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# Cellular extracts from post-mortem human cardiac tissue direct cardiomyogenic differentiation of human adipose tissue-derived stem cells

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**Abstract**

*Background aims.* Human adipose tissue-derived stem cells (hASCs) can be easily (and inexpensively) expanded in culture, and their high plasticity allows their conversion to different cell types. We study the potential capacity of postmortem cardiac tissue to direct cardiac differentiation of hASCs *in vitro*. *Methods.* Cardiac tissue collected from autopsies was used to obtain cell extracts and conditioned medium, and both approaches were tested for cardiac induction. *Results.* Gene expression analyses proved that post-mortem human cardiac tissue maintains genetic integrity. hASCs exposed to the cell extracts or conditioned medium for 2 weeks achieved the appearance of myotube-like structures and were positive for cardiac markers such as sarcomeric α-actinin, cardiac troponin I and T and desmin as proved by immunofluorescence. In addition, differentiated cells showed increased expression of cardiomyocyte-related genes analyzed by reverse transcriptase polymerase chain reaction (*GATA-4*, myocyte-enhancer factor-2c, α-cardiac actin and cardiac troponin I). *Conclusions.* For the first time, post-mortem human cardiac tissue was used to induce hASC differentiation into myocardial-like cells. The methodology described here would serve as a useful model to obtain cardiomyocyte-like cells *in vitro*.

**Key Words:** *cardiomyogenic differentiation, cell extract, conditioned medium, human adipose stem cells, postmortem tissue*

## Introduction

Related heart diseases are the major cause of death throughout the world (1). The development of new cardioactive drugs is a complicated process in which pre-clinical toxicity assays are a critical step. At the present time, cell-based screening assays are used to study disease mechanisms and to test the effect of novel drugs or gene treatments. Different cell sources have been used to obtain human cardiomyocyte-like cells. Studies using human embryonic stem cells (ESCs) have shown great potential for improving drug screening. The effect that some drugs produce in cardiomyocytes was similar to the effect produced in cardiomyocytes derived from ESCs (2,3). In addition, ESCs have proved to be a suitable model to test the development of cardiac arrhythmias as a pharmacologic side effect (4). To avoid ethical restrictions, induced pluripotent stem cells (iPSCs) have become the ideal alternative to ESCs. With the generation of disease-specific iPSC lines from patients with various diseases (5), new expectations have emerged, not only in basic research and regenerative medicine but also in a pharmacologic context. However, the use of ESCs or iPSCs is restricted to a few laboratories around the world, not only because of the difficulties secondary to a very restrictive legislation, which limit their use, but also, and even more important, because of the high cost of ESC and iPSC culture.

Previous studies showed the possibility of obtaining cardiomyocyte-like cells by the use of cell extracts from rat cardiomyocytes (6). More recently, our group demonstrated an improvement of this methodology using human cardiac tissue to direct differentiation of human adipose tissue-derived stem cells (hASCs) toward cardiomyocytes (7). In this study, the cardiac tissue was obtained from patients undergoing coronary artery bypass surgery, with limitations to obtaining large amounts of tissue. Post-mortem human cardiac tissue could be a suitable alternative; it has been demonstrated that robust gene

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expression can be obtained using RNA from autopsy-derived tissue 24 hours after autolysis (8).

Mesenchymal stromal cells transplanted into infarcted myocardium have been shown to acquire the phenotypic characteristics of cardiomyocytes (9,10), suggesting paracrine (i.e., cytokine and chemokine) effects of the cardiac tissue in the implanted cells. Conditioned media, derived by *in vitro* culturing cells dissociated from the heart, have been proven to induce differentiation of bone marrow-derived mesenchymal stromal cells (11).

In this study, we used for the first time post-mortem cardiac tissue to induce hASC differentiation into myocardial-like cells. First, genetic integrity of human cardiac post-mortem tissue was evaluated by real-time polymerase chain reaction (PCR) analyses. Second, two different approaches were implemented: (i) cell extract method and (ii) conditioned medium. To confirm the cardiac phenotype achieved by the hASCs, immunofluorescence analyses were performed. In addition, gene expression was evaluated by reverse transcriptase PCR to determine the expression of several cardiac-specific makers.

## Methods

### *Isolation and culture of hASCs*

The hASCs used in this study were isolated from human adipose tissue and characterized as previously shown (12). Phenotypic characterization and differentiation potential of isolated hASCs are shown in supplementary Figure 1. Cells were cultured with high-glucose Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich), 100 U/mL penicillin and 100 µg/mL streptomycin (Invitrogen, Merelbeke, Belgium) at 37°C in a humidified atmosphere containing 5% $CO_2$.

### *Samples*

Myocardial tissue samples were obtained from five forensic autopsies, ≤12 h post-mortem. Approximately 100 mg of tissue was taken in each case from the lateral wall of the left ventricle and was kept at −80°C until analysis. Each myocardial tissue sample was used individually.

### *RNA extraction and determination of quantity, quality and integrity*

For molecular analysis, 30 mg of each myocardial tissue sample was taken. The frozen tissue samples were disrupted and homogenized using the TissueLyser LT (Qiagen, Hilden, Germany), and subsequently RNA was extracted using RNeasy Fibrous Tissue Mini Kit (Qiagen). RNA quantity and quality were assessed by a NanoDrop 2000c (Thermo Fisher Scientific, Waltham, MA, USA). RNA integrity was evaluated using chip-based capillary electrophoresis with Agilent RNA 6000 Nano Kit (Agilent Technologies, Santa Clara CA, USA).

### *Reverse transcriptase and real-time PCR analysis*

QuantiTect Reverse Transcription Kit (Qiagen) was used for complementary DNA synthesis. Real-time PCR was performed using the QuantiTect SYBR Green PCR kit (Qiagen) on a 7500 device (Applied Biosystems, Foster City, CA, USA). For all samples, a relative quantitative gene expression analysis of cardiac troponin I (*TNNI3*), myosin light chain 3 (*MYL3*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was carried out in triplicate. Primers were commercially supplied by Qiagen (QuantiTect Primer Assay reference numbers: *TNNI3*, QT00084917; *MYL3*, QT00090223; *GAPDH*, QT01192646).

### *Differentiation of hASCs into cardiomyocyte-like cells by cell extract method*

Cell extract method was performed as described before (7). Briefly, post-mortem cardiac tissue was disaggregated by physical and enzymatic methods. Isolated cells were washed twice in cold phosphate-buffered saline (PBS) and then in cold cell lysis buffer (50 mmol/L sodium chloride, 5 mmol/L magnesium chloride, 100 mmol/L *N*-2-hydroxyethylpiperazine-*N*-ethanesulfonic acid, pH 8.2, 1 mmol/L dithiothreitol and 0.1 mmol/L phenylmethylsulfonyl fluoride; all Sigma-Aldrich). Cells were centrifuged at 800*g*, resuspended in 1.5 volumes of cell lysis buffer containing protease inhibitor cocktail (Sigma-Aldrich) and allowed to swell on ice for 45 min. Cells were homogenized by pulse sonication, and the lysate was sedimented at 15,000*g* for 15 min at 4°C. The supernatant was collected and used fresh. For exposure to cell extract, hASCs were harvested by trypsin digestion and washed twice in ice-cold PBS and once in ice-cold Hank's Balanced Salt Solution (Gibco-BRL, Paisley, UK). Cells were pelleted at 300,000 cells/reaction in 1.5-mL tubes and suspended in 488-µL ice-cold Hank's Balanced Salt Solution, placed in a water bath at 37°C for 2 min and a final concentration of 230 ng/mL streptolysin O (Sigma-Aldrich) was added. Samples were incubated for 30 min at 37°C. Cells were centrifuged at 300*g* for 5 min at 4°C in a swing out rotor. The supernatant was removed, and 500 µL extract containing 20 µL 1 mmol/L of each nucleotide triphosphate set (Roche,

Indianapolis, Indiana, USA) was added. Cells were incubated with the extracts for 1 h at 37°C. DMEM/10% FBS containing 2 mmol/L calcium chloride was added, and the cells were transferred to 25 $cm^2$ culture flasks. Dead (floating) cells and the $Ca^{2+}$-containing medium were removed 4 h later and replaced with fresh DMEM containing 10% FBS and antibiotics. Damaged or dead cells, marked with trypan blue (Sigma-Aldrich), represented 15–20% of the total. Cells were cultured for 2 weeks until use. Control cells were either non-permeabilized and non-extract exposed cells or permeabilized cells not exposed to the extracts. Cell viability was assessed by phase contrast microscopy after 2 weeks of extract exposure by counting cells in four different regions of the dish and calculating the average.

### *Differentiation of hASCs into cardiomyocyte-like cells by conditioned medium*

To prepare cardiac tissue-derived conditioned medium, 500–700 mg of post-mortem right ventricle tissue was cut into approximately 1-$mm^3$ pieces, and washed three times with PBS. Tissue fragments were placed into 75 $cm^2$ culture flasks containing 20 mL of DMEM containing 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin and cultured as explants. After 2 days, the medium was collected and centrifuged at 2000*g* for 10 min followed by filtering the supernatant through a 0.22-μm filtration unit (Millipore, Bedford, MA, USA). The medium was stored at 4°C until use. To induce cardiac differentiation of hASCs, $8 \times 10^4$ cells per well were plated on a six-well plate and incubated in cardiac tissue-derived conditioned medium for 2 weeks.

### *Immunofluorescence*

Cells seeded on glass coverslips were washed three times with PBS and fixed with 4% paraformaldehyde in PBS for 20 min at room temperature. Cells were then permeabilized with 0.1% Triton X-100 (Sigma) for 15 min, washed three times with PBS and blocked in 2% blocking buffer solution (Roche) for 1 h at room temperature. Cells were incubated overnight in primary antibody diluted in blocking buffer solution at 4°C, washed three times in PBS and incubated for 2 h with secondary (fluorescein isothiocyanate-conjugated or tetramethylrhodamine isothiocyanate-conjugated; Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibodies diluted 1:200 in blocking buffer solution. Afterward, they were washed three times in PBS, and the coverslips were mounted on slides with 4,6-diamino-2-phenylindole (DAPI)-containing mounting solution (Ultra Cruz mounting medium; Santa Cruz Biotechnology). Controls were performed with non-treated cells. Antibodies used were desmin (1:20, mouse monoclonal; Sigma), human cardiac-specific troponin I (1:100, mouse monoclonal; Research Diagnostics, Flanders, NJ, USA), human cardiac-specific troponin T (1:100, rabbit polyclonal; Abcam, Cambridge, UK) and sarcomeric α-actinin (1:200, mouse monoclonal; Sigma). Photographs were taken with a Leica DM 5500B (Leica Camera AG, Solms, Germany) fluorescent microscope, using Isis software (MetaSystems, Oberkochen, Germany). Figures were processed with Adobe Photoshop 7.0 (Adobe Systems, San Jose, CA, USA).

### *Reverse transcriptase PCR*

After induction of differentiation, total RNA from treated and non-treated hASCs was extracted using an RNeasy Mini Kit (Qiagen). Complementary DNA synthesis was carried out from 1 μg of RNA sample using the Reverse Transcription System (Promega, Madison, WI, USA), and the PCR reaction was performed with ReddyMix PCR Master Mix (Thermo Fisher Scientific). After the initial denaturation (2 min at 94°C), 33 cycles were performed (30 s at 94°C, 50 s for annealing temperature and 1 min at 72°C) for all sets of primers except for β-actin, which was 25 cycles. Primer sequences and annealing temperatures were 5′ATCATGTTTGAGACCTTCAA3′ and 5′CATCTCTTGCTCGAAGTCCA3′ (45°C) for β-actin, 5′ATCTCTGCTGGCCATGAAAC3′ and 5′GATGAGGGAAGGTGGTTTGG3′ (53°C) for cardiac α-actin, 5′CCCTGCACCAGCCCCAATCAGA3′ and 5′CGAAGCCCAGCCCGGTCAACT3′ (64°C) for cardiac troponin I, 5′AGTGGTTTCCGTAGCAACTCCT3′ and 5′TAGTGCAAGCTCCCAACTGACT3′ (62°C) for *Mef2c* and 5′TCCCTCTTCCCTCCTCAAATTCA3′ and 5′GCGTGTAAAGGCATCTG3′ (52°C) for *GATA-4*. The PCR products were visualized on 1% agarose gels containing 0.1 mg/mL ethidium bromide using ultraviolet light.

## Results and discussion

### *RNA integrity and gene expression analysis of human cardiac post-mortem tissues*

First, we evaluated the quality and integrity of the RNA extracted from tissues 5–12 h post-mortem using the RNA Integrity Number (RIN). RIN is an algorithm based on a selection of features that provides information about the RNA integrity: (i) proportion of large molecules compared with smaller ones, (ii) the state of the degradation process and (iii) how far the degradation proceeded. The samples are assigned to 10 different categories ranging from

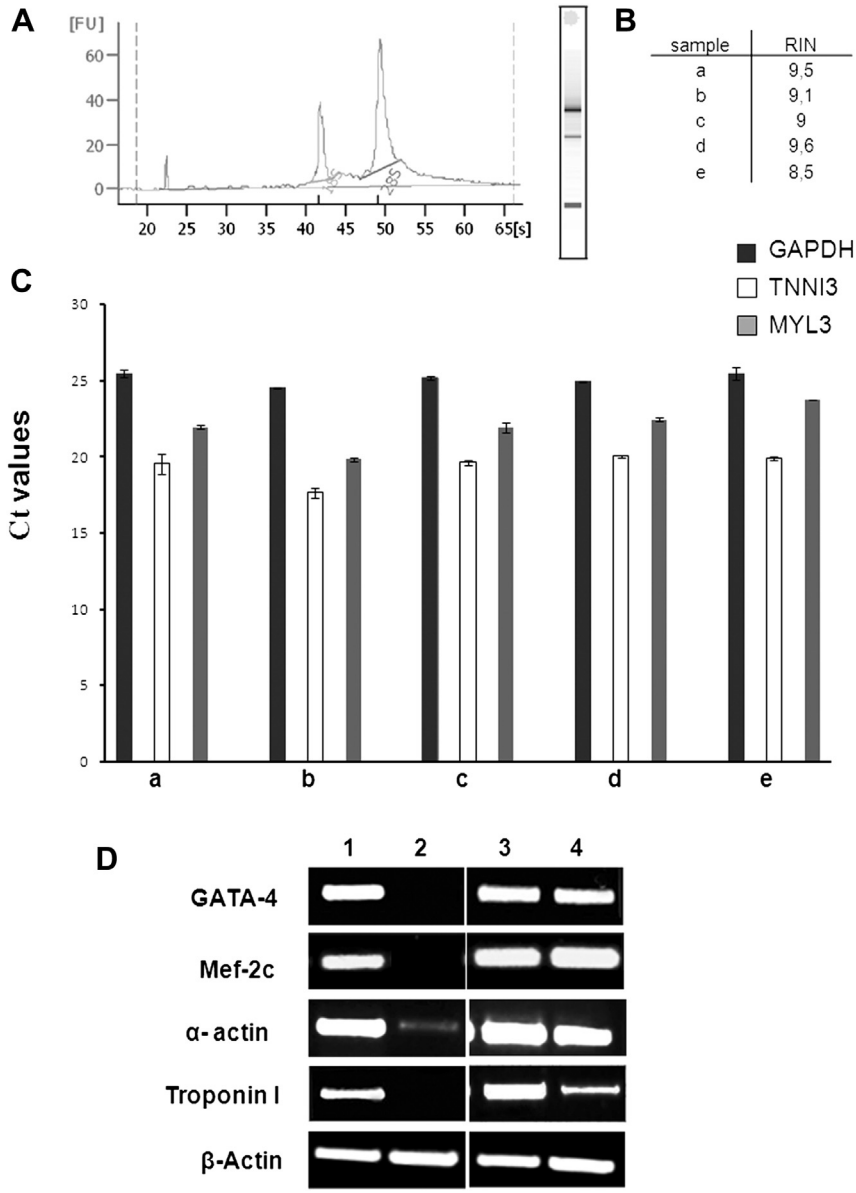


Figure 1. (A) Post-mortem cardiac tissue RNA integrity. Representative RIN graphic for RNA sample with RIN value of 9.5. (B) RIN values for all the samples. (C) Real-time PCR gene expression analysis of *TNNI3*, *MYL3* and *GAPDH* in five samples (a–e) obtained from post-mortem cardiac tissue. (D) Reverse transcriptase PCR analysis of expression of cardiomyocyte marker genes. Isolated cardiomyocytes used as a positive control (lane 1), control hASCs (lane 2), cardiomyocyte-conditioned medium treated cells (lane 3) and cells treated with the cardiomyocyte extracts (lane 4) expressing *GATA-4* (194 bp), *Mef-2c* (230 bp), cardiac α-actin (400 bp) and cardiac troponin I (233 bp). Results are representative of three independent experiments.

1 (worst) to 10 (best) (13). Our samples showed a high degree of RNA integrity with RIN values ranging from 9.5 to 8.5 and with a 260/280 ratio of 2 (Figure 1A). These findings demonstrated the viability of using post-mortem tissue as a reliable source of genetic material. Examination of gene expression levels at the time point of death has become an innovative tool in autopsies because RNA of adequate quality can be extracted from human post-mortem tissue (14,15).

Second, we performed gene expression analysis of cardiac markers such as *TNNI3* and *MYL3* by real-time PCR. The threshold cycle represents the PCR cycle at which an increase in SYBR Green fluorescence above a baseline signal can first be detected. Figure 1B shows threshold cycle values obtained for the genes *TNNI3*, *MYL3* and *GAPDH* in the five different autopsy samples. This result is indicative and demonstrates the maintenance expression of cardiac markers in the post-mortem tissue. Gene expression of beta-glucuronidase (*GUSB*), nitric oxide synthase 3 (*NOS3*), collagen 1 (*COL1A1*) and collagen 3 (*COL3A1*) has been previously shown using messenger RNA extracted from post-mortem

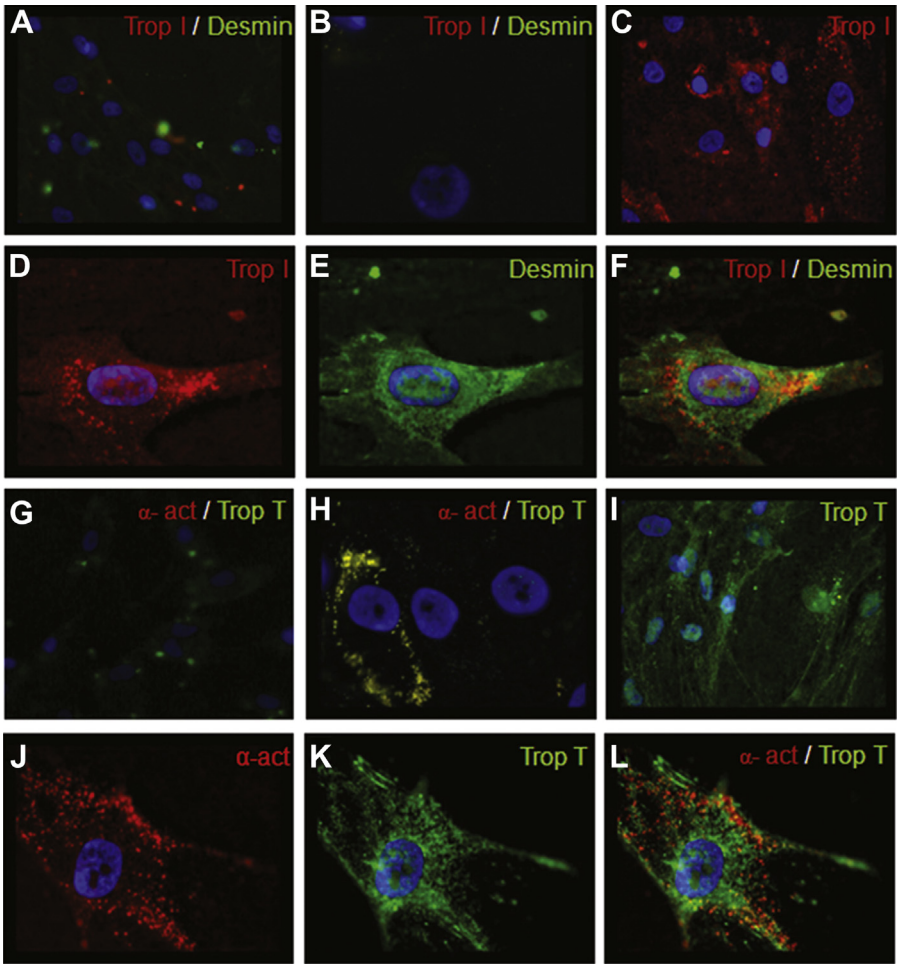


Figure 2. (A–L) Immunofluorescence analysis of cardiac markers in hASCs after extract induction. (C–F and I–L) Cardiomyogenic differentiation of hASCs after 2 weeks of being exposed to cellular extracts obtained from human autopsies. (A, B, G, H) Control non-treated cells. Red label for expression of troponin I (Trop I) and α-actinin (α-act) and green label for expression of desmin and troponin T (Trop T). Nuclei were stained with DAPI. (Original magnification ×40 for A, C, G and I and ×63 for B, D, E, F, H, J, K and L.)

cardiac tissue (16). Autopsy-derived tissue 24 h old seems to maintain genetic integrity and protein integrity and could represent a suitable tissue source for *in vitro* hASCs differentiation toward cardiomyocytes.

### *Expression of cardiac markers in hASCs after differentiation*

In this study, we tested the cardiac differentiation potential of two different methodologies, both based on the use of post-mortem cardiac tissue. Gene expression analysis of messenger RNA levels and immunofluorescence studies revealed expression of cardiac-specific markers in hASCs after exposition to the conditioned medium or to the cardiac cellular extract.

Figure 1C shows cardiomyocyte-related gene expression after cardiomyogenesis induction. Expression of early (*GATA-4* or *Mef2-c*) and late (cardiac α-actin and cardiac troponin I) cardiomyocyte development markers was found in treated cells. *GATA4* and *Mef2c* are cardiac-specific transcription factors, which have been reported to be implicated in cardiac commitment and differentiation (17). In addition, the expression of sarcomeric α-actinin and troponin I, two cardiomyocyte-specific markers, has been proved to be restricted to cardiomyocytes rather than skeletal muscle (18).

Indirect immunofluorescence analyses of human cardiac troponins (I and T), sarcomeric α-actinin and desmin protein distribution revealed clear differences between treated and untreated hASCs (Figures 2, 3). After induction by the cellular extract or by the conditioned medium, we found a high percentage of cells (50–60%) that were positive for troponin I with elongated nuclei, as shown by DAPI staining (Figures 2C,D, 3D). Desmin expression was strongly positive, with a marked filament organization in treated hASCs (Figures 2E, 3C,E) compared with control cells (Figures 2A,B, 3A,B). Although there

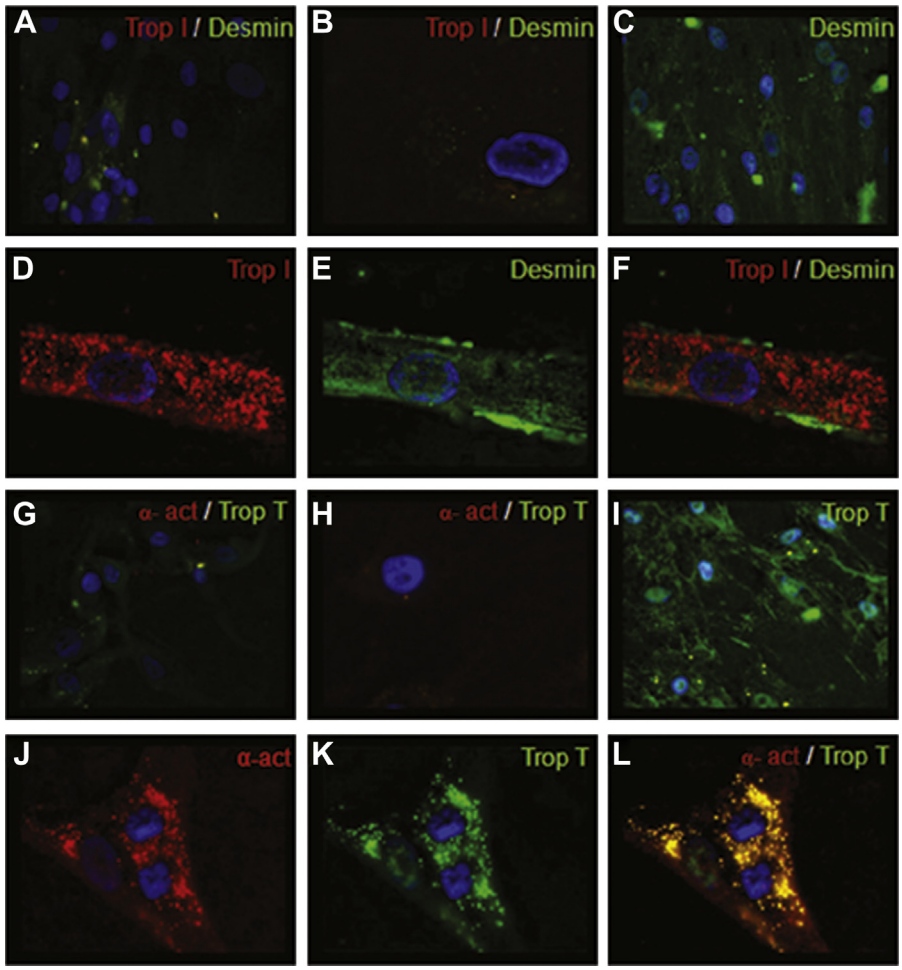


Figure 3. (A–L) Immunofluorescence detection of cardiac differentiation markers of hASCs cultured in conditioned medium. (C–F and I–L) hASCs after 2 weeks of being exposed to human cardiac explant-conditioned medium. (A, B, G, H) Control hASCs. Red label for expression of troponin I (Trop I) and α-actinin (α-act) and green label for expression of desmin and troponin T (Trop T). Nuclei were stained with DAPI. (Original magnification ×40 for A, C, G and I and ×63 for B, D, E, F, H, J, K and L.)

was a weak or non-significant staining for sarcomeric α-actinin in non-induced cells (Figures 2G,H, 3G,H), higher expression of this protein was observed in the exoskeleton of cells treated with both differentiation methods (Figures 2J, 3J). Finally, troponin T was highly expressed in treated cells exposed to the cardiac extract (Figure 2I,K) or cultured in conditioned medium (Figure 3I,K) with almost 65% of positive cells in both methods.

Morphologic changes were also noticeable after treatment with cardiac post-mortem tissue by both methodologies. Closer inspection of Figures 2 and 3 showed that treated cells extended their cytoplasmic processes with adjacent cells and achieved a myotube-like morphology, whereas control cells maintained their spindle-like morphology. In addition, multinucleated cells were detected after the induction (Figure 3J–L).

Cardiac tissue extracts or cell lysates from normal and infarcted myocardial tissue of rats have been proven to contribute to the differentiation of bone marrow-derived stromal cells into cardiomyocyte-like cells (19). These findings raise the hypothesis that soluble signaling molecules produced by cardiac cells after death are ideal inducers of hASC differentiation in myocardial-like cells. There have been 20 proteins including cytokines, growth factors and myocardial related proteins identified to be released into the culture medium by human cardiac explants (20). In our study, we demonstrated that the post-mortem cardiac tissue maintains its integrity and provides the paracrine mechanisms necessary to promote cardiomyogenic differentiation without direct cell-to-cell contact between cardiomyocytes and hASCs.

Previous studies have shown that cells can be isolated from post-mortem tissues (21,22). Viable hepatocytes can be isolated from cadaveric human liver after 24 h of cold ischemia (21). In addition, stem cells isolated from post-mortem tissues (48 h after death) are currently used for experimental and clinical purposes (23–27). Viable and functional

skeletal myogenic cells from humans 17 days post-mortem have been isolated (28).

It has been suggested that cardiomyocytes (29,30) and cardiac fibroblasts (31) secrete cytokines and chemokines in response to various stimuli, such as ischemia or mechanical stress to the heart. Post-mortem cardiac tissue might respond to the stress caused by the natural cardiac arrest and the hypoxic state that precedes natural death liberating factors. Ischemic myocardium produces several cytokines or transcription factors such as vascular endothelial growth factor and stromal cell derived factor that promote and increase stem cell survival (32) and, as has been proven *in vivo* and *ex vivo*, that induce differentiation of mesenchymal stromal cells into cardiomyocyte-like cells (33–35).

In conclusion, the results of the present study show that post-mortem cardiac tissue can induce hASCs to express cardiac-specific contractile proteins *in vitro*. This study confirms that the soluble signaling molecules produced by cardiac cells after death are also ideal inducers of hASC differentiation into myocardial-like cells. The methodology described here would serve as a useful and inexpensive *in vitro* model to obtain cardiomyocyte-like cells that could be used for cardioactive drug toxicity assays.

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## References

1. Mendis S, Puska P, Norrving B, editors. Global atlas on cardiovascular disease prevention and control. Geneva: World Health Organization; 2011.
2. He JQ, Ma Y, Lee Y, Thomson JA, Kamp TJ. Human embryonic stem cells develop into multiple types of cardiac myocytes. Circ Res. 2003;93:32–9.
3. Reppel M, Boettinger C, Hescheler J. Beta-adrenergic and muscarinic modulation of human embryonic stem cell-derived cardiomyocytes. Cell Physiol Biochem. 2004;14:187–96.
4. Caspi O, Itzhaki I, Kehat I, Gepstein A, Arbel G, Huber I, et al. In vitro electrophysiological drug testing using human embryonic stem cell derived cardiomyocytes. Stem Cells Dev. 2009;18:161–72.
5. Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, et al. Disease-specific induced pluripotent stem cells. Cell. 2008;134:877–86.
6. Gaustad KG, Boquest AC, Anderson BE, Gerdes AM, Collas P. Differentiation of human adipose tissue stem cells using extracts of rat cardiomyocytes. Biochem Biophys Res Commun. 2004;314:420–7.
7. Perán M, Marchal JA, López E, Jiménez-Navarro M, Boulaiz H, Rodríguez-Serrano F, et al. Human cardiac tissue induces transdifferentiation of adult stem cells towards cardiomyocytes. Cytotherapy. 2010;12:332–7.
8. Gupta S, Halushka MK, Hilton GM, Arking DE. Postmortem cardiac tissue maintains gene expression profile even after late harvesting. BMC Genomics. 2012;13:26.
9. Wang JS, Shum-Tim D, Galipeau J, Chedrawy E, Eliopoulos N, Chiu RC. Marrow stromal cells for cellular cardiomyoplasty: feasibility and potential clinical advantages. J Thorac Cardiovasc Surg. 2000;120:999–1005.
10. Sun CK, Chang LT, Sheu JJ, Chiang CH, Lee FY, Wu CJ, et al. Bone marrow-derived mononuclear cell therapy alleviates left ventricular remodeling and improves heart function in rat-dilated cardiomyopathy. Crit Care Med. 2009;37:1197–205.
11. Xie XJ, Wang JA, Cao J, Zhang X. Differentiation of bone marrow mesenchymal stem cells induced by myocardial medium under hypoxic conditions. Acta Pharmacol Sin. 2006;27:1153–8.
12. López-Ruiz E, Perán M, Cobo-Molinos J, Jiménez G, Picón M, Bustamante M, et al. Chondrocytes extract from patients with osteoarthritis induces chondrogenesis in infrapatellar fat pad-derived stem cells. Osteoarthritis Cartilage. 2013;21:246–58.
13. Schroeder A, Mueller O, Stocker S, Salowsky R, Leiber M, Gassmann M, et al. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol Biol. 2006;31:7–3.
14. Heinrich M, Matt K, Lutz-Bonengel S, Schmidt U. Successful RNA extraction from various human postmortem tissues. Int J Legal Med. 2007;121:136–42.
15. Vennemann M, Koppelkamm A. mRNA profiling in forensic genetics I: possibilities and limitations. Forensic Sci Int. 2010; 203:71–5.
16. Partemi S, Berne PM, Batlle M, Berruezo A, Mont L, Riuró H, et al. Analysis of mRNA from human heart tissue and putative applications in forensic molecular pathology. Forensic Sci Int. 2010;203:99–105.
17. Kodama H, Hirotani T, Suzuki Y, Ogawa S, Yamazaki K. Cardiomyogenic differentiation in cardiac myxoma expressing lineage-specific transcription factors. Am J Pathol. 2002;161: 381–9.
18. Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, et al. Cardiomyocytes can be generated from marrow stromal cells in vitro. J Clin Invest. 1999;103:697–705.
19. Liu XN, Yin Q, Zhang H, Zhang H, Zhu SJ, Wei YJ, et al. Tissue extracts from infarcted myocardium of rats in promoting the differentiation of bone marrow stromal cells into cardiomyocyte-like cells. Biomed Environ Sci. 2008;21: 110–7.
20. Schittini AV, Celedon PF, Stimamiglio MA, Krieger M, Hansen P, da Costa FD, et al. Human cardiac explant-conditioned medium: soluble factors and cardiomyogenic effect on mesenchymal stem cells. Exp Biol Med (Maywood). 2010;235:1015–24.
21. Erker L, Azuma H, Lee AY, Guo C, Orloff S, Eaton L, et al. Therapeutic liver reconstitution with murine cells isolated long after death. Gastroenterology. 2010;139:1019–29.
22. Machalinski B, Paczkowski M, Kawa M, Paczkowska E, Ostrowski M. An optimization of isolation of early hematopoietic cells from heparinized cadaveric organ donors. Transplant Proc. 2003;35:3096–100.
23. Blazar BR, Lasky LC, Perentesis JP, Watson KV, Steinberg SE, Filipovich AH, et al. Successful donor cell engraftment in a recipient of bone marrow from a cadaveric donor. Blood. 1986;67:1655–60.
24. Kapelushnik J, Aker M, Pugatsch T, Samuel S, Slavin S. Bone marrow transplantation from a cadaveric donor. Bone Marrow Transplant. 1998;21:857–8.

25. Liu X, Zhu Y, Gao W. Isolation of neural stem cells from the spinal cords of low temperature preserved abortuses. J Neurosci Methods. 2006;157:64–70.
26. Palmer TD, Schwartz PH, Taupin P, Kaspar B, Stein SA, Gage FH. Cell culture: progenitor cells from human brain after death. Nature. 2001;411:42–3.
27. Xu Y, Kimura K, Matsumoto N, Ide C. Isolation of neural stem cells from the forebrain of deceased early postnatal and adult rats with protracted post mortem intervals. J Neurosci Res. 2003;74:533–40.
28. Latil M, Rocheteau P, Châtre L, Sanulli S, Mémet S, Ricchetti M, et al. Skeletal muscle stem cells adopt a dormant cell state post mortem and retain regenerative capacity. Nat Commun. 2012;3:903.
29. Kacimi R, Karliner JS, Koudssi F, Long CS. Expression and regulation of adhesion molecules in cardiac cells by cytokines: response to acute hypoxia. Circ Res. 1998;82:576–86.
30. Shen JG, Quo XS, Jiang B, Li M, Xin W, Zhao BL. Chinonin, a novel drug against cardiomyocyte apoptosis induced by hypoxia and reoxygenation. Biochim Biophys Acta. 2000; 1500:217–26.
31. Porter KE, Turner NA. Cardiac fibroblasts: at the heart of myocardial remodeling. Pharmacol Ther. 2009;123: 255–78.
32. Leu S, Kao YH, Sun CK, Lin YC, Tsai TH, Chang LT, et al. Myocardium-derived conditioned medium improves left ventricular function in rodent acute myocardial infarction. J Transl Med. 2011;9:11–29.
33. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation. 2002;105:93–8.
34. Elmadbouh I, Haider H, Jiang S, Idris NM, Lu G, Ashraf M. Ex vivo delivered stromal cell-derived factor-1alpha promotes stem cell homing and induces angiomyogenesis in the infarcted myocardium. J Mol Cell Cardiol. 2007;42:792–803.
35. Das H, George JC, Joseph M, Das M, Abdulhameed N, Blitz A, et al. Stem cell therapy with overexpressed VEGF and PDGF genes improves cardiac function in a rat infarct model. PLoS One. 2009;4:e7325.

## Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.jcyt.2013.06.016