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Research article

First trimester placental mesenchymal stem cells improve cardiac function of rat after myocardial infarction via enhanced neovascularization

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ABSTRACT

Acute myocardial infarction (AMI) is the most critical heart disease. Mesenchymal stem cells (MSCs) have been widely used as a therapy for AMI for several years. The human placenta has emerged as a valuable source of transplantable cells of mesenchymal origin that can be used for multiple cytotherapeutic purposes. However, the different abilities of first trimester placental chorion mesenchymal stem cells (FCMSCs) and third trimester placental chorion mesenchymal stem cells (TCMSCs) have not yet been explored. In this study, we aimed to compare the effectiveness of FCMSCs and TCMSCs on the treatment of AMI. FCMSCs and TCMSCs were isolated and characterized, and then they were subjected to in *vitro* endothelial cell (EC) differentiation induction and tube formation to evaluate angiogenic ability. Moreover, the in *vivo* effects of FCMSCs and TCMSCs on cardiac improvement were also evaluated in a rat MI model. Both FCSMCs and TCMSCs expressed a series of MSCs surface markers. After differentiation induction, FCMSCs-derived EC (FCMSCs-EC) exhibited morphology that was more similar to that of ECs and had higher CD31 and vWF levels than TCMSCs-EC. Furthermore, tube formation could be achieved by FCMSCs-EC that was significantly better than that of TCMSCs-EC. Especially, FCMSCs-EC expressed higher levels of pro-angiogenesis genes, PDGFD, VEGFA, and TNC, and lower levels of anti-angiogenesis genes, SPRY1 and ANGPTL1. In addition, cardiac improvement, indicated by left ventricular end-diastolic diameter (LVEDd), left ventricular end-systolic diameter (LVEDs), left ventricular ejection fraction (LVEF) and left ventricular shortening fraction (LVSF), could be observed following treatment with FCMSCs, and it was superior to that of TCMSCs and Bone marrow MSCs (BMSCs). FCMSCs exhibited a superior ability to generate EC differentiation, as evidenced by in *vitro* morphology, angiogenic potential and in *vivo* cardiac function improvement; further, increased levels of expression of pro-angiogenesis genes may be the mechanism by which this effect occurred.

1. Introduction

Acute myocardial infarction (AMI) is an acute myocardial ischemic necrosis caused by a drastic decrease or interruption in the coronary artery blood supply. Patients who survive AMI suffer from massive loss of myocytes due to necrosis and heart failure [1]. Although tremendous advances in treatment concepts and techniques have been made in recent years, including drugs, coronary artery stent implantation and bypass surgery, the damaged cardiac tissue of AMI patients cannot be effectively repaired [2]. Because of the limitation in AMI treatment, the use of stem cell therapies for treating AMI is increasing.

Bone marrow mesenchymal stem cells (BMSCs) have commonly been used in both basic studies [3,4] and clinical trials [5,6] as useful therapeutic seed cells for AMI. In addition to bone marrow, human placenta mesenchymal stem cells (PMSCs) transplantation were also considered to be an effective therapeutic approach for autoimmune diseases, bone

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repair, and cancer [7,8,9]. Human chorionic mesenchymal stem/stromal cells (CMSCs), also known as PMSCs, were isolated from third trimester placentas and were found to possess stem cell-like properties [10,11,12,13]. Although CMSCs have been widely used in basic research in the past decade, another kind of CMSC from the first trimester placenta has recently received increasing attention [14,15,16]. However, the different roles of first trimester CMSCs (FCMSCs) and third trimester CMSCs (TCMSCs) in the setting of AMI have not yet been explored.

Here, we aimed to evaluate the endothelial cell (EC) differentiation and angiogenesis ability of first trimester placental chorion mesenchymal stem cells (FCMSCs). As we know that when the human embryo develops to 2 weeks, the extra-embryonic mesoderm cells are formed, which, together with the trophoblasts, further form dense chorionic villi and participate in the formation of early placental tissue. The mesenchyme of this part of the extraembryonic mesoderm has a strong ability of forming blood vessels. So, based on the above, we hypothesized that FCMSCs isolated from early chorionic tissue would show a stronger EC differentiation ability and improve cardiac function more than TCMSCs after MI.

2. Methods

2.1. Ethical approval of the study protocol

The Ethics Committee of Soochow University approved the use of FCMSCs, TCMSCs and BMSCs. (Approval No. ECSU-2019000140). Written informed consent was obtained from all donors. The animal study protocol was also approved by the Ethics Committee of Soochow University and was conducted following international guidelines for animal experimentation (Approval No. ECSU-2019000141).

2.2. MSCs isolation

2.2.1. CMSCs

The first trimester placental tissue was obtained by vacuum suction, following termination of uncomplicated pregnancies (7–9 weeks of gestation). The study of FCMSCs was approved by the ethics committee of Soochow University (Approval No. ECSU-2019000140). For the preparation of TCMSCs, third trimester placental tissue was obtained from uncomplicated term pregnancies by cesarean section, and informed patient consent was obtained, along with the approval of the Soochow University Ethics Committee. Furthermore, both first- and third-trimester placental tissue were washed with phosphate buffered saline (PBS) to remove the blood. The chorionic villus samples were minced and digested according to our previous studies [17]. Subsequently, cells were collected and cultured in low glucose Dulbecco's modified Eagle's medium (LG-DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco, CA, USA) and 5 ng/mL bovine basic fibroblast growth factor (bFGF). CMSCs were passaged upon reaching approximately 80% confluence. After 14 days of culture, CMSCs developed a homogeneous fibroblastic morphology. Finally, CMSCs were collected and cryopreserved in liquid nitrogen for further experiments. All CMSCs used were between passages 1 and 5.

2.2.2. BMSCs

The Ethics Committee of Soochow University approved the use of BMSCs (Approval No. ECSU-2019000140). Written informed consent was obtained from all donors. MSCs were derived from bone marrow aspirates of healthy adult donors. The BMSCs preparation method was described previously [18]. After culturing for 7 days, BMSCs exhibited a spindle-shaped morphology. All BMSCs used were between passages 1 and 5.

2.3. Flow cytometry

The immunophenotypes of MSCs were analyzed by flow cytometry [3]. MSCs were harvested and incubated for 30 min at 4 °C with monoclonal antibodies against CD34, CD73, CD90, CD105 and HLA-DR or the appropriate isotype controls (eBiosciences or BD Biosciences). The expression of CD73, CD90, CD105 are regarded as the Gold standard for phenotypic identification of mesenchymal stem cells, low expression of HLA-DR indicated the low immunogenicity of cells, CD34 is one of the markers of specific expression in hematopoietic stem cells. Later, the surface antigens of MSCs were detected using a Gallios flow cytometer and were analyzed using Kaluza software (Beckman Coulter). In addition, the expression of CD31 and vWF in both FCMSC-ECs and TCMSC-ECs was also analyzed by flow cytometry.The CD31 and vWF are specially expressed on vascular endothelial cells.

2.4. Cell culture

Human umbilical vein endothelial cells (HUVECs) were maintained in endothelial basal medium-2 (EBM-2) containing endothelial growth medium-2 (EGM-2 SingleQuots Kit, Lonza). The medium was changed every 3 days. HUVECs were used between passages 1 and 5.

2.5. Endothelial cell differentiation induction

For endothelial cell differentiation induction, MSCs from passage 3 were cultured in SIGMA MCDB 131 medium (Sigma Chemical Co., USA) containing 5% FBS, 50 ng/ml VEGF and 20 ng/ml IGF-1 (Pepro Tech, USA). Briefly, 50,000 FCMSCs, TCMSCs, BMSCs or HUVECs were cultured on a 6-well plate. Here, HUVECs were set as positive controls, which had the strongest ability to differentiate into endothelial cells and form capillary-like structures. Furthermore, MSCs were incubated at 37 °C and the culture medium was replaced with new medium every 2 days [19].After 14 days, these MSCs were designated MSC-ECs.

2.6. Non-quantitative and quantitative reverse transcription PCR

Total cellular RNA was extracted from the above cells that underwent different treatments with TRIzol reagent (Invitrogen). For nonquantitative experiments, the RevertAid First Strand cDNA Synthesis Kit (Fermentas) and PCR Master Mix (Promega) were employed for cDNA synthesis and gene product expansion, respectively, according to the manufacturer's instructions. The products of reverse transcription-polymerase chain reaction (RT-PCR) were detected by electrophoresis in agarose gels. The primers for Sox-2, Nanog, Oct4, FLT-1, KDR, and GAPDH were listed in Table 1. In addition, real-time PCR was performed using an iScript™ One-Step RT-PCR Kit with SYBR® Green (#1708893, Bio-Rad, CA, USA) according to the manufacturer's instructions. Data were collected with an iQ5 Real-time PCR Detection system (Bio-Rad, Hercules, CA, USA). Relative gene expression was analyzed by the $2^{-\Delta\Delta CT}$ method [20]. The primers for multiple angiogenesis-related genes PDGFD, TEK, VEGFA, SPRY1, TNC, MFAP5, HBEGF, ANGPTL1 and GAPDH were listed in Table 2.

2.7. In vitro angiogenesis assay

Tube formation assays were used to assess neovascularization ability. In brief, trypsinized FCMSC-ECs, TCMSC-ECs or HUVECs were harvested, resuspended (1×10^4 cells), and seeded on pretreated Matrigel. After cells were incubated at 37 °C for 6 h, forming tube structures could be observed under an Olympus microscope [21]. The average tube length was quantified using AngioTool software. FCMSCs, TCMSCs and HUVECs from passages 3 were used here.

2.8. Rat model of acute myocardial infarction

For the generation of an MI model, 8-week-old male Wistar rats (n = 45) were used. Rats weighing 250–280g were anaesthetized with intraperitoneal ketamine (100 mg/kg) and xylazine (10 mg/kg) [21,22], and a small incision was made in the fourth intercostal space. After that, the

Table 1. Gene Expression (RT-PCR) primers used in this study.

Gene	Forward primer (5′- 3′)	Reverse primer (5′- 3′)
Sox2	ATGACCAGCTCGGAGACCTACAT	TCTGGTAGTGCTGGGACATGTGAA
Nanog	CTTGCCTTGCTTTGAAGCATCCGA	CTGCAGAAGTGGGTTGTTTGCCTT
Oct4	CGAGCAATTTGCCAAGCTCCTGAA	TTCGGGCACTGCAGGAACAAATTC
FLT-1	GAAAACGCATAATCTGGGACAGT	GCGTGGTGTGCTTATTTGGA
KDR	AACGTGTCACTTTGTGCAAGA	TTCCATGAGACGGACTCAGAA
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC

heart was then gently brought out through the window. Moreover, the left main descending coronary artery (LCA) was ligated at a site 3 mm from its origin. After ligation, 2×10^6 MSCs or saline were immediately intramyocardially injected into the border zone of MI model rats: 1) saline (MI group, n = 9); 2) FCMSCs (FCMSCs group, n = 12); 3) TCMSCs (TCMSCs group, n = 12); 4) BMSCs (BMSCs group, n = 12). In brief, the duration of the experiment was about 60mins. After that, the animal health and behavior were monitored every day. At 2 and 4 weeks after MI, all rats were assessed by dimensional Doppler ultrasound (PHILIPS 5500, Netherlands) to evaluate the left ventricular end-diastolic diameter (LVEDd), left ventricular end-systolic diameter (LVEDs), left ventricular ejection fraction (LVEF) and left ventricular shortening fraction (LVSF). LVEDd, LVEDs, LVEF and LVSF are common indicators of cardiac function, when cardiac function decreases LVEDd and LVEDs increase in different degrees, while LVEF and LVSF decrease. Finally, after final imaging was performed at week 4, the subjects were killed for tissue harvesting and histologic evaluation. Histologic evaluation was observed over the 4 weeks following implantation. All animals were euthanized by CO_2 inhalation [23,24]. All procedures were approved by the Ethics Committee of Soochow University (Approval No. ECSU-2019000141) according to the Guide for the Care and Use of Laboratory Animals.

2.9. Masson's trichrome staining

Heart tissues from MI model rats with or without treatment with TCMSCs or FCMSCs cells were isolated 4 weeks after treatment. The tissues were then fixed in 10% formalin at room temperature. After fixation, heart tissues were embedded in paraffin. After that, the paraffin-embedded samples were sectioned at a thickness of 5 μm. Finally, the tissue sections were stained with a Masson's trichrome staining kit (Sigma, St. Louis, MO, USA) to assess the fibrosis of hearts according to the manufacturer's protocol.

2.10. Immunofluorescence

Before staining, the FCMSC-ECs and TCMSC-ECs were pre-inoculated on the microscope cover glass slides (Fisherbrand). Then, the cells were incubated with PBS containing 5% bovine serum albumin to block nonspecific protein binding sites. After that, the cells were incubated overnight at 4 °C with a specific antibody against mouse anti-human CD31 (1:3000, Cell Signaling Technology). Moreover, incubation with HRP-conjugated secondary antibodies (Life Technologies, Duren, DE) was carried out for 30 min at room temperature. Finally, DAPI (Dojindo Laboratories, Kumamoto, Japan) was used to stain the nuclei, and images were collected by fluorescence microscopy (Nikon Eclipse Ni, Tokyo, Japan).

2.11. Statistical analyses

All statistical values were assessed by using SPSS v18 (SPSS, Chicago, IL, USA). Data are reported as the means ± standard deviation (SD). Multiple groups were assessed through one-way analysis of variance followed by the Bonferroni test. A p-value <0.05 was considered to be significant.

3. Results

3.1. Morphological and phenotypic comparison between FCMSCs and TCMSCs

To study the role of CMSCs, we first isolated FCMSCs and TCMSCs and analyzed their morphological and phenotypic differences. As shown in Figure 1A, primary FCSMCs and TCMSCs had the similar morphology with the mesenchymal stem cells. Moreover, flow cytometry analyses revealed that both FCMSCs and TCMSCs had no surface expression of CD34 and HLA-DR, but they expressed CD73, CD90 and CD105 (Figure 1B). In addition, the stem cell marker evaluation revealed that unique pattern of Sox-2 and Oct-4 expression was found on primary FCMSCs, whereas weak expression of Sox-2, Nanog and Oct-4 on primary TCMSCs. After cell passage, the FCMSCs initiated the expression of Sox-2, Nanog and Oct-4, which was similar to the feature of human embryonic stem cells (Figure 1C). These results indicated that FCMSCs might possess a more primitive feature compared with TCMSCs. In the views of the multilineage differentiation potential, both FCMSCs and TCMSCs were able to differentiate into fat and bone (Figure S1).

3.2. Endothelial cell differentiation ability comparison between FCMSCs and TCMSCs

Since FCMCs and TCMSCs were obtained, we further explored their potential for functional endothelial cell differentiation. Because VEGF promotes angiogenesis by two important receptors, FLT-1 and KDR, we first examined the expression of FLT-1 and KDR, and the results showed

Table 2. Gene Expression (real-time RT-PCR) primers used in this study.

Gene	Forward primer (5′- 3′)	Reverse primer (5′- 3′)
PDGFD	TTGTACCGAAGAGATGAGACCA	GCTGTATCCGTGTATTCTCCTGA
TEK	TCCGCTGGAAGTTACTCAAGA	GAACTCGCCCTTCACAGAAATAA
VEGFA	AGGGCAGAATCATCACGAAGT	AGGGTCTCGATTGGATGGCA
SPRY1	GAGAGAGATTCAGCCTACTGCT	GCAGGTCTTTTCACCACCGAA
TNC	TCCCAGTGTTCGGTGGATCT	TTGATGCGATGTGTGAAGACA
MFAP5	GGGTCAATAGTCAACGAGGAGA	CTGTAGCGGGATCATTCACCA
HBEGF	ATCGTGGGGCTTCTCATGTTT	TTAGTCATGCCCAACTTCACTTT
ANGPTL1	CAGGGCCAATCTGTGTCAACA	CAGAACATCTATCTCCCGCTTC
GAPDH	CTGGGCTACACTGAGCACC	AAGTGGTCGTTGAGGGCAATG

that the expression of both FLT-1 and KDR could be found on primary FCMSCs, passaged FCMSCs and primary TCMSCs but not passaged TCMSCs and BMSCs (Figure 2A). We also induced differentiation into endothelial cells, and the images revealed that in comparison to HUVECs, a similar morphology could be found in FCMSC-ECs but not in TCMSC-ECs or BMSC-ECs (Figure 2B). Furthermore, flow cytometry and immunofluorescence analyses confirmed the higher expression of the endothelial cell markers CD31 and vWF in FCMSCs than in TCMSCs (Figure 2C and D). Moreover, an in vitro tube formation experiment revealed that significantly improved tube formation could be achieved by FCMSC-ECs compared to TCMSC-ECs (Figure 2E). Based on these findings, we demonstrated that FCMSCs could be induced to form functional endothelial cells.

3.3. Mechanisms involved in the endothelial cell differentiation ability of FCMSCs and TCMSCs

To further explore the possible mechanism involved in the endothelial cell differentiation process of FCMSCs, we examined multiple angiogenesis-related genes involved in tube formation. The results showed that induction to differentiate from FCMSCs into endothelial cells resulted in significantly elevated PDGFD, VEFGA, SPRY1 and TNC and decreased levels of TEK and MFAP5, whereas induction to differentiate into endothelial cells in TCMSCs increased the levels of PDGFD, TEK, VEGFA, SPRY1, HBEGF and ANGPTL1 and decreased the levels of TNC and MFAP5 (Figure 3). These data suggested that FCMSCs could produce differential angiogenic factors involved in angiogenesis.

3.4. Effects of cardiac improvement by FCMSCs and TCMSCs in vivo

According to previous studies, effective angiogenesis may lead to cardiac improvement in the setting of myocardial infarction. Therefore, we analyzed the cardiac improvement effects of FCMSCs and TCMSCs in vivo in Table 3. The results of cardiac improvement, as indicated by LVEDd, LVEDs, LVEF and LVSF, were better following treatment with FCMSCs than they were for TCMSCs and BMSCs (Figure 4A–D). Moreover, Masson's trichrome staining analysis showed that many necrotic myocardial cells were replaced by collagen fibers in the infarcted area of

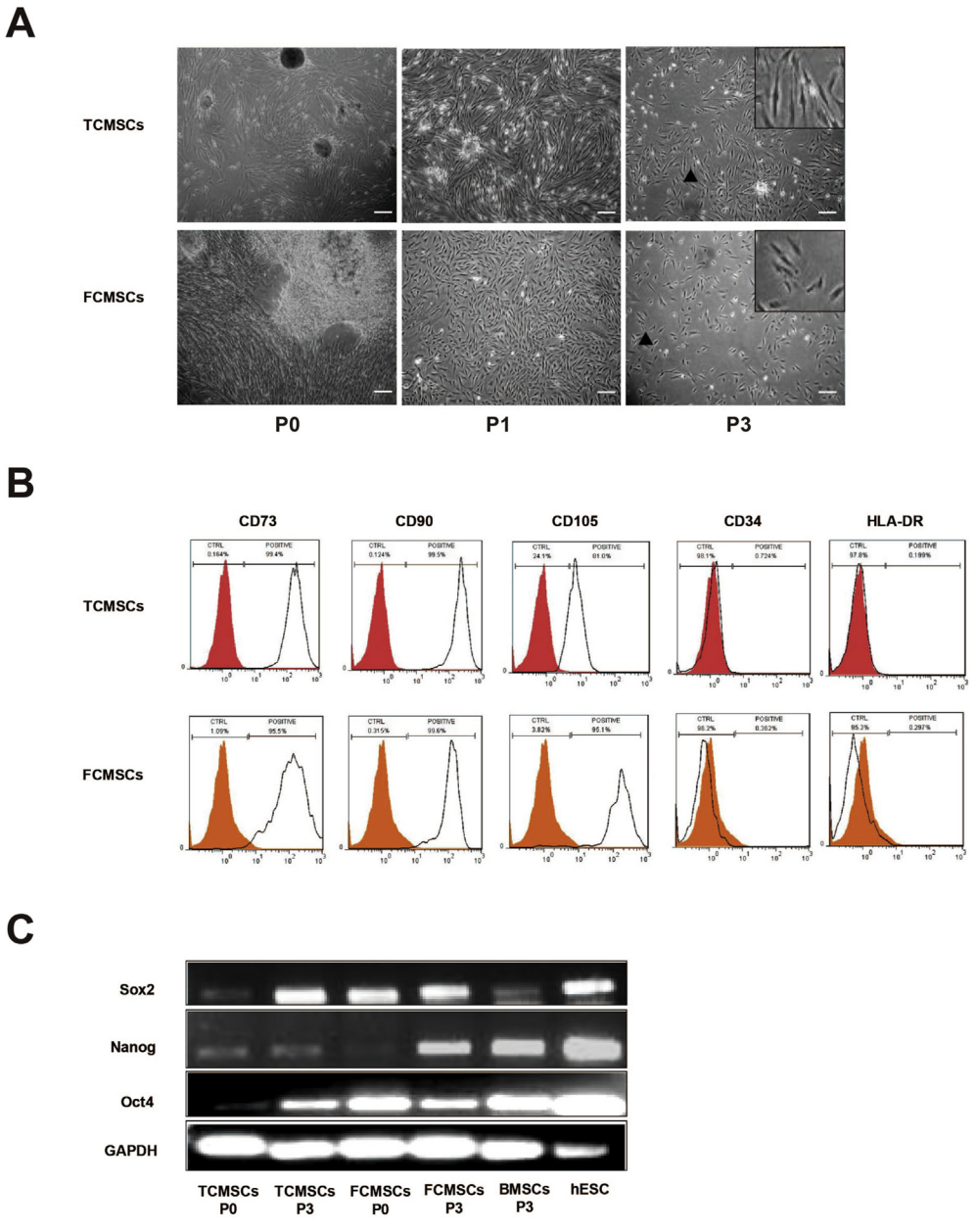

Figure 1. Morphology and phenotype comparison of human first (F) and third trimester (T) placental chorion mesenchymal stem cells (CMSCs). A. Microscopy analyses of the morphology of human FCMSCs and TCMSCs. The insets show the represent amplified pictures of the FCMSCs and TCMSCs cells (indicated by black arrows) (left: P0 means primary cells, middle: P1 means passaged cells in one time, right: P3 means passaged cells in three times), scale bars, 40 μm. B. Flow cytometry analysis of the expression of surface markers, CD73, CD90, CD105, CD34 and HLA-DR, on FCMSCs and TCMSCs. C. Expression of the stem cell markers Sox2, Nanog and Oct4 in human FCMSCs and TCMSCs.

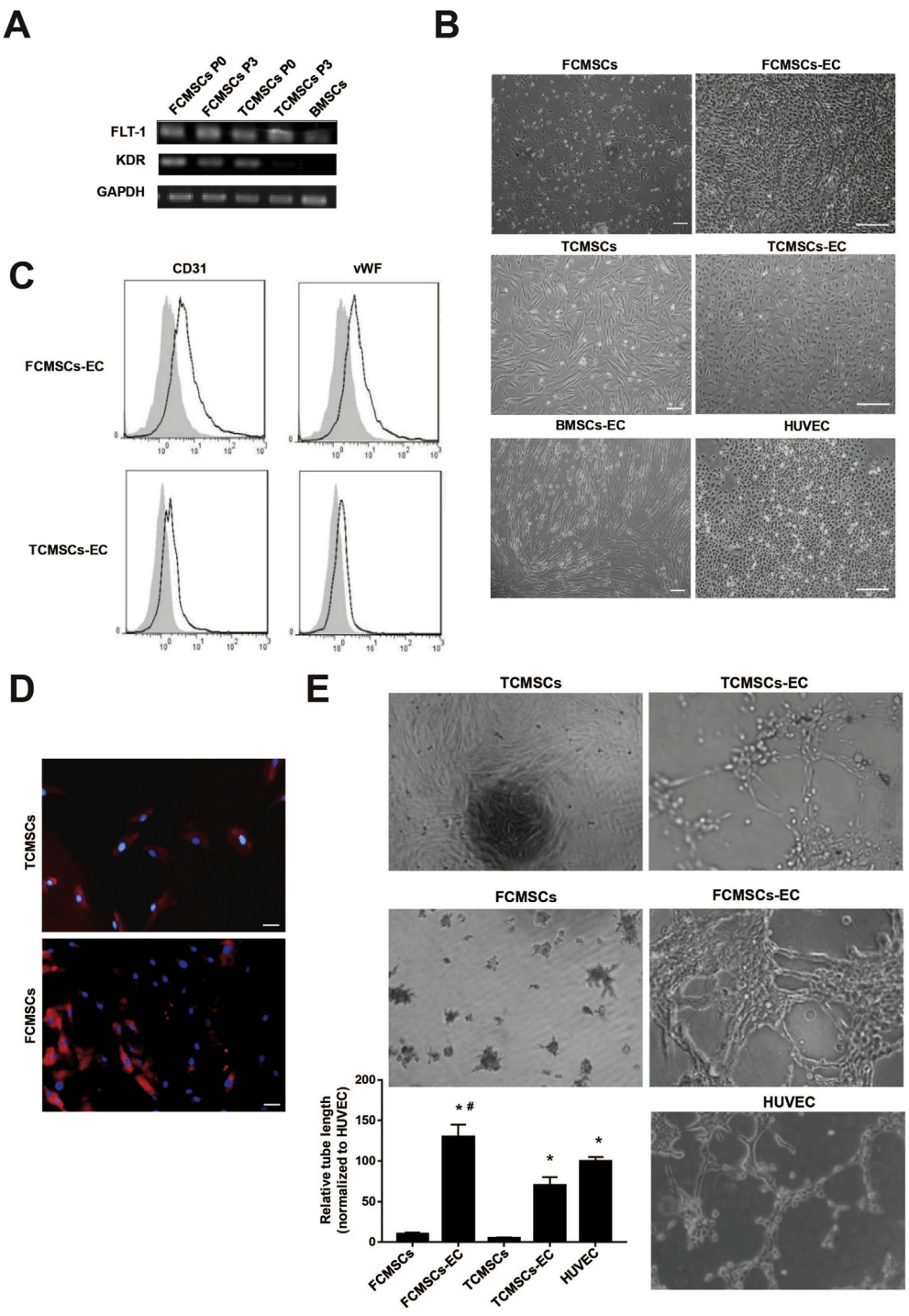

Figure 2. VEGF receptor expression, endothelial cell differentiation ability and surface expression of endothelial-specific markers on human first (F) and third trimester (T) placental chorion mesenchymal stem cells (CMSCs). A. Expression of the two VEGF receptors KDR and FLT-1 in FCMSCs and TCMSCs was determined by RT-PCR. B. Microscopy analyses of the morphology of FCMSCs and TCMSCs after endothelial cell differentiation induction, Scale bars, 40 μm. C, D. Flow cytometry and immunofluorescence evaluation of the expression of the endothelial cell markers vWF and CD31 in FCMSCs and TCMSCs after endothelial cell differentiation induction, Scale bars, 20 μm. E. Angiogenesis ability of FCMSCs and TCMSCs. Significant tube formation was observed with FCMSCs, which is similar to that of HUVECs, but not TCMSCs. $^{*}P < 0.05$ compared to MSCs without endothelial cell differentiation induction; $^{\#}P < 0.05$ compared to the HUVEC group.

the control group. There were fewer remaining myocardial cells, and they were disordered. Compared with normal myocardial cells, there were more elastic fibers in the aggregation of collagen fibers and more elastic fibers in the epicardium of the treatment groups (Figure 5A). The collagen fibers and elastic fibers in the infarcted area were significantly reduced in both the FCMSCs and TCMSCs groups (Figure 5B and C), especially in the FCMSCs group (Figure 5C). We further assessed the expression of human vascular endothelial cell markers in response to FCMSCs transplantation in a rat MI model. As shown in Figure S2, human CD31-and vWF-positive cells were found in the myocardial infarction area of rats after transplantation.

4. Discussion

Because of divergent developmental requirements, the fetus and placenta grow more rapidly in the first trimester than they do in the subsequent trimesters. Therefore, the function between early placental MSCs and later pregnancy MSCs is different. However, no studies have compared the roles of MSCs from different developmental origins [25]. Here, we explored the effect of gestational age over this temporal window. In brief, we compared the phenotypic and functional characterization (EC differentiation) of MSCs derived from the regions of chorionic villi of the human first and third trimester placenta and evaluated the efficacy of these placenta-derived MSCs in a rat model of MI. Our results

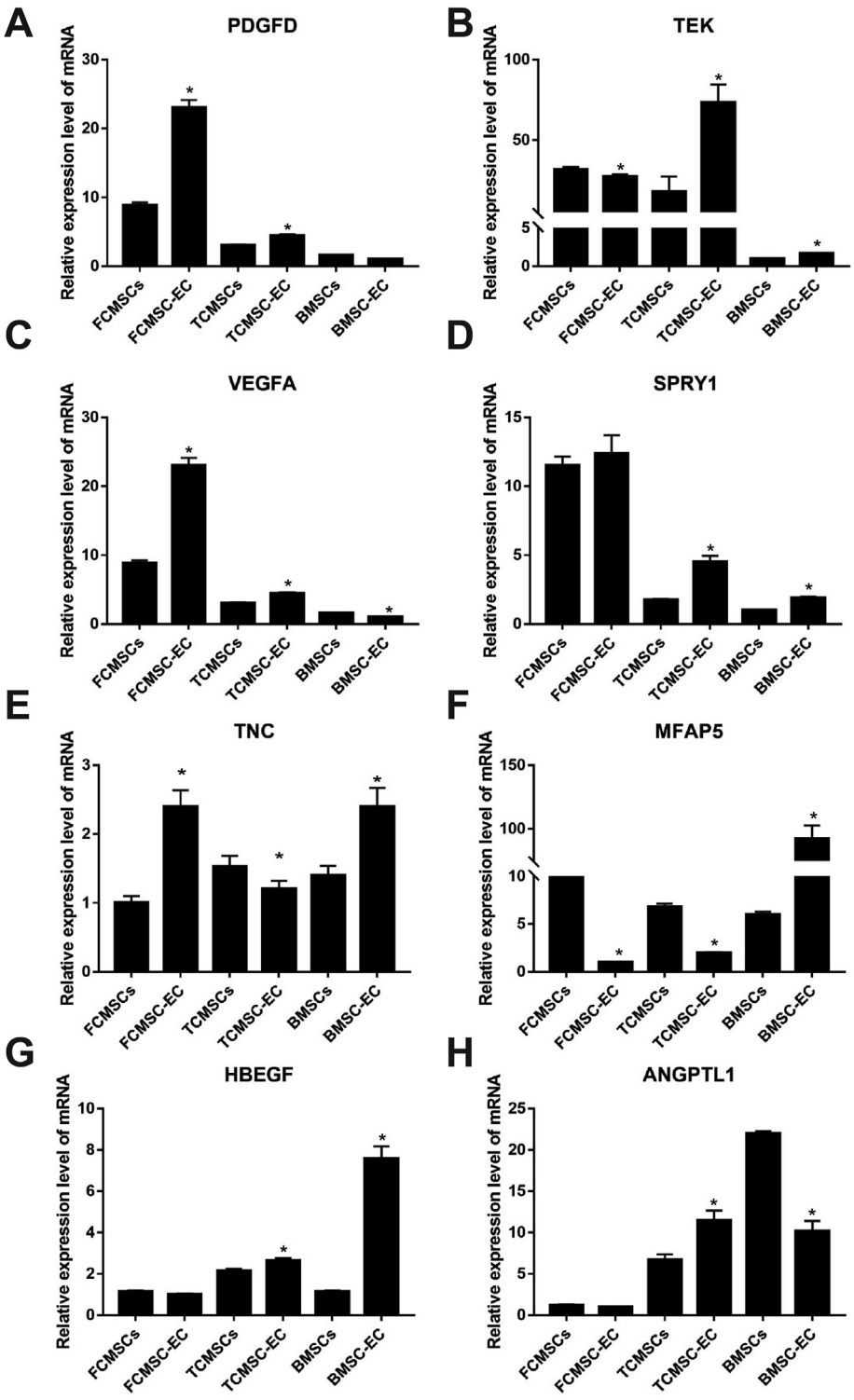

Figure 3. Real-time quantitative PCR analyses of the expression of proangiogenesis- and antiangiogenesis-related genes, PDGFD (A), TEK (B), VEGFA (C), SPRY1 (D), TNC (E), MFAP5 (F), HBEGF (G) and ANGPTL1 (H). $P < 0.05$ compared to MSCs without endothelial cell differentiation induction.

showed that compared to TCMSCs, primary FCSMCs were smaller, but they had a similar pattern of MSCs surface markers. After EC differentiation induction, FCMSC-ECs showed morphology that was more like ECs and exhibited higher CD31 and vWF when compared to TCMSC-ECs. Moreover, significantly better tube formation could be achieved by FCMSC-ECs compared to TCMSC-ECs. Furthermore, FCMSC-ECs expressed higher levels of pro-angiogenesis genes, PDGFD, VEGFA, and TNC, and lower levels of anti-angiogenesis genes, SPRY1 and ANGPTL1. In addition, superior cardiac improvement, as indicated by LVEDd, LVEDs, LVEF and LVSF, could be achieved by FCMSCs compared to TCMSCs and BMSCs.

According to previous studies, both amniotic membrane-derived mesenchymal stem cells (AMSCs) and chorionic-derived mesenchymal stem cells have multipotent differentiation potential, as they can be induced to differentiate into different kinds of cells under different experimental conditions; for example, they can generate endodermal

Table 3. MSCs improved cardiac function in a rat model of MI.

Group	LVEDd (mm)	LVEDs (mm)	LVEF (%)	LVSF(%)
MI	8.41 ± 0.36	6.81 ± 0.42	45.08 ± 5.01	22.18 ± 7.01
FCMSCs	6.80 ± 0.36*#&	4.92 ± 0.22*#&	65.08 ± 6.21*#&	34.12 ± 5.21*#&
TCMSCs	7.61 ± 0.26*	5.23 ± 0.31*	55.08 ± 6.13*	30.12 ± 6.21*
BMSCs	7.42 ± 0.46*	5.43 ± 0.26*	57.08 ± 5.31*	29.12 ± 5.91*

A rat model of myocardial infarction was established. After ligation, 2×10^6 FCMSCs, TCMSCs or BMSCs was injected into 3 locations on the border of the infarcted area. There are four groups: 1) saline (MI group, n = 9); 2) FCMSCs (FCMSCs group, n = 12); 3) TCMSCs (TCMSCs group, n = 12); 4) (BMSCs group, n = 12). At 4 weeks, cardiac improvement was indicated by left ventricular end-diastolic diameter (LVEDd), left ventricular end-systolic diameter (LVEDs), left ventricular ejection fraction (LVEF), and left ventricular shortening fraction (LVSF) that was superior in FCMSCs compared to TCMSCs and BMSCs. $^*P < 0.05$ compared to the MI group; $^{\#}P < 0.05$ compared to the BMSCs group; $^{\&}P < 0.05$ compared to the TCMSCs group.

cells such as pancreatic and liver cells [26], mesodermal cells such as cartilage, bone, fat [27,28], cardiomyocytes, and ectodermal cells such as neurons [29]. Further studies showed that the biological characteristics of MSCs derived from the two sources above were not exactly the same. AMSCs have good neurobiological characteristics and can be used to repair cerebral ischemia well in rats. However, CMSCs can easily form capillary-like structures in vitro, which is an ideal seed cell for stem cell transplantation to repair ischemic injury diseases [30]. Currently, most CMSCs used in the laboratory are from full-term placental chorionic membranes. At the beginning of the second week of embryonic development, the chorionic villus contains only trophoblast cells, and it is called the primary stem villus. Then, at the end of the second week or the beginning of the third week of embryonic development, until the extra-embryonic mesoderm structure grows inside the trophoblast, the villus is called the secondary stem villus. Finally, the secondary stem villus develops into the tertiary stem villus only when the inside extraembryonic mesoderm differentiates into vascular networks and connective tissue communicating with the blood vessels in the embryo, which then further develops into the chorionic membrane of the placenta. The extraembryonic mesoderm and the connective tissue that developed from the

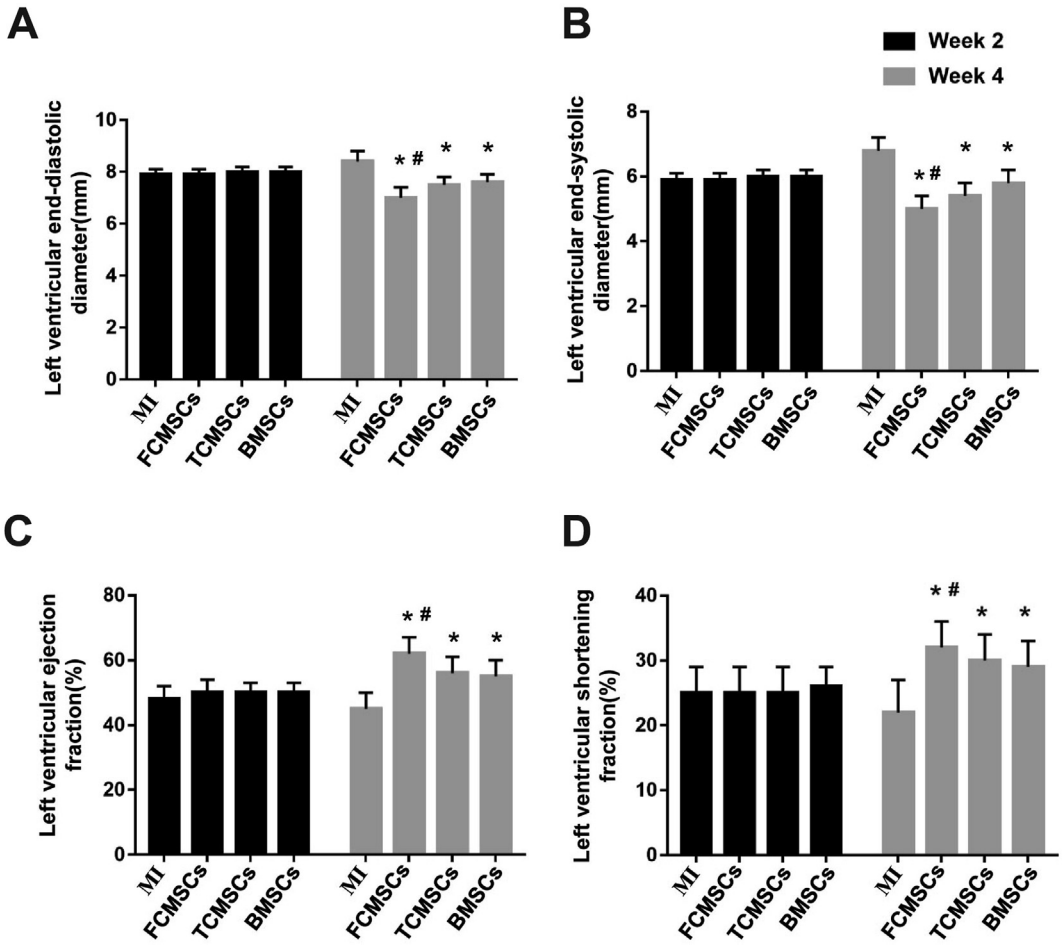

Figure 4. Effects of cardiac improvement by human first (F) and third trimester (T) placental chorion mesenchymal stem cells (CMSCs) in vivo. A rat model of myocardial infarction was established. After ligation, 2×10^6 FCMSCs, TCMSCs or BMSCs was injected into 3 locations on the border of the infarcted area. There are four groups: 1) saline (MI group, n = 9); 2) FCMSCs (FCMSCs group, n = 12); 3) TCMSCs (TCMSCs group, n = 12); 4) (BMSCs group, n = 12). At 4 weeks, cardiac improvement was indicated by left ventricular end-diastolic diameter (LVEDd, A.), left ventricular end-systolic diameter (LVEDs, B.), left ventricular ejection fraction (LVEF, C.), and left ventricular shortening fraction (LVSF, D.) that was superior in FCMSCs compared to TCMSCs and BMSCs. $^*P < 0.05$ compared to the MI group; $^{\#}P < 0.05$ compared to the BMSCs group.

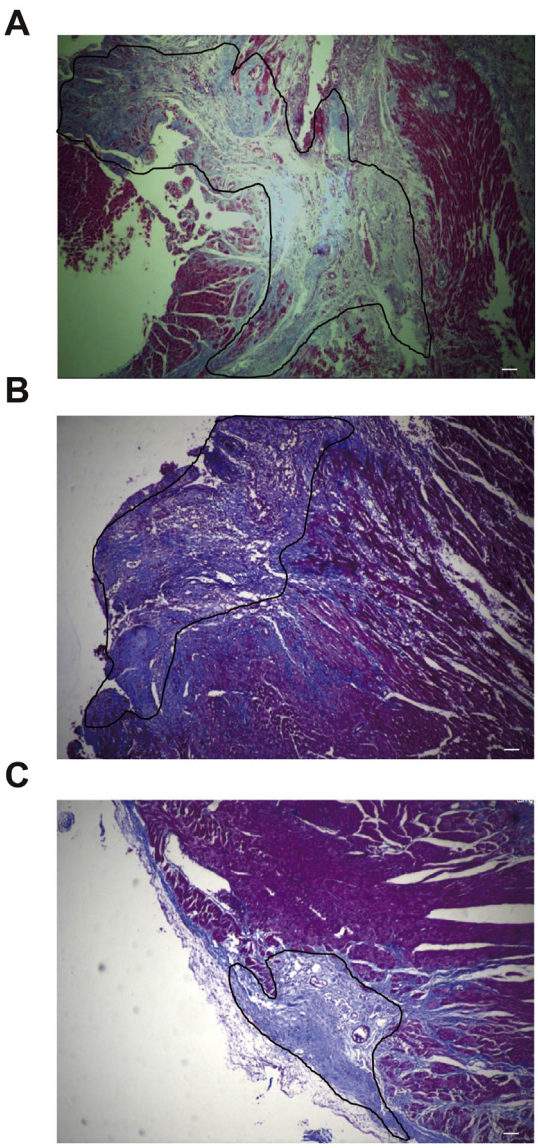

Figure 5. Human first (F) and third trimester (T) placental chorion mesenchymal stem cells (CMSCs) ameliorate myocardial infarction. Heart histological changes were assessed 4 weeks after CMSCs transplantation as described in the Methods. Representative images of Masson's trichrome-stained sections of heart tissues for MI group (A), TCMSCs group (B), and FCMSCs group (C) are shown, indicating that treatment with FCMSCs strongly ameliorates myocardial infarction-induced collagen fibers and elastic fibers in the infarcted area. The Black dashed circle: the area of the myocardial infarction. Scale bars, 5μm.

extraembryonic mesoderm are the real sources from which chorionic MSCs are isolated [31]. According to the development of chorionic villi, we speculated that compared with TCSMCs, FCSMCs would have a more significant ability to differentiate into endothelial cells and form blood vessels. Our experimental results also confirmed that in addition to having an immunological phenotype similar to TCMSCs, FCSMCs have a stronger ability to differentiate into endothelial cells and form capillary-like structures, which was used to help to alleviate a model myocardial infarction injury.

Passipieri et al [32] compared the function of cells derived from the regions of chorionic villi (cv) and chorionic plate (cp) in the human term placenta and found that cp-derived MSCs had greater clonogenic potential and higher expression of genes related to cell cycle progression, which improved cardiac function via a mouse model of MI. Recently, the human term placental tissue-derived MSCs, or exosome from cultures of these cells, have been reported to enhance angiogenesis [33,34]. Here, we performed quantitative analyses of the pro-angiogenesis and anti-angiogenesis genes in FCMSCs and TCMSCs. The platelet-derived growth factor (PDGFD) and vascular endothelial growth factor (VEGFA) play an important role in angiogenesis. The receptor TEK are newly discovered information pathways that play an important role in regulating physiological and pathological angiogenesis [35]. Moreover, the TNC is a macromolecular glycoprotein in extracellular matrix, which is closely related to the occurrence and development of cardiovascular disease and its complications. However, the SPRY1 is a protein coding gene, which can be used as an inhibitor of receptor tyrosine kinase signal transduction. The inhibition of its expression or function may promote cell proliferation and angiogenesis [36]. Besides, it has been reported that the ANGPTL1 interacts with integrin α1β1 to suppress HCC angiogenesis and metastasis by inhibiting JAK2/STAT3 signaling [37]. Altogether, the results showed that FCMSC-ECs expressed higher levels of pro-angiogenesis genes, PDGFD, VEGFA, and TNC, and lower levels of anti-angiogenesis genes, SPRY1 and ANGPTL1. Furthermore, the expression of the two VEGF receptors FLT-1 and KDR indicated the possible importance of the VEGF-mediated pathway.

Park et al [38] also found that both first- and third-trimester placental MSCs had very similar surface expression patterns of CD44, CD73, CD90, CD105 and so on. In our study, we also confirmed the immunological expression in both sets of cells. However, they illustrated that there was a significant difference in the expression levels of pluripotency-coupled genes in the two cell populations. As a result, first trimester placental MSCs (fPMSCs) exhibited more prominent differentiation into ectodermal tissues than third trimester placental MSC tPMSCs. Here, we also observed that both FCMSCs and TCMSCs had distinct functions in differentiation. FCMSC-derived ECs (FCMSC-ECs) exhibited morphology that was more similar to that of ECs and had higher CD31 and vWF levels than TCMSC-ECs under the condition of EC differentiation. Notably, human CD31-and vWF-positive cells were found in the myocardial infarction area of rats after FCMSC transplantation. This result just suggested that FCMSCs may enhance neovascularization to improve MI cardiac function. But it was not a direct evidence of angiogenesis in vivo. All the results of this study suggested that FCMSCs may enhanced neovascularization to improve cardiac function after myocardial infarction. However, we have little direct evidence of angiogenesis in vivo such as Vessel density of MI rat heart. For the inadequacies in this manuscript, we would make a further study of the subject in another project.

In conclusion, we demonstrated here that compared to other cells, FCMSCs exhibited a superior ability to differentiate into ECs, as evidenced by in vitro morphology, tube formation assays and in vivo cardiac function improvement in MI; increased levels of pro-angiogenesis gene expression may be involved in the mechanism.

Declarations

Author contribution statement

Fang Li, Yunyun Xu: Conceived and designed the experiments; Wrote the paper.

Xinran You, Shuichang Yu, Hansi Liang: Performed the experiments.

Ying Li, Xiaohan Hu, Jinnan An: Conceived and designed the experiments; Performed the experiments.

Yi Fu, Xia Zhang: Analyzed and interpreted the data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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