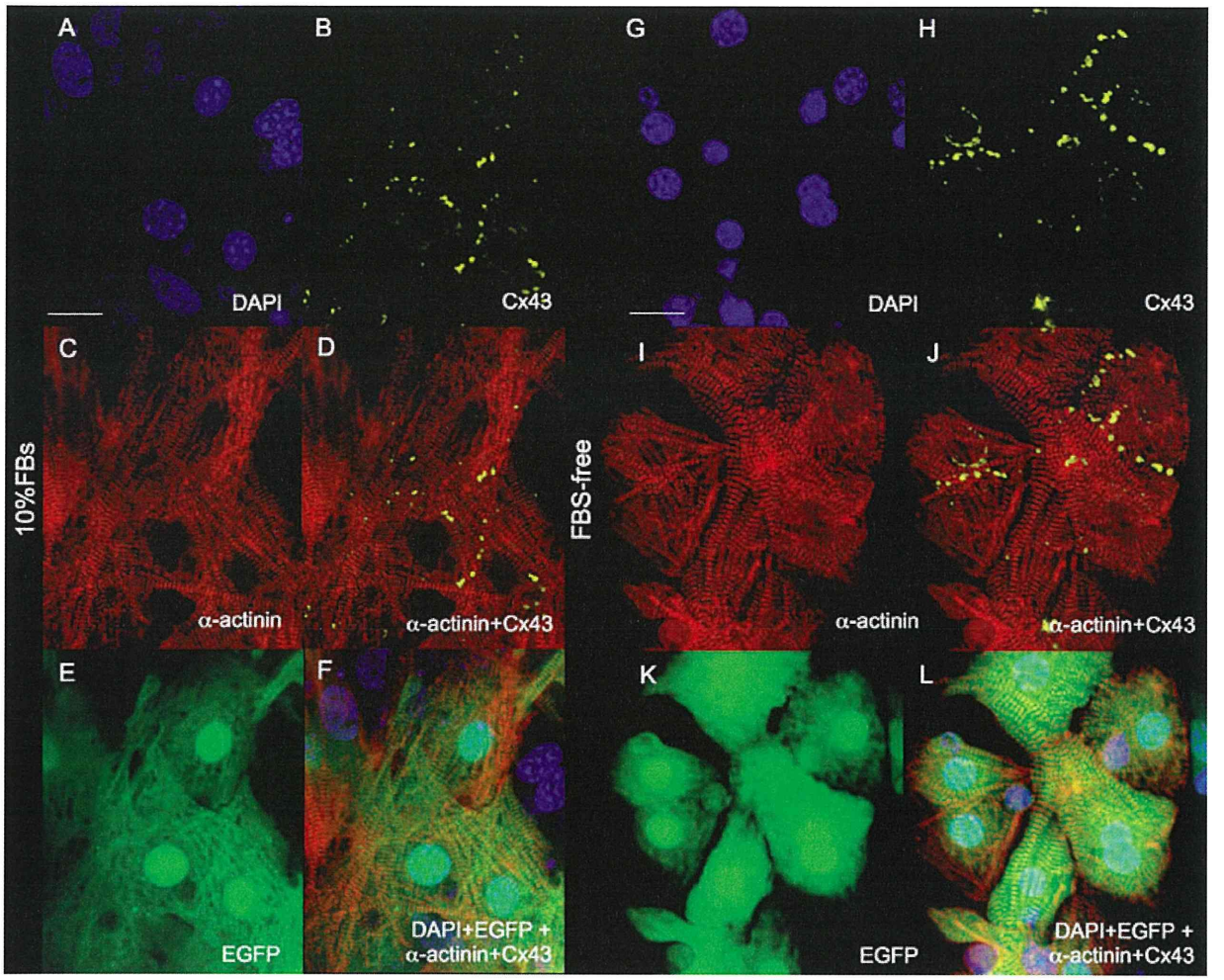


FIG. 5. Confocal microscopic view of EMC214-derived cardiomyocytes immunostained with anti-sarcomeric α-actinin and connexin 43 antibody. Immunocytochemistry of transdifferentiated EMC214s with anti-sarcomeric α-actinin (α-actinin) and connexin 43 (Cx43) antibody. The cells were stained with DAPI (blue), anti-α-actinin (red), and Cx43 (yellow) antibody. Both EMC214s cultured with 10%FBS containing DMEM or FBS-free ACCITT medium expressed α-actinin and Cx43. Scale bar denotes 20 μm.

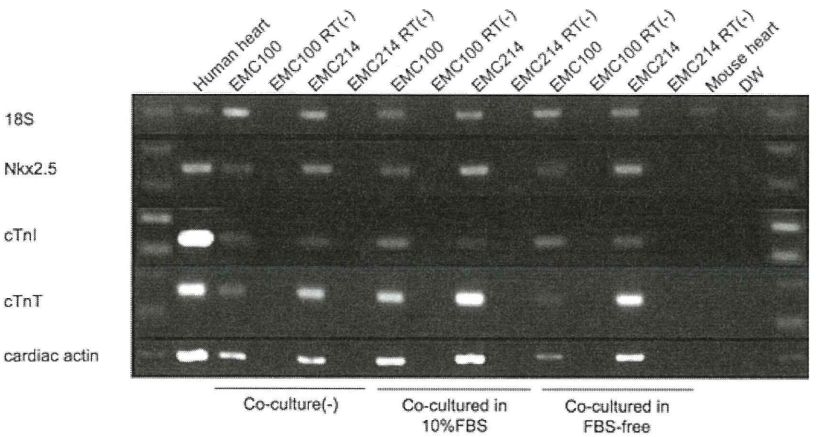


FIG. 6. Expression of cardiomyocyte-specific genes of EMC100 and MC214. RT-PCR was performed with PCR primers with specificity for human genes encoding cardiac proteins but not for the corresponding murine genes. Human heart and mouse heart cells were used as a positive control and negative control, respectively. Most human cardiac genes were constitutively expressed in the default state of EMCs and were not affected by FBS-free ACCITT medium.

Mechanism of improvement in cardiomyogenic transdifferentiation in FBS-free medium

FBS-free ACCITT medium was defined in the early 1990s (19,26) for culturing isolated adult rat ventricular myocytes. Since about 70% of the energy source for ATP production in the adult cardiomyocyte is free fatty acid, ACCITT medium contains L-carnitine, which is of central importance in mitochondrial fatty acid metabolism, in order to maintain an adult cardiomyocyte phenotype in situ. In the present study, both physiological and histological phenotypes in the differentiated cardiomyocytes from EMCs were more matured and maintained for a longer period in FBS-free medium than in 10% FBS-containing medium. Cardiomyogenic transdifferentiation efficiency of EMC100s and MMCs was significantly improved. The lack of statistical significance in the improvement of cardiomyogenic transdifferentiation efficiency of EMC214s may be due to extremely high cardiomyogenic transdifferentiation ability of EMC214s in 10%FBS medium. Consequently, the effect of FBS-free medium may be saturated in our system. In addition to adult myocytes in culture, FBS-free ACCITT may provide a suitable condition for maturation of EMC-derived and MMC-derived cardiomyocytes.

In order to define the essential element for facilitating cardiomyogenic transdifferentiation in FBS-free medium, we have tried to culture with FBS-free ACCITT medium, eliminating each element one by one; however, each element in ACCITT is essential for maintaining healthy feeder cardiomyocyte conditions, and the essential elements for facilitating cardiomyogenic transdifferentiation in ACCITT are still unclear. Alternatively, it is possible that 10% FBS inhibits cardiomyogenic transdifferentiation of EMCs and MMCs in the co-culture system. The mechanisms of improvement in cardiomyogenic transdifferentiation in FBS-free medium are still unclear. However, supplemental composition in ACCITT may be a clue to define factors that facilitate cardiomyogenic transdifferentiation in human mesenchymal cells. Further experiments should be done.

Establishment of a FBS-free cardiomyogenic transdifferentiation assay system in vitro

In our co-cultivation system, administration of the supernatant of murine cardiomyocyte culture did not cause cardiomyogenic transdifferentiation. Thus, the key factor for cardiomyogenic transdifferentiation may not be a humoral factor, but may be due to interaction between the mesenchymal cells and murine cells. Furthermore, the concentration of the factor secreted from the murine cardiomyocyte may not diffuse far; therefore, concentration of the specific factor could not be detected in the net supernatant of the culture medium. By use of a FBS-free cardiomyogenic transdifferentiation assay system, we may able to detect small concentration changes in the specific factors in the culture that may be masked when serum-containing medium is used in the assay system.

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New innovative instruments facilitate both direct-vision and endoscopic-assisted mini-mitral valve surgery

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Objective: The efficacy of new, innovative, original instruments, including a left atrial retractor, silicon annuloplasty ring sizer, modified Cosgrove aortic clamp, and reusable clip for fixing knots of polytetrafluoroethylene (Gore-Tex; WL Gore & Associates, Inc, Flagstaff, Ariz) suture, to allow surgical exposure in an ideal operative setting of mini-mitral valve repair surgery was verified.

Methods: Since 1998, a great deal of innovation has contributed to establishing mitral valve repair via right minithoracotomy as a routine surgical approach for mitral valve insufficiency in 252 cases. During the last 2 years, a newly launched left atrial retractor system attachable to the minithoracotomy spreader has been used. An additional retractor for the posterior wall of the left atrium was attached to the minithoracotomy spreader. The retractor moves flexibly and can be fixed in any favorable position to realize optimal exposure of the mitral valve. A 5 blade size was available depending on the left atrial size and target legion. By using the smallest size, even papillary muscles were exposed easily and clearly. Furthermore, a flexible silicon ring sizer, which could easily pass thorough a narrow working port without tissue damage, was used for sizing the annuloplasty ring. For the surgical technique, multiple chordal reconstructions by the loop technique with polytetrafluoroethylene (Gore-Tex CV-5 sutures) were applied. A reusable clip for fixing knots made it easy to tie the Gore-Tex suture in the correct position without slipping.

Results: No operative mortality occurred. There were 2 conversions to sternotomy for correction of aortic dissection (1) and for coronary artery bypass grafting (1). There were 2 early reoperations for failure of mitral valve repair. The mean aortic crossclamp time was 163.5 ± 41.6 minutes. Annuloplasty with a ring or band was performed in all cases except one. The loop technique was used in 173 cases. Among them, a combination of the loop technique and resection and suture technique was used in 56 cases.

Conclusions: Newly innovated mini-mitral valve surgical instruments and techniques facilitate both direct-vision and endoscopic-assisted approaches and accomplish a favorable surgical outcome even in the complex pathology of mitral valve insufficiency. (J Thorac Cardiovasc Surg 2011;■:1-4)

Endoscopic-assisted mitral valve surgery via minithoracotomy (mini-mitral surgery) has recently evolved and rapidly spread because it is less invasiveness and has a cosmetic advantage. Although mini-mitral surgery is well known as safe and feasible, and is associated with a high repair rate, a low perioperative morbidity, and an excellent durability of repair,[1-3] this technique is still limited to specific surgeons[4] because special setting, instruments, and surgical training are essential for this technique. However, mini-mitral surgery realizes great surgical exposure of pathologic lesions in the mitral valve, and complex surgical procedures can be performed as in the median-sternotomy approach if the surgeon knows how to prepare for the ideal operative setting with optimal instruments. In regard to the technical aspect, special training with endoscopic surgical instruments and surgical strategy, which is different from the sternotomy case and arranged for mini-mitral surgery, is necessary to achieve favorable surgical results. The efficacy of new, innovative, original instruments and an operative setting in mini-mitral surgery were verified, including a left atrial retractor, silicon annuloplasty ring sizer, modified Cosgrove aortic clamp, and reusable knot clip to facilitate fixing knots of polytetrafluoroethylene (PTFE) (Gore-Tex; WL Gore & Associates, Inc, Flagstaff, Ariz) suture in an ideal position.

MATERIALS AND METHODS

From 1998 to April 2011, 252 patients underwent mitral valve repair via a right minithoracotomy for treatment of mitral valve regurgitation. Several innovations have contributed to establishing mitral valve repair via right minithoracotomy as a routine surgical approach. The mean age of patients was 51.1 ± 13.9 years, and 36.9% were female. During the last 2 years, a newly launched left atrial retractor system attachable to the minithoracotomy spreader was developed and has been used.

In routine mini-mitral surgery, a right anterolateral minithoracotomy of 6 to 8 cm was made thorough the fourth intercostal space. In cases with

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Abbreviation and Acronym
PTFE = polytetrafluoroethylene

difficult exposure, the fourth costal bone was cut to make a larger working space. This bone was repaired with a hydroxyapatite-poly-L-lactide plate (Osteotrans MX; Takiron Co Ltd, Osaka, Japan) at the end of the surgery, and pseudoarthrosis at the rib-costal cartilage was prevented.[5] Cardiopulmonary bypass was established with the femoral artery (16F–20F FEM-Flex II arterial cannula; Edwards Lifesciences, Irvine, Calif), femoral vein (20F–24F VFEM venous cannula, Edwards Lifesciences), and right jugular vein (16F FEM-Flex II arterial cannula, Edwards Lifesciences) cannulation under transesophageal echocardiography guidance. The heart was arrested with antegrade cold blood cardioplegia infusion through a root cannula under direct crossclamping of the ascending aorta with a modified Cosgrove flexible clamp. Infusion of the cardioplegia was repeated every 30 minutes with topical cooling. The mitral valve was exposed thorough the interatrial grove approach with a left atrial retractor attached to a minithoracotomy spreader and an additional retractor for the posterior wall of the left atrium attached to the spreader. Multiple chordal reconstructions by the loop technique[6,7] with PTFE Gore-Tex CV-5 sutures were applied for correction of prolapsed leaflet rather than the resection and suture technique since 2005.[8] We routinely prepare the PTFE loops in the same length in almost all cases and adjust the efficient length of the neochordae with the secondary loop for the mitral leaflet depending on the size of the left ventricle and the part of the prolapsed leaflet (loop-in-loop technique). A reusable clip as a substitution for the neurosurgery clip was used to tie the PTFE suture firmly in the correct position without slipping. A flexible silicon ring sizer was used to choose the annuloplasty ring size so it can pass easily thorough a narrow working port without tissue damage. All manipulations in the heart were performed under endoscope assistance. However, surgeons did not always use endoscopic vision and used direct vision when they were comfortable with it.

RESULTS

No operative mortality after the surgery occurred (Table 1). There were 2 intraoperative conversions to median sternotomy for correction of retrograde aortic dissection (1) and for coronary artery bypass grafting to the left anterior descending artery area (1). There were 2 early reoperations for mitral valve repair failure and 3 reoperations for bleeding. No severe cerebral complication occurred. Since the new retractor was launched in 2010, there has been no conversion to median sternotomy and reoperation for bleeding among 53 cases. The mean aortic crossclamp time was 163.8 ± 42.0 minutes. Annuloplasty with ring or band was done in all but 1 case. The loop technique was used in 173 cases (68.6%). Among them, a combination of the loop-technique and resection-and-suture technique was used in 56 cases with complicated mitral valve pathology. After 2010, the loop technique was used in 47 cases (88.7%).

TABLE 1. Surgical outcome between 1998 and April 2011 (n = 252)

Age, y	51.1 ± 13.9
Male/female	159/93
Operative mortality	0
Conversion to sternotomy	2
Early reintervention	
Failure of mitral valve repair	2
Surgical bleeding	3
Aortic crossclamp time	163 ± 42.0
Loop technique	173 (68.6%)
After 2010	47/53 (88.7%)
Loop technique+resection and suture	56 (22.2%)

DISCUSSION

The newly developed left atrial retractor system, including a minithoracotomy spreader, a left atrial blade, and an additional retractor for the posterior wall of the left atrium, is specialized for mini-mitral surgery and can solve all kinds of difficulties in exposure of the mitral valve. The advantage of this mini-valve system is the flexibility. It moves flexibly to various positions and has good positioning of retractors for optimum exposure depending on the situation and the surgeon's choice. A 5 blade size is available depending on the left atrial size and target legion. To adapt the size of the blade to the pathologic legion, the left atrial retractor can be exchanged easily to the other size under fixed optimal exposure. By retraction of the anterior mitral leaflet toward the anterior wall of the left ventricle with the smallest size blade deeply inserted, even papillary muscles can be exposed easily and clearly on the straight view through the small working port. This function enables comfortable manipulation to the papillary muscle in chordal reconstruction with the loop technique. The exposure of the P2 to P3 area of the mitral valve usually is difficult because of elevation of the left atrial wall. To solve this problem, an additional retractor attachable to the minithoracotomy retractor can push the left atrial wall away from the surgeon's sight. In case of unfavorable exposure of the P1 area, the additional retractor can be used alternatively for retraction of the anterolateral side of the left atrial wall.

Correct sizing of the annuloplasty ring is an important and critical part of the mitral valve repair but is difficult through a small working port. A homemade flexible silicon ring sizer was developed as the same size as the official sizer of the Physio II mitral annuloplasty ring (Edwards Life Science). This can easily pass thorough a narrow working port without tissue damage and is helpful for correct sizing under endoscopic vision.

To repair a prolapsed leaflet in the mitral valve, the gold standard has been the resection-and-suture technique of the prolapsed leaflet, which has demonstrated excellent long-term results.[9] In contrast, the "respect rather than resect" approach proposed by Perier and colleagues,[10] which respects leaflet tissue as an important component of the coaptation surface and preserves leaflet without resection, is widely accepted because it allows a larger coaptation area than the resection-and-suture technique and has an advantage in dynamic distribution of forces and stress on valve components and the left ventricle. Especially in cases

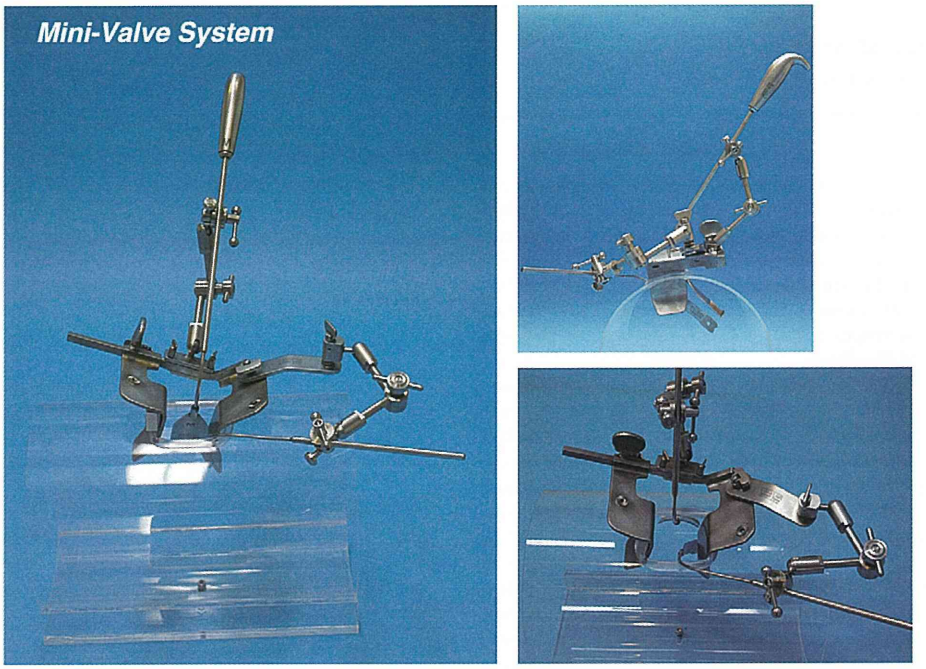

FIGURE 1. Mini-valve system is composed of a mini-thoracotomy spreader with a tilted blade, a left atrial retractor that moves flexibly, and an additional blade for the posterolateral wall of the left atrium.

with broad prolapse of the posterior leaflet or with anterior leaflet prolapse, multiple chordae reconstruction using PTFE suture is essential. The loop technique is a modification that normally uses 4 premade PTFE loops to facilitate chordal reconstruction. In mini-mitral surgery with a limited working area, multiple chordal reconstructions with individual PTFE sutures are technically difficult and even dangerous because of the risk of injury in the papillary muscle. The loop technique is a feasible solution to preserve as much leaflet area as possible and to repair the broad prolapsed leaflet in mini-mitral surgery. The loop-in-loop technique avoids the surgical manipulation in the deep working area at the base of the left ventricle and facilitates multiple reconstructions of neochordae. The technique also enables reattachment of the neochordae when residual leakage is found in the saline injection test. Recently, 88.7% of mitral valve repairs were done with the loop technique, and the variation of the repair method is increasing to treat various type of mitral valve pathology.

For all these advantages in the loop technique, many surgeons still hesitate to adapt this technique because surgeons feel uncomfortable when they tie slippery PTFE (Gore-Tex) sutures many times. To solve this problem and help surgeons tie many knots with PTFE suture in the correct position, a reusable clip as a substitution for the neurosurgery clip is used to tie the PTFE suture firmly without slipping. The length of the second loop to fix the premade loops with PTFE suture is determined after filling the left ventricle with saline and appropriately positioning the clip, which can easily slide to the best position when the leakage disappears.

Although this study shows longer mean aortic cross-clamp times than a previous study on mitral valve repair, the surgical outcome is excellent with no surgical mortality. This is supported by careful myocardial protection with antegrade cold-blood cardioplegia infusion under transesophageal echocardiography monitoring of the aortic valve and aortic root pressure during infusion. During cardioplegia, surgeons and anesthesiologists should ensure the aortic valve is incompetent, the cardioplegic solution is running into the coronary artery, and the aortic root pressure is sufficiently high. To afford an incompetent aortic valve, the left atrial retractor should be released during cardioplegia. The mini-valve system can aid in easy release and repositioning of the left atrial retractor after repeated cardioplegic shots. This secure strategy in myocardial protection may help beginner surgeons using the mini-mitral approach to achieve good results in cases of complex mitral valve pathology.

CONCLUSIONS

In an optimal operative setting, mitral valve repair via minithoracotomy is a feasible and durable procedure with minimal mortality and morbidity. The feasibility of multiple PTFE chordae reconstructions is an important strategy especially when trying to preserve leaflets. To reconstruct multiple chordae with PTFE suture, the loop technique is essential in mitral valve repair via minithoracotomy. New

innovative instruments and a refined surgical setting in mini-valve surgery facilitate both direct-vision and endoscopic-assisted approaches even in complex pathology and contribute to the acceptance of mini-mitral valve surgery as a routine surgery.

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000 New innovative instruments facilitate both direct-vision and endoscopic-assisted mini-mitral valve surgery

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Newly innovated mini-valve systems, including a mini-thoracotomy spreader and left atrial retractors, and specific surgical techniques designed for working in a limited space facilitate mitral valve surgery via mini-thoracotomy. The special setting realizes a favorable surgical outcome even in the complex pathology of mitral valve insufficiency.

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CASE REPORT

Repair of traumatic tricuspid insufficiency via minimally invasive port access

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Abstract We report on a successful tricuspid valve plasty using port-access minimally invasive cardiac surgery (MICS) for severe traumatic tricuspid insufficiency caused by blunt chest trauma suffered 15 years previously. A combination repair procedure, consisting of cleft closures, plication of the anteroseptal commissure, and ring annuloplasty, was necessary to achieve valve competence and proved possible via port access without difficulty. Port-access MICS is an alternative approach for tricuspid valve surgery.

Key words Traumatic tricuspid insufficiency · Port access · Minimally invasive cardiac surgery

Introduction

Minimally invasive mini-thoracotomy is now used regularly in cardiac surgery to avoid potential median sternotomy-related complications. Surgical trauma to the patient can be reduced without compromising quality or safety and leads to faster recovery.[1,2] In addition, a small submammary incision results in improved cosmesis, especially for female patients. We herein describe a patient with severe traumatic tricuspid insufficiency (TI) that was corrected via this approach.

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Case report

A 46 year-old woman whose case had been followed for several years at another hospital with a diagnosis of severe traumatic TI was referred to our hospital for a cardiac operation because she had become more breathless during exercise. She had a history of blunt chest trauma due to a traffic accident 15 years previously that had resulted in 24 rib fractures. On admission to our hospital, electrocardiography showed an incomplete right bundle branch block with normal sinus rhythm. Echocardiography revealed severe TI, chiefly resulting from failed valves and chordae ruptures of the anterior tricuspid leaflet, plus right ventricular dilatation.

Operative technique

Cardiac exposure was obtained through a right anterior mini-thoracotomy with a 7-cm submammary incision by means of port-access minimally invasive cardiac surgery (MICS), as has been previously reported.[3,4] Briefly, the femoral artery and vein were used to establish cardiopulmonary bypass (CPB) using Fem-Flex II cannulas (Edwards Lifesciences, Midvale, UT, USA) via a small groin incision. A soft vent catheter was inserted via the right upper pulmonary vein. Aortic cross-clamping was applied using a modified Cosgrove flex clamp via the port, and cardiac arrest was achieved by antegrade cardioplegia using a root cannula and topical cooling. The tricuspid valve (TV) and subvalvular apparatus were observed after a tracheal tube was inserted into the superior vena cava via right atriotomy to establish total CPB. Several chordae ruptures, with a large cleft on the anterior leaflet of the TV, were observed. Cleft closure

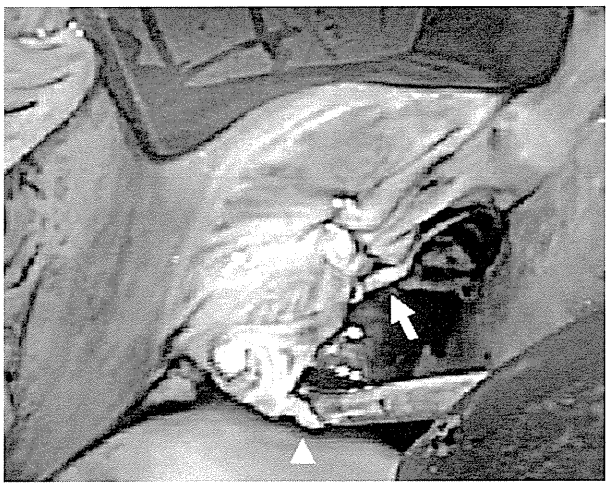

Fig. 1 Intraoperative findings show massive tricuspid regurgitation, chiefly due to failed valves and chordae ruptures of the anterior leaflet on the tricuspid valve. A large cleft on the anterior leaflet was closed by means of a continuous suture (*arrow*). A severely failed valve with chordae rupture (*arrowhead*) remains, however, especially around the anteroseptal commissure (indicated by a hook)

from the free edge with a continuous suture was performed (Fig. 1). Plication of the partial anteroseptal commissure was performed due to extremely poor coaptation, taking care to avoid creating TV stenosis. Ring annuloplasty was also carried out using a 28-mm Edwards MC3 annuloplasty system (Edwards Lifesciences, Irvine, CA, USA). A water test showed a dramatic reduction of TI. However, a mild degree of TI remained due to a small cleft in the center of the septal leaflet of the TV. TI disappeared after cleft closure using the same technique. The postoperative course was uneventful, and the patient was discharged 7 days after the operation. Postoperative echocardiography revealed trivial TI.

Discussion

There are few reports regarding TV surgery using port-access MICS, but this technique enables good exposure. Advantages of the port-access approach include reduction of surgical trauma, due in part to less blood loss, and earlier recovery to full activity, as well as avoidance of median sternotomy-related complications, such as deep wound infection, compromised cosmesis, and possible injury to the right ventricle during repeated sternotomy.[1–3] Limitations of this approach include concomitant cardiac or aortic procedures other than mitral disease or simple congenital defects. A patient with a high likelihood of dense lung adhesions is also contraindicated for this approach due to technical difficulties. Based on our experience of more than 400 cases using this approach, we currently prefer using a flexible metal clamp instead of balloon aortic occlusion to secure clamping and to avoid balloon migration. Transesophageal echocardiography is also essential to evaluate whether there is aortic insufficiency or left ventricular distention during the infusion of cardioplegic solution, as when using mini-thoracotomy the left side of the heart cannot be seen. In the present case, there were minor concerns about lung adhesions due to the multiple rib fractures, but they proved trivial and the procedure was performed without difficulty.

Traumatic TI is a relatively rare complication of blunt chest trauma; but due to the increasing sophistication of imaging facilities, reports on the discovery of this particular disease have become more common in the literature. Traumatic TI due to complex lesions, often consisting of ruptured chordae, torn leaflets, and cusp retraction, is still a challenging surgical problem, being different from TI caused by simple annular dilatation secondary to mitral disease.[5] Several techniques have been reported—including artificial chordae implants, papillary muscle reconstruction, double orifices, clover technique, and bicuspidization—that avoid prosthesis-related complications and long anticoagulation treatment and that preserve the geometry and function of the right ventricle.[5,6] Repair is preferable to replacement with prostheses. We believe that the above techniques can be applied via port access in addition to the conventional approach.

Conclusion

We have presented a successful TV repair using port-access MICS for severe traumatic TI caused by blunt chest trauma. Combination repair procedures were feasible via a less invasive port-access approach, making this technique a successful alternative to traumatic TV surgery and other TV procedures.

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