A comparative study of the influence of two types of PHEMA stents on the differentiation of ASCs to myocardial cells

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Abstract. In the present study, subcutaneous fat was obtained from adult women that had undergone conventional liposuction surgery. A comparative study was performed to investigate the effect of transparent and white poly-β-hydroxyethyl methacrylate (PHEMA) stents, which have different surface and cross-sectional morphological characteristics, on the differentiation of adipose-derived stem cells (ASCs) into myocardial cells. The cell counting kit-8 assay revealed that cell growth increased at varying rates among the different treatment groups. The absorbance of the experimental transparent PHEMA treated group increased in a time-dependent manner with the duration of incubation. The highest levels of proliferation were observed in the transparent PHEMA group. In addition, the transparent PHEMA treated group exhibited the strongest cell adhesion ability, which was significantly different to that of the white PHEMA group ($P<0.01$ and $P<0.05$ for Matrigel and fibronectin assay, respectively). Comparisons between the two stent materials with the inducer control group revealed statistically significant differences in the rate of ASC differentiation ($P<0.05$). The level of differentiation was the greatest in the transparent PHEMA group, and was significantly different to the white PHEMA group ($P<0.05$) and the blank control group ($P<0.01$). The results suggest that the inducers 5-aza-2-deoxycytidin and laminin, and material microstructure stents effectively promote the proliferation, growth and adhesion of ASCs. However, the transparent material microstructure may be a more suitable candidate for ASC-associated injections. The present study provides further evidence that a PHEMA stent structure, comprised of a high number of matrixes and a low water content, induces a high level of ASC differentiation to myocardial cells.

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Introduction

Ischemic cardiomyopathy (ICM) involving damage to cardiac structure and function induced by irreversible myocardial cell necrosis is a leading cause of morbidity and mortality worldwide as a result of modern lifestyle (1). The currently available primary treatments include drug interventions, percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG) and heart transplantation (2). Although these methods lower the fatality rates and improve the quality of life of patients, a number of limitations and defects still exist. For example, CABG has been observed to lower the rates of mortality and myocardial infarction, however, it increased the incidence of stroke. In addition, the differences in quality-of-life were smaller than expected as many of the patients who were initially treated with PCI required a repeat revascularization procedure (3). However, regenerative medicine is continuously developing, and transplant therapy using muscle tissue has become a primary focus within the field. The aim of this approach is to implant appropriate seed stem cells into specific tissue engineering materials *in vitro*. Integrated mechanical, chemical and biological signals would then be applied to stimulate and construct functional myocardial tissue. Finally, the tissue would be implanted into the patient to repair or replace the damaged cardiac muscle.

Selecting the appropriate seed cells is the most important step during the construction of myocardial tissues. Adipose-derived mesenchymal stem cells (ASCs) have been successfully isolated from human adipose tissue by Zuk *et al* (4) using widely available materials. The culture period is long, however, the cells produced exhibit strong proliferation abilities. In addition, this method is ethically approved, and the stem cells have the potential to differentiate into multiple germ layers, which can be induced to differentiate into cardiomyocytes directly (5,6). In addition, ASCs exhibit the same immunosuppressive effects and paracrine signaling abilities as bone mesenchymal stem cells (7-9).

Stent materials and the culture microenvironment are equally important in myocardial tissue engineering. Previous studies have demonstrated that the spatial microstructure of stent materials has a significant impact on the proliferation and differentiation of seed stem cells (10,11). The ideal stent material for tissue engineering is a natural tissue, and the cultivation environment for cell stent planting should be similar to the microenvironment of human myocardial tissues in order to enhance stem cell adhesion and proliferation, as well as their differentiation into myocardial cells (6,7). Therefore, the concept of using extracellular matrix (ECM) in myocardial tissue engineering has been proposed, and associated studies have gained a great deal of attention (12-14). Consequently, the present study compared the effect of two types of poly-β-hydroxyethyl methacrylate (PHEMA) stents (transparent and white PHEMA), on ASC proliferation, adhesion and their differentiation into cardiomyocyte-like cells.

Materials and methods

Reagents. Dulbecco's modified Eagle's medium (DMEM) was purchased from Hyclone; GE Healthcare Life Sciences (Logan, UT, USA); fetal bovine serum (FBS) was purchased from Gibco; Thermo Fisher Scientific, Inc. (Waltham, MA, USA); cell counting kit (CCK)-8 solution was purchased from Yeasen Biotech (Hong Kong) Co., Ltd., (Hong Kong, China); the type I collagen enzyme, decitabine (5-aza-2'-deoxycytidine) and laminin (LN) were purchased from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany); 2-hydroxyethyl methacrylate (HEMA) was purchased from Rohm & Haas Company (Philadelphia, PA, USA); ethylene glycol dimethacrylate (EGDMA) was purchased from Tokyo Kasei Kogyo Co., Ltd., (Tokyo, Japan); ammonium persulfate (APS) was purchased from Ajax Finechem; Thermo Fisher Scientific, Inc.; N,N,N',N'-tetramethylethylenediamine (TEMED) was purchased from Sigma-Aldrich; Merck KGaA; the GATA binding protein 4 (Gata4; cat. no. GTX113194), NK2 homeobox 5 (Nkx2.5; cat. no. GTX133155), cardiac troponin T (cTnT; cat. no. GTX28295), connexin-43 (Cx43; cat. no. GTX11369), myogenic differentiation (MyoD; cat. no. GTX100885), α-smooth muscle actin (α-SMA), desmin (cat. no. GTX103557) and β-actin (cat. no. GTX110564) antibodies were purchased from GeneTex, Inc. (Irvine, CA, USA); horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG and HRP-conjugated goat anti-mouse IgG secondary antibodies (heavy and light chain; cat. no. 106003) were purchased from Neobioscience Technology Company (Shenzhen, China).

Preparation of the PHEMA porous hydrogel stent and morphological analysis. As described by Lou *et al* (15), 1.5 ml of HEMA monomer (Rohm & Haas Company) was injected into a small cylindrical polystyrene mold with a diameter of 15 mm. The monomer was polymerized at 50˚C for 20 h, before the mixture was poured into a Soxhlet extractor. Ionized water was used to elute residual monomers and oligomers for 48 h at room temperature (18-20˚C). The crosslinking agent EGDMA (Tokyo Kasegi Kogyo Co., Ltd.), the APS initiator (Ajax Finechem; Thermo Fisher Scientific, Inc.), TEMED (Sigma-Aldrich; Merck KGaA) and deionized water were added to the two polymer types, which were prepared in a HEMA sponge, to conduct polymerization. To prepare transparent PHEMA, 101.5 μl EGDMA, 80 μl APS, 40 μl TEMED and 6 g of deionized water were added. For white PHEMA, 36.5 μl EGDMA, 80 μl APS, 40 μl TEMED and 15 g of deionized water were added. The percentage of water in the transparent PHEMA was 29.9 and 74.8% in white PHEMA. Morphological analysis was performed on the surface and on cross-sections of the two polymer types using scanning electron microscopy (magnification, x1,000).

Advanced ASC separation and cultivation methodology. A total of 5 female patients (age, 27±2 years) from The First Affiliated Hospital, Sun Yat-sen University (Guangdong, China) were enrolled in January 2012. Using the syringe negative pressure method as described by Panfilov *et al* (16), 50 ml human abdominal subcutaneous suction fat fluid was collected from the excess tissues excised during plastic and reconstructive surgery (excessive inflation fluid, auxiliary ultrasonic emulsification or resonance technology were not required). A total of 50 ml phosphate-buffered saline (PBS) was added, followed by thorough mixing and centrifugation at 1,200 x g for 10 min at 37˚C. The supernatant was removed and transferred into a fresh centrifuge tube (50 ml) with 5-fold the volume of Collagenase I (0.075%; concentration of working liquid; Sigma; Merck KGaA). The adipose tissue was then cut into small pieces. The tube was subsequently sealed at 37˚C and mixed at 200 x g for 30 min at 37˚C. Digestion was terminated by adding the same volume of DMEM containing 10% FBS and the solution was centrifuged at 1,200 x g for 10 min at 37˚C. The supernatant was removed for incubation at room temperature for 5 min, followed by centrifugation 1,200 x g for 5 min at 37˚C. The supernatant was removed and 10X volumes of medium was added. A nylon cell strainer (Corning Life Sciences, Corning, NY, USA) with a pore diameter of 100 mm was used to filter the tissue block. Cells were incubated in 10-cm culture dishes (inoculation density of 30-50%) at 37˚C and 95% relative humidity in culture medium (DMEM containing 10% FBS) to a final volume of 10 ml. The medium was refreshed following 24 h, then once every 2.5 days. Cells were passaged when the cell density reached 80-90%. When the cell density of the passage (P) 1 generation reached 80-90%, cells were cryopreserved for induction of differentiation. P2 generation cells were used immediately for the following experiments. All procedures were conformed to the principles outlined in The Declaration of Helsinki. The study protocol was approved by the Human Ethics Committee of The First Affiliated Hospital, Sun Yat-sen University (Guangzhou, China). Written informed consent was obtained for the collection and utilization of tissue samples from all subjects included in the present study.

Cell culture. Human LN (1.2 mg/ml) and decitabine (10 mmol/ml) were added to the pores of the white and transparent PHEMA to induce differentiation of the ASCs into myocardial cells. Following incubation for 2 h at 37˚C, PBS was used to wash the PHEMA stents. Stent materials were not added to the remaining two pores. To each hole, $5x10^4$ ASCs were applied. DMEM (2 ml) containing 10% FBS were added to the blank control pores. For the inducer group, DMEM (2 ml) containing 10 μmol/ml 5-Aza-2'-deoxycytidine and LN (1.2 μg/ml) + 10% FBS was added the following day and incubated for 24 h at 37˚C with 90% humidity. For the

stent+inducer group, DMEM (2 ml) containing 10 μmol/ml 5-Aza-2'-deoxycytidine and LN (1.2 μg/ml) on white PHEMA or transparent PHEMA + 10% FBS was added. The concentration of LN and 5-Aza-2'-deoxycytidine added to the cells was described by van Dijk *et al* (17).

Cell proliferation analysis. Digestive enzymes were added into the 4 sample holes. A total of 100 μl cell solution (~3,000 cells) was added to each hole. Following 24 h, 10 μl CCK-8 solution and 90 ml complete medium were applied. The cells were then incubated for a further 1 h, and the absorbance was measured at 450 nm.

Test for cell adhesion. To test cell adhesion capabilities, 10 g/l bovine serum albumin (BSA), 50 mg/l Matrigel (dilution, 1:8) or 10 mg/l fibronectin (FN) were added into 96-well plates, with 50 μl in each well. The cells were incubated at 4˚C with 90% humidity overnight. BSA was used as the control base. Excess liquid in the culture plate was removed. A total of 50 μl serum-free culture medium containing 10 g/l BSA was added into each well and incubated in a water bath at 37˚C for 30 min. A total of 4 ml of 0.25% digestive enzymes were added into the 4 sample holes and the cell density was adjusted to $1x10^5$ cells/ml. The cell suspension (100 μl) was inoculated in the coated 96-well plate; 3 parallel samples were used for each group. A total of 10 g/l medium containing BSA was used for control culture at 37˚C for 1 h and the nutrient solution was removed. The CCK-8 method was used to determine the absorbance at 450 nm. With the absorbance value of adherent cells in the BSA group as the reference, the adherence rates of the Matrigel group and FN group were calculated. Adhesion rate was calculated using the following formula: Adhesion rate (%) = $[(OD_{Matrigel\ group}$ or $OD_{FN\ group}$ / $OD_{BSA\ group}) -1] x100\%$.

Western blot analysis to determine the direction of ASC differentiation. ASCs from the 4 sample groups were cultured for 2 weeks at 37˚C with 90% humidity. The cells were lysed in lysis buffer [150 mM NaCl, 50 mM Tris-HCl (pH 8.0), 0.1% SDS, 1% Triton X-100] containing protease and phosphatase inhibitors (Roche Diagnostics, Basel, Switzerland). Cell lysate protein content was determined using a bicinchoninic acid protein assay kit (Sigma-Aldrich; Merck KGaA). An equal amount of whole cell extracted protein (10 μg) was subjected to 12% SDS-PAGE gel, transferred to PVDF membranes and blocked by non-fat milk in an incubator (26˚C, 40 x g, 2 to 4 h). The blocking mixture was then discarded, and a hybrid solution containing primary antibodies (GATA4, Nkx2.5, cTnT, desmin, Cx43, MyoD, α-SMA) was added and incubated at 4˚C overnight. A secondary antibody hybrid solution (1:10,000) was added the following day, and membranes were incubated at 26˚C for 1 h (40 x g). An electrochemiluminescence kit (Thermo Fisher Scientific, Inc.) and Kodak gel imaging system 2200 (Kodak, Rochester, NY, USA) were used to collect and analyze images.

Statistical analysis. SPSS software (version, 13.0; SPSS, Inc., Chicago, IL, USA) was used for data processing. Data are presented as the mean ± standard deviation. Student's t-test was used for comparisons between groups, and a one-way analysis of variance with the Bonferroni post hoc test were used to compare differences among >3 groups. $P<0.05$ and $P<0.01$ were considered to indicate statistically significant differences.

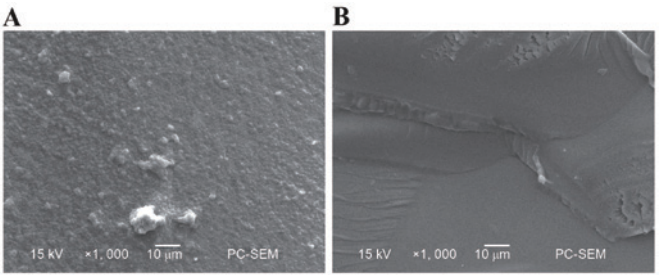

Figure 1. Comparative electron microscope images of the surface morphology between white and transparent PHEMA polymers. (A) White PHEMA polymers (Monomers, 25.2%; Water, 74.8%) displayed an uneven surface, while (B) transparent PHEMA polymers (Monomers, 70.1%; Water, 29.9%) displayed an even surface (magnification, x1,000). PHEMA, poly-β-hydroxyethyl methacrylate; PC-SEM, personal computer-scanning electron microscopy.

Results

Morphological analysis of the PHEMA polymer. The two types of polymers exhibited different surface and cross-sectional morphological characteristics. Differences in pore structure between white and transparent PHEMA polymers are shown in Figs. 1 and 2. The white PHEMA is a milky white polymer with noticeable porous structures. By contrast, the transparent PHEMA is a little translucent and is similar to homogeneous, non-porous hydrogels.

Cell proliferation analysis. Cell proliferation increased to varying extents among all experimental groups, as determined using the CCK-8 assay (Fig. 3). The absorbance of each experimental group increased in a time-dependent manner (Fig. 3). When compared with the control group, the white and transparent stent treated groups demonstrated significantly increased proliferation rates at 48, 72 and 96 h ($P<0.05$ and $P<0.01$; Fig. 3). The transparent PHEMA treated group exhibited higher rates of proliferation when compared with the white PHEMA treated group at 72 and 96 h ($P<0.05$). Therefore, the highest proliferation rate was observed in the transparent PHEMA polymer group at 96 h. The results demonstrated that, under the identical culture conditions, inducers and material microstructures effectively promote the proliferation and growth of ASCs. In addition, the transparent PHEMA polymer microstructure demonstrated the greatest proliferation promotion ability.

Cell adhesion analysis. Cells cultured on Matrigel and FN-coated surfaces demonstrated marked differences in adherence capabilities (Table I). The transparent material group demonstrated the greatest cell adhesion ability, which was significantly greater when compared with the white material group ($P<0.01$; Table I). The results demonstrated that the inducers and material microstructures effectively promoted the adhesion of ASCs, when compared with the controls. In addition, the microstructure composed of transparent material may present the most suitable candidate for vaccinations of ASCs.

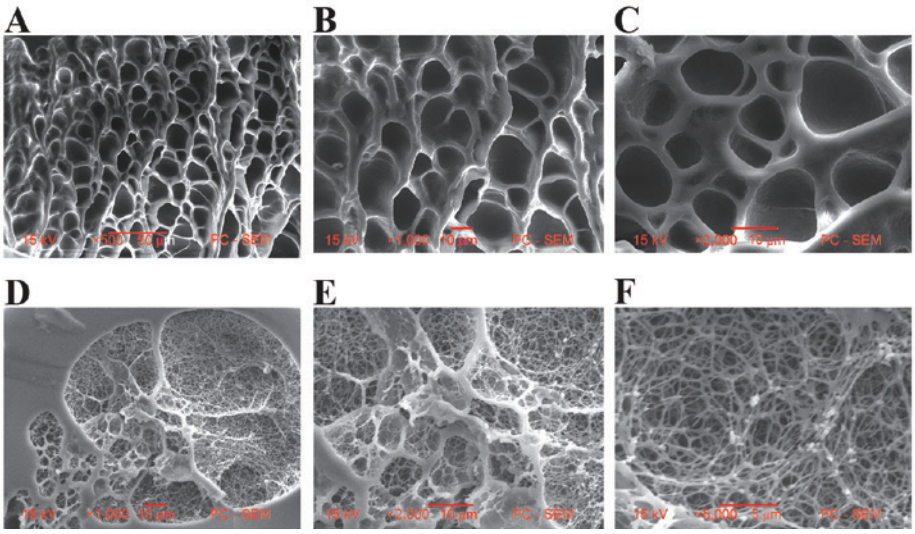

Figure 2. Comparative electron microscope images of the cross-sectional morphologies of white and transparent PHEMA polymers. Images of the porous network structure of white PHEMA polymer samples (Monomers, 25.2%; Water, 74.8%) at (A) x500, (B) x1,000 and (C) x2,000 magnifications. The porous network structure containing nanofibers of transparent PHEMA polymer samples (Monomers, 70.1%; Water, 29.9%) at (D) x1,000, (E) x2,000, (F) x5,000 magnifications. PHEMA, poly-β-hydroxyethyl methacrylate; PC-SEM, personal computer-scanning electron microscopy.

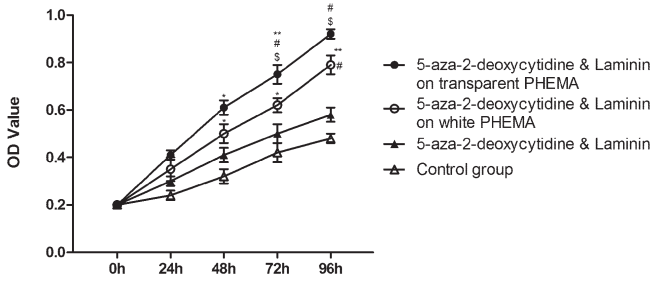

Figure 3. Cell proliferation at different time-points across the 4 groups. Cell proliferation was determined using the cell counting kit-8, and is represented by the OD values at 0, 24, 48, 72 and 96 h, respectively. *P<0.05 and **P<0.01 vs. the control group at each time-point; $^{\#}$P<0.05 vs. 5-aza-2'-deoxycytidine and laminin group; $^{\$}$P<0.05 vs. white PHEMA group. PHEMA, poly-β-hydroxyethyl methacrylate; OD, optical density.

Western blot analysis to determine the direction of differentiation of the ASCs. Western blotting was used for the semi-quantitative detection of specific proteins expressed in myocardial stem cells and myocardial cells. Fig. 4 demonstrates that differentiated ASCs expressed the myocardial proteins cTnT, Cx43, desmin, GATA-4 and Nkx-2.5. The results demonstrated that a limited number of ASCs in the blank control group, which were treated without inducers and stent materials, appeared to have differentiated into myocardial stem cells and myocardial cells (Fig. 4). When compared with the blank control group, a statistically significant increase in the expression of myocardial-specific proteins in the inducer-treated control group was observed (P<0.05; Fig. 4). In addition, when compared to the inducer-treated control group, a statistically significant difference in the rate of differentiation between the two stent material structures under the same experimental conditions was observed (P<0.05; Fig. 4). The results suggest that the PHEMA stent structure effectively promoted ASCs to differentiate into myocardial cells. Compared with the other groups, the differentiation rate was highest in the transparent PHEMA group (Mon 70.1%, Wat 29.9%), and was significantly different to the white PHEMA group (Mon 25.2%, Wat 74.8%; P<0.05) and the blank control group (P<0.01; Fig. 4). The percentage increase in the expression of specific myocardial proteins relative to the controls were as follows: GATA-4, 5.23%; Nkx-2.5, 5.66%; cTnT, 36.35%; desmin, 42.57%; and Cx43, 5.78%. The percentage increase in the expression of the myocyte-specific protein, MyoD and the smooth muscle-specific protein, α-SMA were 1.03 and 1.07%, respectively, relative to the controls. These results suggest that PHEMA stent structures with a high number of matrixes and a low water content may promote the differentiation of ASCs to myocardial cells.

Discussion

The development and application of myocardial tissue engineering may provide a novel approach for the clinical treatment of ICM; however, there are currently limitations with regard to the low survival rate of stem cells and the low differentiation rate of cardiomyocyte-like cells following transplantation of seed stem cells (18,19). ASCs are known to be one of the most appropriate type of seed cells for myocardial tissue engineering (20). In order to identify appropriate seed cells, the focus of myocardial tissue engineering research has altered to focus on the construction of a bionic model of myocardial ECM (21,22). This primarily uses technology to integrate stent materials and biologically active substances, as well as stimulate mechanical or chemical signals to create a suitable environment for the survival of stem cells and the differentiation of myocardial cells (23). HEMA is an artificial polymer material used widely in the field of clinical medicine (24). It demonstrates effective biocompatibility, degradability, and resistance to high temperature, acid and alkali hydrolysis. In addition, HEMA possesses a certain level of mechanical strength, elasticity and plasticity (22,25-28). Furthermore, the hydrogel form, comprised of the hydrophilic polymer, serves an important role in clinical applications including tissue

Table I. Adhesion rate of adipose stem cells following different treatments.

Treatment group	Adhesion rate (%)	
	Matrigel	FN
Control group	28.06±0.35	33.74±1.24
5-aza-2-deoxycytidin & LN	36.17±1.50[a]	48.36±1.35[c]
5-aza-3-deoxycytidin & LN on white PHEMA	58.39±2.26[a]	65.99±2.30[c]
5-aza-4-deoxycytidin & LN on transparent PHEMA	72.88±1.64[a,b]	78.95±1.53[c,d]

Adhesion rate was calculated using the following formula: Adhesion rate (%)=[($OD_{Matrigel\ group}$ or $OD_{FN\ group}$/$OD_{BSA\ group}$)-1]x100%. [a]P<0.01 vs. Control group of cells coated with Matrigel; [b]P<0.01 transparent PHEMA group vs. white PHEMA group of cells coated with Matrigel; [c]P<0.01 vs. Control group of cells coated with FN; [d]P<0.05 transparent PHEMA group vs. white PHEMA group of cells coated with FN. FN, fibronectin; BSA, bovine serum albumin; PHEMA, poly-β-hydroxyethyl methacrylate; LN, laminin; OD, optical density.

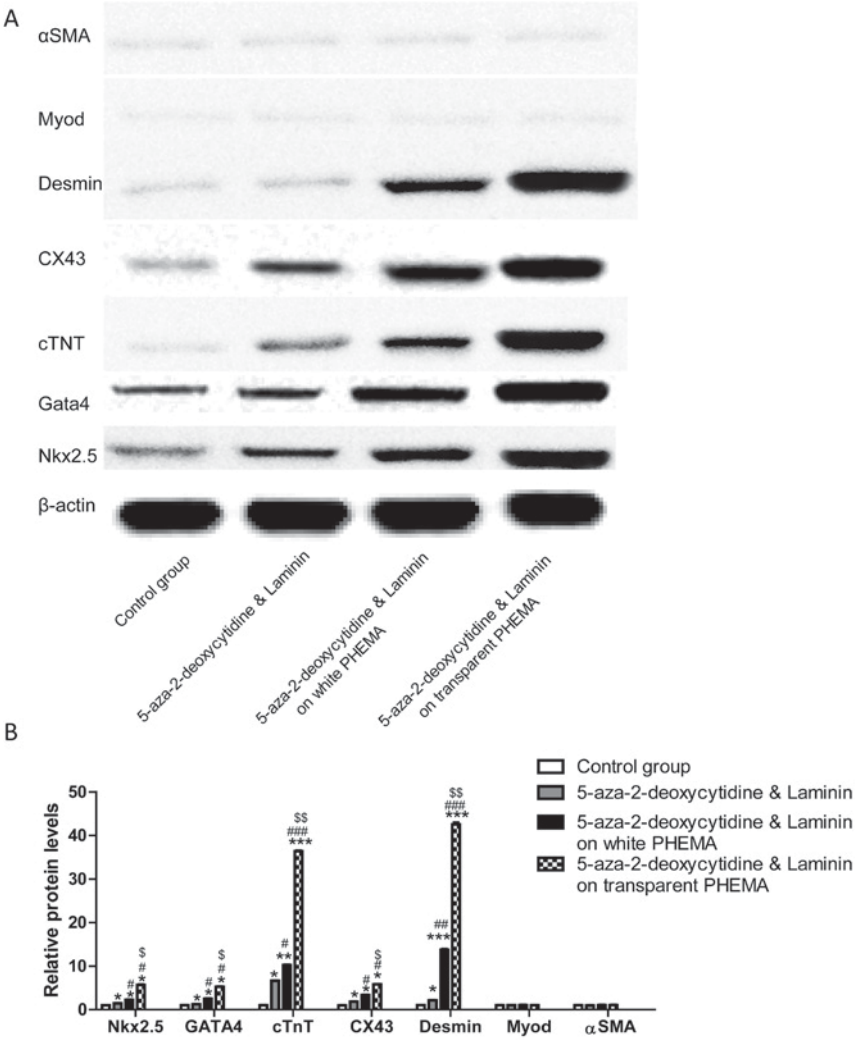

Figure 4. Level of cardiomyocyte-like cell differentiation across the 4 groups. (A) Western blotting analysis of protein expression levels and (B) quantification of the results. The percentage increase in the protein expression levels of differentiation markers relative to control in the adipose-derived stem cells in the transparent group were as follows: GATA-4, 5.23%; Nkx-2.5, 5.66%; cTnT, 36.35%; desmin, 42.57%; Cx43, 5.78%. There were no differences observed in the expression of MyoD and α-SMA among groups. *P<0.05, **P<0.01 and ***P<0.001 vs. control group; #P<0.05, ##P<0.01 and ###P<0.001 vs. 5-aza-2'-deoxycytidine and laminin group; $P<0.05 and $$P<0.01 vs. white PHEMA group. Gata4, GATA binding protein 4; Nkx2.5, NK2 homeobox 5; cTnT, cardiac troponin T; Cx43, connexin-43; MyoD, myogenic differentiation; α-SMA, α-smooth muscle actin; PHEMA, poly-β-hydroxyethyl methacrylate.

regeneration, heart transplantation and skin grafts (22,29-31). Its three-dimensional rubber structure and high-level of water retention are very similar to that observed in human tissues. The present study used HEMA substrates to form PHEMA

hydrogel stents. On the one hand, the hydroxyl and carboxyl groups increase the hydrophilic properties of the polymer, however, the hydrophobic methyl groups and the main stem maintain the hydrolytic stability of the polymer and support a certain degree of mechanical strength in the matrix (32,33). In addition, the PHEMA hydrogel form possesses an ideal porous structure. The characteristics of the induction of photopolymerization to phase-separation may be generated by a one-step polymerization reaction.

Previous studies have demonstrated that 5-azathioprine (5-Aza) promotes the differentiation of ASCs to cardiomyocyte-like cells during cultivation (34,35). Planat-Bénard *et al* (6) and Rangappa *et al* (36) successfully induced ASC differentiation into active myocardial cells using 5-Aza. Decitabine is an analogue of 2'-deoxycytidine, and demonstrates a 30-fold higher level of inhibitory activity on DNA methylation when compared with 5-Aza (37,38). Previous studies have indicated that two natural ECM components, LN and FN, serve an important role in the growth and differentiation of ectomesenchymal stem cells *in vitro* (39-41). LN and FN are highly expressed in the normal myocardium following myocardial infarction (42). Van Dijk *et al* (17) confirmed that FN enhanced the cell adhesion rate of ASCs, while LN improved the differentiation rate of ASCs to cardiomyocyte-like cells. Notably, the data demonstrated that under the co-induction of decitabine and LN, the differentiation rate of ASCs to myocardial cells was as high as 61%. A previous study has revealed that stem cell proliferation decreases following exposure to the inducer 5-Aza (43). The present study utilized a novel type of decitabine and LN to induce and effectively promote the proliferation of ASCs.

The specific myocardial transcription factors, Nkx2.5 and GATA-4, serve an important role in the early embryonic development of the heart (44). Bai *et al* (35) and Gassanov *et al* (45) revealed that Nkx2.5 induces the transcription of a series of genes in a downstream signaling pathway, by combining the zinc finger structure at the end of GATA-4c, thereby inducing the expression of a large number of myocardial transcription factors (46,47). Therefore, Nkx2.5 is able to control the original myocardial tube formation and cyclization, as well as myocardial cell differentiation. In addition, the expression of Nkx2.5 is one of the earliest characteristics of the differentiation of cardiac precursor cells. In the present study, ASCs may have differentiated into cardiomyocyte-like cells following induction, as an overexpression of desmin, cTnT, Cx43, Nkx2.5 and GATA4, which are markers of cardiomyocyte-like cells, was observed. The blank control group exhibited increased transcription factor and protein expression of Nkx2.5 and GATA4, demonstrating that free differentiation of ASCs to cardiomyocyte-like cells had occurred without induction. When compared with the blank control group, the level of differentiation in the inducer control group was significantly different, suggesting that the inducer effectively promotes ASC differentiation into cardiomyocyte-like cells. Positive expression of the transcription factors, Nkx2.5 and GATA-4, in the PHEMA groups were higher than that observed in the control group. The results indicated that under the same experimental conditions plus exposure to inducers and chemical factors produced via ASC paracrine signaling mechanisms, the PHEMA stent microstructure promotes the differentiation of ASCs into cardiomyocyte-like cells, and the expression of cardiac differentiation-associated transcription factors. The protein expression levels of cardiac transcription factors were highest in the transparent PHEMA group (Mon 70.1%, Wat 29.9%). In addition, the expression levels of the specific myocardial proteins, cTnT, desmin and Cx43 were highest in the transparent PHEMA group, and the expression levels of the specific proteins MyoD and α-SMA in myocardial stem cells were lower, suggesting that it may be easier to produce the cardiomyocyte-like phenotype in the transparent PHEMA microstructure. Although the western botting data was not consistent with the high differentiation rate observed by van Dijk *et al* (17), it was higher than the positive rate observed by Gaustad *et al* (48).

Cx43 is one of the most important proteins present in the gap junction channels located between mammalian ventricular muscle cells (49). It serves an important role in cardiac development, normal cardiac electrical activity, heart diastolic movement and the differentiation of stem cells to myocardial cells (50). In addition, Cx43 has been observed to be a functional marker of stem cell differentiation to cardiomyocyte-like cells (51). Shulz and Heusch (52) noted that the positive and negative expression of Cx43 greatly influenced the pathophysiological processes associated with ischemic heart disease and atherosclerosis. Thomas *et al* (53) demonstrated that when the content of Cx43 was reduced by 50%, ventricular conduction velocity was subsequently reduced by 38%, which induces intraventricular blocks and leads to arrhythmia and sudden death. This demonstrated that the microstructure of transparent PHEMA may be advantageous to the formation of Cx43 between cardiomyocyte-like cells and further formation of electrical coupling. However, the present study failed to observe the synchronous pulse of cardiomyocyte-like cells under the microscope. If regular mechanical traction and electrical stimulation that simulate diastole are used to increase ASC mechanical signaling, it is possible that the differentiation rate of cardiomyocyte-like cells may be greatly improved.

The present study only investigated two forms of PHEMA stents. The most suitable proportion of matrix-to-water in the PHEMA stent necessary for the differentiation of cardiomyocyte-like cells requires further investigation, and will be explored in future experiments.

In conclusion, inducers and material stent microstructures effectively promote the proliferation, growth and adhesion of ASCs. In addition, the transparent material microstructure was revealed to be a more suitable candidate for ASC vaccinations. The experiments provide additional evidence to suggest that in PHEMA stents, a structure with a high number of matrixes and a low water content, increases the rate of ASC differentiation to myocardial cells.

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