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Cardiogel as an Instructive Microenvironment for in vitro Differentiation of Bone Marrow- Derived Mesenchymal Stem Cells into Cardiomyocytes

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Abstract

Background: Stem cell therapy has been developed as an effective treatment method for the heart failure. Also, extracellular matrix has shown the positive effects in stem cell differentiation and myocardial tissue organization. Cardiogel is a native cardiac extracellular matrix (ECM) derived from cardiac fibroblasts. In the present study the role of cardiogel is examined for its cardiomyogenic potential on mouse bone marrow- derived mesenchymal stem cells (BM-MSCs).

Method: The BM-MSCs were isolated from six-week-old mice. Cardiac fibroblasts were collected from neonatal heart mice and the cells were seeded on 0.2% gelatin pre-coated plates for up to 21 days. Then, the decellularization was performed via enzymatic digestion. For cardiomyocyte differentiation, the BM-MSCs were plated on matrix-coated plates (Cardiogel, CCP), Matrigel-coated plates (MCP) and gelatin-coated plates (GCP) as a control group at a density of 1×10^4 cells per cm^2 in CM containing 3 µM 5-azacytidine.

Results: The results proved that cardiogel is capable to promote the cardiomyogenic differentiation of BM-MSCs. Cardiogel had a great influence on cellular adhesion, gene expression and cardiomyocyte differentiation compared to MCP and GCP. Gene expression analysis showed that cardiac specific markers were expressed in BM-MSCs has grown in CCP that represented the cardiac-specific differentiation.

Conclusion: Our results suggest that cardiogel is an effective ECM that can improve the cardiomyogenic differentiation of BM-MSCs, and it can be used as a possible therapeutic approach in cardiac tissue regeneration.

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Introduction

The extracellular matrix (ECM) is an important component for the promotion of cell adhesion, proliferation and differentiation as well as maintenance of tissue homeostasis throughout the whole organism. All cells exist in a specialized environment that their biological activities are regulated by this environment that is called ECM (1). Biological scaffold derived from the ECM tissues are effectively used in different aspects of tissue engineering, same as artificial scaffolds (2).

In the heart tissue, cardiac fibroblasts synthesize the ECM which is called Cardiogel. This substrate is composed of laminin, fibronectin, Type I and III collagen, proteoglycans and growth factors (3). Cardiogel is essential for inducing more functional beating myocytes from mesodermal stem cells. Also, cardiogel provides tissue-specific stem cell niches which are promising inducers for cardiac regeneration (4-6).

Myocardial infarction (MI) reduces normal cardiomyocytes in the heart. One approach to compensate this decline after MI is cell transplantation. Several animal studies have recommend different types of cells for transplantation in cardiac diseases (7).

Among different stem cells of the adult person, bone marrow is an invaluable source of stem cells. The bone marrow-derived mesenchymal stem cells (BM-MSCs) may have the potential for application in regeneration of the heart tissue because they have differentiation capacity into the cardiomyocyte (8). Recent studies have shown that the stem cells play a key role concerning tissue repairs after transplantation via the secretion of a variety of factors (9-14). In addition, BM-MSCs are readily available and easily expanded in vitro (15). The important point about the BM-MSCs is that BM-MSCs are immunologically compatible, so, they can be used easily for allogeneic organs (16). Despite these advantages, the BM-MSCs need to be combined with other materials or structures in clinical cellular cardiomyoplasty. The source of the BM-MSCs is limited and their differentiation capacity decrease with age. ECM components significantly affect the growth of cardiomyocytes. Moreover, ECM components can induce the development of physiological activity and morphological differentiation. This indicates the ability of ECM components for differentiation of mesenchymal stem cells into cardiomyocytes (17).

Recently, several reports have demonstrated that the combination of stem cells and artificial extracellular matrix such as Matrigel can improve the differentiation of stem cells into heart tissue. Although this type of treatment is promising, but there are so many problems for fabricating engineered heart tissue from stem cells such as differentiation into different cells of heart tissue and the high percentage of transplanted cells death (18-21).

Several studies have introduced the attempts of repairing the failing heart with the use of stem cells. This study was undertaken to further investigate and expand on cellular adhesion, cell viability, gene expression and morphological differentiation of bone marrow- derived mesenchymal stem cells to cardiomyocyte.

Material & Methods

Isolation, culture and expansion of BM-MSCs

The BM-MSCs were isolated from femur and tibiae of six-week-old mice. Femur and tibiae were dissected and the ends of the bones were cut away and the bone marrow was flushed out with culture medium (Dulbecco's modified Eagle's medium, DMEM/F12, Invitrogen Inc., Carlsbad, CA)

supplemented with 10% fetal bovine serum (GIBCO), and 1% w/w penicillin/streptomycin (GIBCO). The cells were seeded in 25 Cm^2 plastic cell flasks with culture medium and then incubated at 37 ºC in a humidified atmosphere containing 95% air and 5% CO2. On the third day, non-adherent cells and debris were removed by changing culture medium and fresh medium was added to flasks to allow further growth. The adherent cells grown to 80-90% confluency were detached by 0.05% trypsin and 0.02% EDTA for 5–10 min at 37 ˚C. After centrifugation, cells were resuspended in new flasks with mentioned culture medium. After the third to fifth passage, the cells were trypsinized and used for following steps (22).

Adipogenic and Osteogenic Differentiation

To test the BM-MSCs nature the differentiation potential of stem cells into adipocyte and osteocyte were analyzed. Adipogenic differentiation assay was performed by culture of bone marrow derived cells in medium supplemented with 10% FBS and 100 nM dexamethasone (Sigma- Aldrich) for 21 days (d) weeks. For analysis of osteogenic differentiation, the bone marrow derived cells were cultured in medium containing 10 nM β- glycerophosphate, 80 µg/ml ascorbic acid and 10 nM dexamethasone (Sigma- Aldrich) for 21 d. Then, the cultured cells were fixed by 4% paraformaldehyde (PFA, Sigma-Aldrich), and stained with Oil red (Sigma- Aldrich) and Alizarin Red S (Merck) for visualization of adipogenic and osteogenic differentiation, respectively.

Flow Cytometry Analysis

The homogeneity of the BM-MSCs was quantified by flow cytometry. After third to fifth passage, the cells were treated with trypsin/EDTA for 5 min to form single cells and suspended in PBS. Then, the flow Cytometry machine (FCM, BD FACS Caliber, Becton Dickinson, San Jose, CA, USA) was used to analyze the BM-MSCs markers CD90, CD105 and hematopoietic stem/progenitor cell marker CD34.

Preparation of Cardiogel

Isolation of Cardiac Fibroblasts

The heart of neonatal (3 d) mice were dissected, minced and enzymatically disaggregated by incubation in 0.25% trypsin-EDTA (Sigma- Aldrich) and 0.1% collagenase (Sigma, C7661) for 30 minutes (min) at 37 ºC with gentle shaking. The supernatant containing single cells was centrifuged at 2500g for 5 min at 4º C and the pellet was resuspended in the DMEM/F12 medium containing 15% FBS, 1% w/w penicillin/streptomycin, 2mM L-glutamine (Invitrogen) and 0.1mM non essential amino acids (Invitrogen) that was known as the Cardiac Medium (CM). To pereplating, the mixed cell population was seeded on 1% gelatin-coated plates and incubated at 37 °C in 95% air, 5 % CO2 for 45 min, then, the non-adherent cells were removed by changing the medium and the plates were washed two times with PBS. The adherent cells were cultured in CM at 37 °C in 5 % CO2.

Extracellular Matrix Deposition and Decellularization

For extracellular matrix (ECM) deposition, cardiac fibroblasts were re-plated on 0.2% gelatin pre-coated plates at a density of 1×10^4 cells/cm^2 in CM for up to 21 d. After reaching to adequate confluency, decellularization was performed by aspirating the medium and rinsing with PBS. Fibroblasts were removed from the underlying matrix by incubation in a 37 °C incubator with 1 ml pre-warmed extraction buffer (0.25% Triton X- 100 and 10 mM NH4OH in PBS) for 1-2 min. Then,

the plates were washed off several times with chilled PBS until the matrix was denuded of cells. Plates were observed by using an inverted microscope (Nikon Eclipse TS100, Melville, NY, USA), to ensure that the complete cell lysis happened and no intact cells were remained. Finally, the matrix-coated plates were stored at 37 °C in PBS containing 100 U/ml penicillin, 100 g/ml streptomycin, and 0.25 g/ml fungizone for use afterward. Control plates were coated with 0.2% gelatin.

Induction of Cardiogenic Differentiation

For cardiomyocyte differentiation, the BM-MSCs were plated on matrix-coated plates (Cardiogel, CCP), Matrigel-coated plates (MCP) and gelatin-coated plates (GCP) as a control group at a density of 1×10^4 cells per cm^2 in CM containing 3 µM 5-azacytidine (Sigma- Aldrich). The duration of the experiment was 12 d and the medium was refreshed every two days.

MTT Assay

Cell viability was evaluated based on mitochondrial function of living cells by reduction of the tetrazolium salt (MTT, 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2Htetrazolium bromide) at 4 different time points (3^{rd}, 6^{th}, 9^{th} and 12^{th} day) in 96-well microplates (Falcon). Briefly, 5×10^3 BM-MSCs per 200 ml medium was seeded on different coated plates (matrix-, matrigel- and gelatin- coated plates) in CM containing 3 µM 5-azacytidine for 12 days at 37 °C in 5% CO2. Then, the wells were washed with PBS, and 50 µl of 1 mg/ml MTT solution (Sigma-Aldrich) constituted in PBS was poured to each well and the cells were incubated for 3 hours at 37 °C. After incubation, the culture medium were removed and 50 µl of 100 % Dimethyl sulfoxide (DMSO) were mixed, then placed on a shaker for 10 min (23). The results were analyzed by spectrometric absorbance at 570 nm (Haratizadeh, et al; 2016) (PerSeptive Biosystems, Framingham, Massachusetts, USA).

Cell Adhesion Evaluation

The cellular adhesion assays were performed by a modified protocol described by (Vohra, *et al, 2008).*

The BM-MSCs were seeded onto matrix-, matrigel- and gelatin- coated plates in CM containing 3 µM 5-azacytidine in concentration of 1×10^4 cells per well of a 6-well plate and incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37 ºC. When the cells reached to approximately 70% confluency, the wells were washed three times with PBS and trypsinized with 0.25% Trypsin-EDTA at three time intervals (0.5, 2.5 and 5 min). Then, the plates were washed several times with PBS and the cellular adhesion was measured by the MTT test.

Morphological features of cardiomyocyte differentiated of BM-MSCs

For detailed observation of the morphological changes, cells in the last day of differentiation were rinsed with PBS, then cells were fixed with ethanol for 5 min at room temperature (RT), and stained with Giemsa (Merck) for 2 min. Photomicrographs were taken with a Nikon digital camera coupled to an inverted microscope (Nikon, Eclipse-TS100).

Immunofluorescence staining

For immunofluorescent staining, the BM-MSCs (1×10^4 cells per cm^2) were cultured on different coated plates in CM containing 3 µM 5-azacytidine for 12 d. PBS at PH 7.4 was

used for washing stem cells. Then, 4% PFA was used for fixing cells at room temperature for 30 minutes. 0.025% Triton X-100 was provided for permeabilizing the cells for 10 min at RT. Unspecific binding of the antibody was prevented by incubating the cells in 10% goat serum for 30 min. Incubation with cTnI primary antibody (Rabbit polyclonal, ab47003, abcam system) diluted 1:1000 in TBST [Tris buffered saline (TBS), 1% BSA and 0.1% Tween-20 (all Sigma)] was done for 60 min at RT. Then, three washes with PBS were performed and incubation with the FITC-conjugated Goat polyclonal secondary antibody to Rabbit IgG (ab975, abcam system, diluted 1:100 in TBST) were done for 45 min in the dark at RT, then cells were washed eight times with PBS. Nuclei were counterstained by 3,3'-diaminobenzidine (DAPI, sigma) staining.

To verify the complete decellularization of ECM, Fibroblasts were removed by incubation with pre-warmed extraction buffer (nonenzymatic). Then, decellularized ECM was fixed with 4% PFA for 20 min and washed with PBS. The plates were mounted with DAPI to confirm removal of the cells.

In addition, decellularized ECM was fixed with 4% PFA for 20 min at RT and then blocked with 10% goat serum. The plates were incubated with primary antibody against laminin (Rat monoclonal to Laminin, ab11576, abcam system). Further incubation with the FITC- conjugated sheep anti-Rat polyclonal secondary antibody (ab53435, Invitrogen) were performed for 45 min in the dark at RT. Then, the plates were washed 3 times in PBS. Images were taken with an Olympus phase contrast microscope (BX51, Olympus, Tokyo, Japan) (24-26).

Immunoflourescence Staining

Immunocytochemistry staining was rendered by the streptavidin–peroxidase procedure. The subcultured cells were washed with PBS and fixed in 4% PFA for 30 min and premeabilized with 0.2% Triton X-100 for 5 min. Blockage of the nonspecific binding was performed by the primary antibody, and then cells were incubated with goat serum for 30 min at RT. Afterwards, cells were treated with 3% H_2O_2 in PBS, and incubated with myogenin (Dako) and F-actin (Dako) for 45 min.

The primary antibodies were incubated using DAB (HRP DAB Kit, Genemed Biotechnologies, Inc). Cells were evaluated under an inverted light microscope (Nikon, Eclipse-TS100) and brown cytoplasmic stain was considered as positive reaction.

RT-PCR

Total RNA extraction from cell cultures was performed by Trizol Reagent (Invitrogen Life Technologies), and then transcribed into cDNA by using reverse transcriptase kit (Fermentas), according to the manufacturers' instructions. RT-PCR was performed to evaluate the expression of Nkx2.5, α-MHC, Osteocalcin, Aggrecan and GAPDH genes by using SYBR Green master mix (ABI, Step one plus, USA). Primers were designed by AlleleID software version 7.6 (Primer Biosoft, Palo Alto, USA). Primer sequences are shown in table 1. The temperature profile in qRT-PCR amplification was (1) 95°C for 10, (2) 40 cycles of 95°C, 30 seconds; 60°C, 45 seconds; and 72°C, 45 seconds and (3) 72°C for 7 min for the final extension. The ΔΔCt method was used for rating the relative quantification of gene expression.

Table 1. The sequence of primers

GENE	SIGNIFICANCE	PRIMER SEQUENCE	PRODUCT SIZE (BP)
Nkx2.5	Cardiac marker	5′- CCAAGGACCCTAGAGCCGAA -3′ (F) 5′- ATAGGCGGGGTAGGCGTTAT -3′ (R)	461bp
α-MHC	Cardiac marker	5′- CTGCTGGAGAGGTTATTCCTCG -3′ (F) 5′- GGAAGAGTGAGCGGCGCATCAAGG -3′ (R)	301 bp
Osteocalcin	Osteogenic marker	5′- AGTCACCAACCACAGCATCC -3′ (F) 5′- TTTGTCCCTTCCCTTCTGCC -3′ (R)	327 bp
Aggrecan	Chondrogenic marker	5′-CTGGAGACAGGACTGAAATC-3′ (F) 5′-CTCCATTCAGACAAGGGCTT-3′ (R)	297 bp
GAPDH	INTERNAL MARKER	5′- AGCCACATCGCTCAGACACC--3′ (F) 5′- GTACTCAGCGGCCAGCATCG -3′ (R)	302 BP

Statistical analysis

Statistical analysis was conducted using one-way analysis of variance (ANOVA) test, followed by a Tukey *post-hoc* test using SPSS software for Windows, version 23. Differences between samples were considered statistically significant at $P<0.05$.

Results

Morphological characteristics of BM-MSCs

To make sure the removal of any contamination with adherent hematopoietic cells, the BM-MSCs were cultured and passaged (P) 3-5 times (Figure 1A, B, C &D). To examine the multipotent differentiation potential, the cells were cultured under mentioned adipogenic conditions for 21 days. The BM-MSCs began changing into ovoid morphology and intracellular lipid droplets were formed in differentiated cells, as approximately 75% of the cells were positive to oil red O staining. Osteogenic differentiation was analyzed by culturing cells in a specific medium with known osteogenic factors. Deposition of a mineralized extracellular matrix in the culture was observed by Alizarin red staining. The data confirm that these cells are a subset of BM-MSCs (Figure 1 E and F).

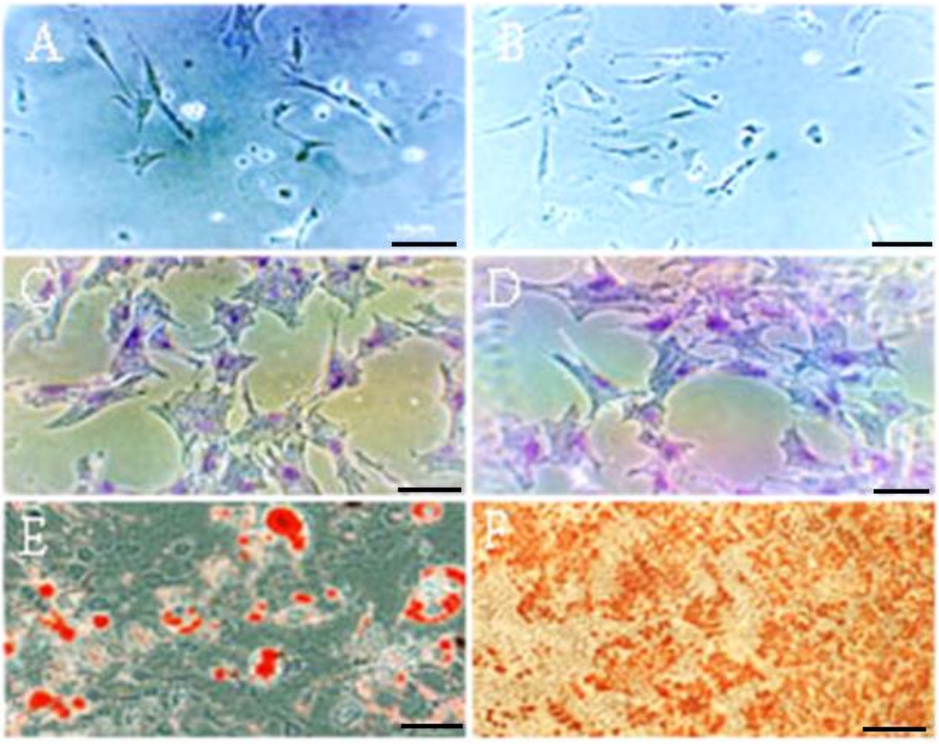

Figure 1. Morphology of the BM-MSCs. (A and B) spindle fibroblast-like cells (P3), (C and D) Giemsa staining proved that BM-MSCs were mononuclear, (E) is alizarin red staining for osteogenesis and (F) is Oil Red O staining for adipogenesis. Scale bars; 50µm.

By flow cytometric analysis, the BM-MSCs expressed mesenchymal stem cell markers CD105 (87.42%) and CD90 (83.10%), whereas they were negative for hematopoietic stem cell marker CD34 (0.4%) less than 5%. This result also revealed that the isolated cells have the basic properties of the MSCs (Figure 2).

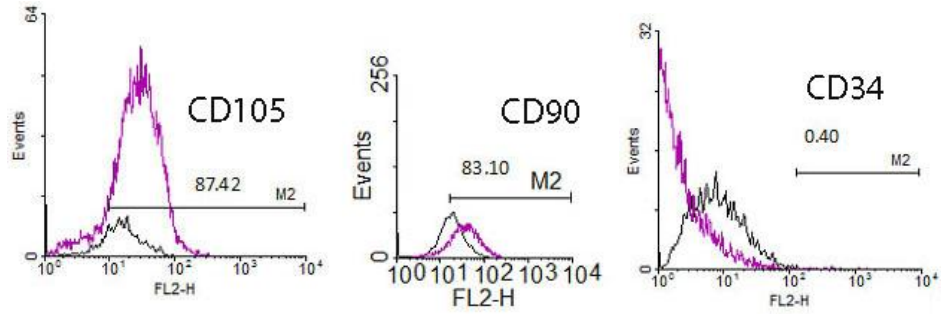

Figure 2. Flow cytometry of cell markers in the BM-MSCs. Mesenchymal stem cell markers CD105 (87.41%) and CD90 (83.10%) and hematopoietic stem cell marker CD34 (0.4%) and CD34.

The complete decellularization of the ECM derived from cardiac fibroblasts was first shown by staining nuclei with DAPI (Figure 3 A and B). In addition, the ECM components of cardiac fibroblasts were tested by immunofluorescent staining of laminin (Figure 3 C).

Figure 3. Verification of ECM substrate decellularization. The pictures were stained by DAPI before (A) and after (B) decellularization. (C) Immunofluorescent staining of acellular matrix against laminin (green). Scale bars; 50µm.

Based on the interaction between cellular components and matrix surface components, adhesiveness assay was performed. For this purpose, cells into the various substrates were trypsinized for time periods (0.5, 2.5 and 5 min) to remove the non-adherent cells. Total number of adherent cells was analyzed by using the MTT test. Our results demonstrated that cell survivability was gradually decreased after varying time periods of trypsin treatment. However, after exposing cells to 5 min trypsin treatment, the viability rate of cells in CCP was 2.5 fold higher than other substrates. Also, the viability rate of cells in CCP was more as compared to other substrates in all different time periods, indicating that the greatest degree of attachment was observed on CCP (Figure 4).

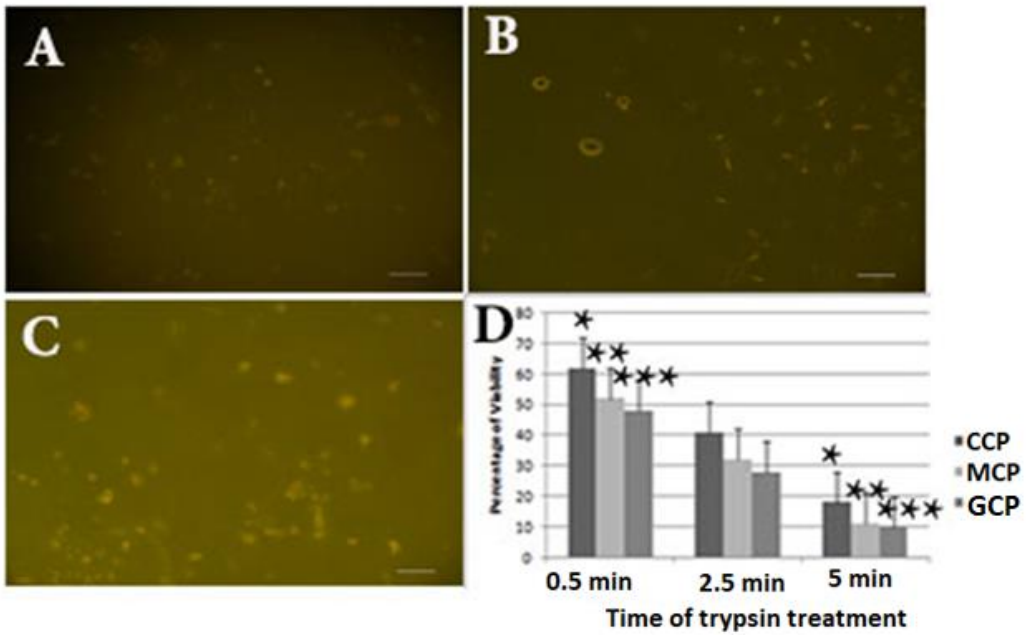

Figure 4. Adhesiveness assay after trypsinisation. Pictures of BM-MSCs seeded onto (A) gelatin- coated plates (GCP) as a control group, (B) matrigel- coated plates (MCP) and (C) matrix- coated plates (CCP) at a density of 1 × 104 cells per well of a 6-well plate in CM containing 3 µM 5-azacytidine after trypsinisation for 1 min. Scale bars; 50µm. (D) Presents the histogram of the viability percentage of adherent BM-MSCs on GCP, MCP and CCP after enzymatic treatment for 0.5, 2.5 and 5 min. The cellular adhesion index was measured by the MTT test ($p<0.05$).

The viability of BM-MSCs gradually decreased from 3rd to 12th days, but there was no significant difference between the different coated plates (substrates). However, the viability percentage of BM-MSCs in the CCP was more than other substrates at all days (Figure 5).

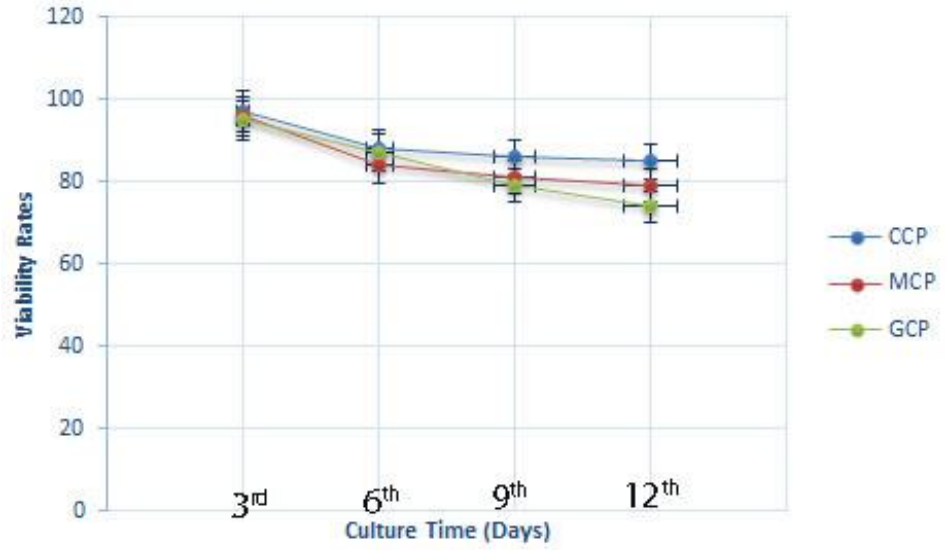

Figure 5. The comparison between the mean percent of viability rates in the BM-MSCs treatment with 5-azacytidine at days 3, 6, 9 and 12. There was no significant difference between the different coated plates ($p<0.05$).

The results of morphological analysis showed that in the presence of substrates, treated cells were differentiated gradually and transformed to cardiomyocytes. Differentiated cells expanded their processes and created myotube like organization through formation of connections to adjusted cells. In addition, these cells became thinner and longer and formed clusters of 8-10 cells aligned parallel to each other. Also, differentiated cells seem striated with eccentric nucleus and end branched.

Our immunofluorescent results showed that cTnI positive cells were observed only in cardiogel/CCP cultured (Figure 6 B and C). Additionally, some Myogenin and F-actin positive cells were observed (Figure. 6 D) (Figure. 6 E & F).

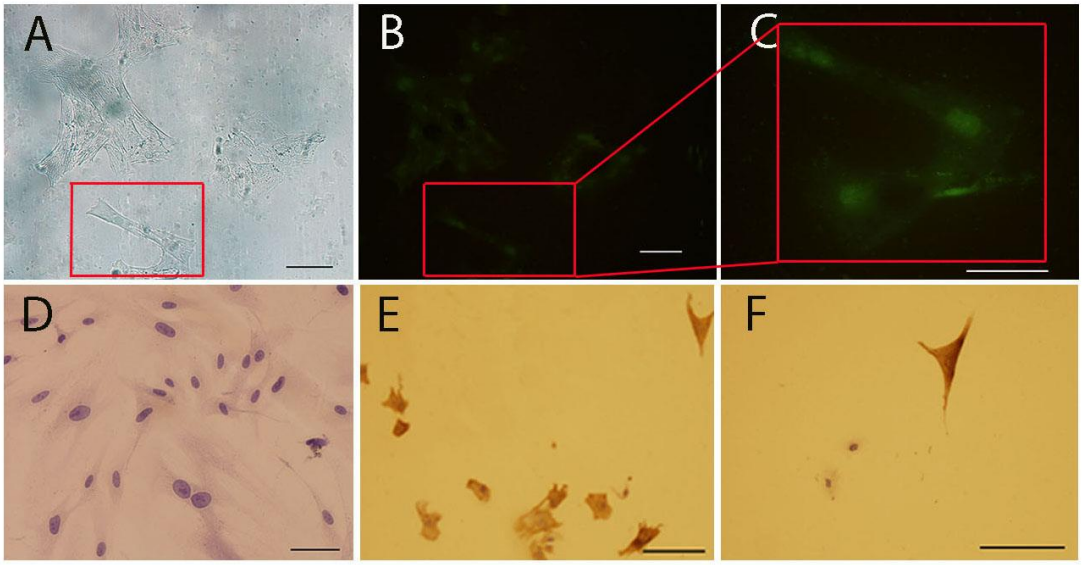

Figure 6. Immunophenotype of cultured BM-MSCs on matrix- coated plates in CM containing 3 μM 5-azacytidine. Immunocytochemical staining with FITC-labeled secondary antibody for expression of cardiac marker cTnI; phase contrast (A), flourocent microscopy (B) and higher magnification microscopy image of the encircled zone indicated by the inset in (A and B) illustrating morphology and cTnI expression pattern. The treated BM-MSCs demonstrated positive reaction with myogenin (D), and also, with F-actin in clusters (E) and in a single cell (F). Scale bars; 50μm in A, B, D and E and 20μm in C and F.

Using RT-PCR, we assessed the expression of cardiac specific, osteogenic and chondrogenic markers in the three different substrates. Early cardiac transcription factor Nkx2.5 was expressed in CCP and MCP. While, α-MHC (cardiac myosin heavy chain) which is known to be mainly a marker for mature cardiac cells that interacts with actin was expressed only in CCP. The expression of aggression, which is considered as the chondrogenic marker, was supported in MCP and GCP (Figure. 7).

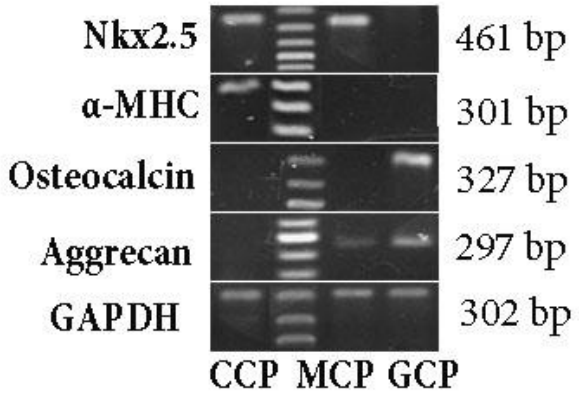

Figure 7. RT-PCR analysis of cardiac-specific markers (Nkx2.5 and α- MHC), osteogenic marker (osteocalcin) and chondrogenic marker (aggrecan) on matrix- coated plates (cardiogel, CCP), matrigel- coated plates (MCP) and gelatin- coated plates (GCP).

Discussion

Cell–matrix interactions play a crucial role in stem cell differentiation, as well as the regulation of the structure and function of individual cells and tissues. Cardiogel, the native ECM of the heart, is secreted by cardiac fibroblasts, and includes collagen (type I and III) and adhesive glycoproteins (laminin and fibronectin). We hypothesized that Cardiogel can provide instructive microenvironment which may enhance BM-MSCs specification toward cardiomyocyte. In our study, cultured BM-MSCs on Cardiogel have exhibited more specific features into functional cardiomyocytes than the control group. We found that Cardiogel can affect cellular adhesion, cell viability and gene expression of BM-MSCs during cardiomyocyte differentiation. In our study, the viability rate of cells in CCP was 2.5 fold higher than other substrates (MCP and GCP) due to the higher degree of cell attachment in this group. Morphological differentiation such as expansion of cell processes and creation of myotube like organization happened in differentiated cells. In RT-PCR assay was observed Nkx2.5 (early cardiac transcription factor) expressed in CCP and MCP, while, α-MHC (cardiac myosin heavy chain) was expressed only in CCP. However, osteogenic marker (osteocalcin) was only expressed in GCP, and the expression of chondrogenic marker (aggrecan) happened in MCP and GCP.

Several studies have indicated that the nature of the ECM in which cells are cultured on it can affect the fate of one kind of cells to various differentiated cells (27). ECM components have regulatory effets on cell proliferation and differentiation (28). Cardiogel in the body differs from the invitro synthesized Cardiogel, because the culture condition can change the type and amount of proteins which is produced by cardiac fibroblasts. In addition, the orientation of matrix components in culture environment can affect differentiated cardiomyocytes phenotype (3). Our results confirmed the influence of ECM on the differentiation efficiency of BM-MSCs.

Different studies have shown the important role of the ECM, as an effective component of the stem cell niche, on stem cell proliferation, self-renewal and differentiation (29). Besides, ECM stiffness has noticeable effects on stem cell differentiation (30). The stiffness of the substrate from soft to hard condition can affect the fate of MSC. For example, soft substrates may promote differentiation into the neurogenic lineage, but stiff substrates may promote differentiation into the osteogenic lineage (31). Also, hydrostatic pressure (HP) can regulate stem cell fate especially in cartilage tissue production. It has been reported that HP increases chondrogenic gene expression and matrix production in MSCs (32).

Previous studies demonstrated that cultured BM-MSCs on Cardiogel ECM had higher proliferation and differentiation abilities. By the same token, they had a higher survival rate under oxidative stress. Cultured BM-MSCs on Cardiogel ECM had a higher resistance rate to proteolytic disassociation (33). Evidence shows that culture in Cardiogel coated plates would increase cellular adhesion compared to the non-coated plates (34, 35). In our study, after exposing cells to trypsin for 5 minutes, the viability rates in CCP was 2.5 fold higher than GCP and MCP. This effect of Cardiogel which can increase cellular adhesion of stem cells can be used in tissue engineering particularly for transplanting stem cells into the damaged tissue. Our results confirmed the positive effects of culturing stem cells in Cardiogel coated plates, as a promising therapeutic approach for curing heart failure by increasing viability rates, enhancing proliferation and differentiation abilities and producing differentiated cardiomyocytes (36).

Previous studies demonstrated that Cardiogel can promote differentiation of murine embryonic stem cells into working cardiomyocytes (37). During normal cardiogenesis in the body, myofibrils firstly spread in irregular arrays, which matured into parallel arrays, but in vitro studies reported that M-band is not recognizable during long-term culturing of human-ES-derived cardiomyocytes (38). There are several reports that highlighted the effects of cell microenvironment such as ECM structure on the viability and differentiation of cultured cells. For instance, cardiac matrix can organize multicellular clusters with intercellular desmosomal attachment similar to the cardiac intercalated disc from hESC, but skeletal muscle matrix can organize larger myotubes (39).

In cardiogenesis, Nkx2.5, GATA-4 and MEF2C were identified as main regulators in cardiac development (8). The expression of cardiac transcription factor Nkx 2.5 was increased in the CCP and MCP. This confirmed the influences of Cardiogel and Matrigel in stimulating cardiogenesis. Other important cardiogenic genes are cardiac alpha-myosin heavy chain (α-MHC) and cardiac beta-myosin heavy chain (β-MHC) which are known as cardiac structural genes (13). In our study, α-MHC which is known as a main marker of mature cardiac cells was expressed only in CCP.

In summary, the present study has indicated that Cardiogel can promote the cardiomyogenic differentiation of BM-MSCs. Cardiogel had positive effects on cellular adhesion, cell viability, cardiac gene expression and cardiomyocyte differentiation compared to the matrigel and gelatin- coated plates. Osteogenic and chondrogenic markers didn't express in BM-MSCs cultured in CCP, while cardiac specific markers expressed in BM-MSCs cultured in CCP which represented the cardiac-specific differentiation. The current study indicates that Cardiogel has a wide impact in tissue engineering and stem cell biology and can be used for replacing damaged regions or lost myocardial tissues to restore cardiac function.

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Compliance with ethical guidelines

In accordance with the Care and Use of Laboratory Animals Committee, Statement on Ethical Conduct in Animal Research, ethical approval was granted through the Mater Research of Mazandarn Medical University Ethics Committee.

Conflict of interest

The authors declare that they have no conflict of interest.

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