molecular pharmaceutics

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Induced Pluripotent Stem (iPS) Cells Inhibit Apoptosis and Fibrosis in Streptozotocin-Induced Diabetic Rats

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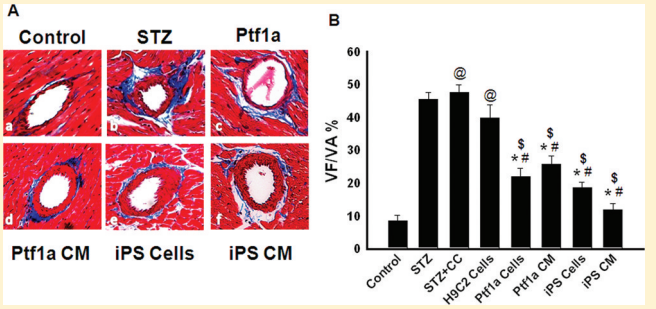
ABSTRACT: Recent data suggests that transplanted bone marrow stem cells improve cardiac function in streptozotocin induced diabetic cardiomyopathy (SIDC). However, whether modified embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, or factors released from these cells can inhibit apoptosis and fibrosis remains completely unknown. Therefore, we hypothesized that transplanted ES cells overexpressing pancreatic transcription factor 1 a (Ptf1a), a propancreatic endodermal transcription factor, iPS cells, or their respective conditioned media (CM) will attenuate cardiac remodeling and improve cardiac function in SIDC. Experimental diabetes was induced in male Sprague−Dawley rats (8−10 weeks old) by intraperitoneal injections of streptozotocin (STZ) (65 mg/kg body weight). Animals were divided into different groups including control, STZ, stem cells, and CM. Histology, TUNEL, caspase-3 activity, sarcomeric α-actin, and DHE stainings were performed to assess cardiac apoptosis, fibrosis, and oxidative stress. Animals transplanted with ES cells, iPS cells, or both CM showed a significant ($p < 0.05$) reduction in apoptosis compared with STZ treated animals. Furthermore, our data also shows that active apoptosis was present in cardiac myocytes as confirmed with combined stainings with TUNEL, sarcomeric α-actin, and active caspase-3 antibodies. Increased oxidative stress as evidenced by DHE staining was significantly ($p < 0.05$) reduced following stem cell or CM transplantation. Moreover, stem cells or CM also attenuated increased interstitial and vascular fibrosis in SIDC hearts. Echocardiography analysis showed a significant ($p < 0.05$) improvement in fractional shortening in stem cell and CM transplanted groups compared with respective controls. In conclusion, our data suggest that transplanted stem cells or their CM inhibit apoptosis, reduce fibrosis, and improve cardiac function in STZ-treated diabetic rats.



KEYWORDS: *heart, fractional shortening, type 1, embryonic stem cells, induced pluripotent stem cells*

INTRODUCTION

Diabetes is a devastating disease affecting an estimated 170 million people worldwide.[41] The World Health Organization (WHO) predicts that this number will more than double to an astounding 366 million people by the year 2030.[41] Type 1 diabetes is an autoimmune disease characterized by destruction of insulin-producing beta cells of the islets of Langerhans in the pancreas.[4,41] Normally, insulin functions to regulate blood glucose levels and to increase cellular absorption of glucose by enhancing membrane expression of glucose receptors.[13] Loss of insulin production leads to high circulating blood glucose, or hyperglycemia, which causes many complications, including retinopathy, nephropathy, neuropathy, impaired wound healing, osteoporosis, and cardiomyopathy.[4,11,18,21] Moreover, diabetic cardiomyopathy (DCM) alters many pathways mediated by oxidative stress and hyperglycemia,[6] which leads to an increase in cellular death via apoptosis and necrosis.[16,36] In the diabetic heart, apoptosis is well documented by various investigators.[1,5] Following apoptotic cell death, adverse ventricular remodeling occurs, which involves interstitial and vascular fibrosis.[10,19] Fibrosis alters cardiac structure and geometry as well as initiates stiffening of the heart that further significantly reduces cardiac function, which leads to congestive heart failure and death.[3,7,38] The exact mechanisms of increased fibrosis in diabetic cardiomyopathy are still being elucidated.[7] Various treatment strategies are being developed to treat diabetic cardiomyopathy including intensive insulin therapy, islet and β-cell transplantation, copper chelators, glycated cross-link breakers, and advanced glycation end product inhibitors.[1,20,24−26]

Recent studies have demonstrated that stem cell strategies could be potential options to treat various cardiac diseases.[16,30,32] More recently, the effects of bone marrow derived stem cells have been examined in diabetic cardiomyopathy.[43] This data suggests an increase in angiogenesis, with improved cardiac function. However there was limited evidence of enduring engraftment of stem cells in the diabetic heart following bone marrow stem cell transplantation. Based on these observations we postulate that beneficial effects in this model may also be due to autocrine or paracrine factors

Received: May 23, 2011
Accepted: October 11, 2011
Published: October 11, 2011

secreted from the transplanted stem cells. However, there are no studies which demonstrate the effects of modified ES cells overexpressing pancreatic transcription factor 1 a, cardiac iPS cells, and their CM on STZ-induced diabetes in rats. Accordingly, we hypothesize that transplanted Ptf1a or iPS cells and their CM will inhibit cardiac remodeling and improve cardiac function in the SIDC rat model.

MATERIALS AND METHODS

Ptf1a Cells. Ptf1a, transfected ES cells were a kind gift from Mark Magnuson of Vanderbilt University as reported in Burlison et al.[9] Ptf1a ES cells were cultured and maintained with similar cell culture conditions as we previously published.[32]

Generation of iPS Cells. iPS cells were generated via transducing H9c2 cells (cardiomyoblasts) as we published recently.[42] In brief, the pBluescript SK(−) vector from Stratagene was used to clone the 4 human stemness factors c-Myc, Klf4, Oct3/4, and Sox2. H9c2 cells were transduced with four stemness factors as mentioned above. Expression of the 4 human stemness factors was verified by RT-PCR and Western blot analysis. Additionally, iPS cells were stained for Oct 3/4 and alkaline phosphatase to verify pluripotency. Transduced H9c2 cells, now referred to as iPS cells, were cultured in iPS growth medium (GM) containing 50% mouse embryonic fibroblast-CM, 50% embryonic stem cell-growth medium (Dulbecco's modified Eagle's medium), supplemented with streptomycin, penicillin, nonessential amino acids, glutamine, ES qualified fetal bovine serum, leukemia inhibitor factor (LIF), β-mercaptoethanol, 10 μg/mL bFGF, and 10 μg/mL activin A.

Generation of Conditioned Media. As we described previously using ES cells, CM was generated by plating 1.6×10^6 iPS cells or Ptf1a-ES cells in GM.[33] The following day, growth medium was removed and replaced with differentiation medium (DM), which lacks LIF and β-mercaptoethanol. Forty-eight hours later, medium was removed, filtered to remove any cellular debris, and labeled as Ptf1a or iPS CM.

Streptozotocin-Induced Diabetes in the Rat. All procedures using animals were approved by the University of Central Florida Institutional Animal Care Use Committee. Male Sprague−Dawley rats 8−10 weeks old were purchased from Charles River, Wilmington, MA. Animals were provided an irradiated rodent diet from PicoLab, Brentwood, MO, and autoclaved water *ad libitum*. Animals were divided into eight study groups including control, STZ, STZ + cell culture medium (CC), STZ + H9c2 cells, STZ + Ptf1a ES cells, STZ + Ptf1a CM, STZ + iPS cells, and STZ + iPS CM. In all groups, prior to injections, blood sugar was measured using a One-touch Ultra glucose meter. Following glucose determination, all groups except control were injected with STZ intraperitoneally (ip) on day 1 (D1) and D2 at a dose of 65 mg/kg, as reported previously. In the control group, only sodium citrate vehicle buffer was injected ip, as this solution was used to prepare STZ. Twenty-four hours post the last STZ injection, blood glucose was measured again. All animals with circulating blood glucose exceeding 250 mg/dL were considered diabetic and continued in the study. On D3, D4, and D5, animals were injected intravenously with Ptf1a-ES cells or iPS cells at 1×10^6 cells/1 mL/day or Ptf1a-ES or iPS CM 2 mL/day. Control animals were given 1 mL/day saline iv. At D28 after the last injection of the various treatments, rats were sacrificed under isoflurane followed by cervical dislocation. Hearts were removed, the top half placed in RNA later, and the bottom half in 4% paraformaldehyde (PFA) for further analysis.

Determination of Apoptosis. Hearts preserved in 4% PFA were washed 3 times with PBS and transferred to 70% ethanol. Hearts were dehydrated in a Leica tissue processor sequentially in 70, 80, 95, and 100% ethanol, followed by incubation in Citrosol, and paraffin. Paraffin blocks were prepared using a TissueTek embedding machine. Five micrometer (5 μm) sections were cut transversely from the paraffin blocks. Sections were deparaffinized and rehydrated as we reported previously.[32] Slides were then washed with distilled water, followed by PBS for 6 min. TUNEL staining for apoptotic nuclei was done with TMR Red In Situ Cell Death Detection kit from Roche, (Burlingame, CA) following the manufacturer's instructions as described in the kit. Vectashield (Indianapolis, IN) mounting medium containing DAPI was used to stain total nuclei. Percent TUNEL stained positive nuclei = number of TUNEL positive nuclei/total nuclei × 100. Apoptotic nuclei were quantified by counting the total number of TUNEL positive nuclei on an entire section from six different rat hearts per experimental group.

Sections were also colabeled with primary antibody either against active anti-caspase-3 rabbit polyclonal (1:50 dilution, Santa Cruz Biotechnology and cell signaling) or sarcomeric cardiac α-actin antibody (1:20; Sigma) for 1 h at 37 °C in a humidified chamber to determine presence of active caspase-3 in cardiac myocytes. Sections were washed and incubated with secondary antibody Alexa 635 or Alexa 488 (Molecular Probes). Rat heart sections were covered with Antifade Vectashield mounting medium containing DAPI (Vector Laboratories) to stain all nuclei present in the heart section. Sections were examined with a confocal microscope.

Caspase-3 Activity. To confirm apoptosis using an additional method, we performed a caspase-3 activity assay, which is a hallmark of apoptosis. In brief, tissue from hearts preserved in RNAlater (Ambion) was homogenized in RIPA buffer with protease inhibitor cocktail (Sigma), PMSF, sodium fluoride, and sodium orthovandate. Homogenates were centrifuged for 10 min at 13000*g*. Supernatant was removed and stored at −20 °C for later use. Tissue homogenates were assayed for protein concentration by Bradford assay using Bio-Rad Protein Assay Dye Reagent, and Bio-Rad Protein Assay Standard 11, bovine serum albumin. Biovision caspase-3 colorimetric activity assay kit was used as per manufacturer's directions, using 200 μg of each protein sample. Developed bluish color was measured in a microtiter plate reader (Biorad) at 405 nm. The caspase-3 graph was plotted as arbitrary units.

Oxidative Stress Determined by DHE Staining. Dihydroethidium (DHE) fluorescence staining was performed on deparaffinized rat heart sections with 1 μM/mL DHE dye obtained commercially for 25 min at room temperature. Red staining positively indicating oxidative stress was examined and captured on an Olympus and confocal microscope. Using NIH Image J program, red staining fluorescence intensity was quantified in 4−6 randomly selected regions in the SIDC heart sections in 6 animals/group as we previously reported.[15] Average corrected integrated density was calculated, and a fluorescence intensity graph was plotted as arbitrary fluorescence units (AFU) as reported.[15]

Fibrosis Quantification. Heart tissue was blocked and embedded as previously described.[33] Sections were stained with Masson's trichrome. To quantify interstitial fibrosis, sections were first qualitatively assessed, and then quantified directly

dx.doi.org/10.1021/mp2004675|*Mol. Pharmaceutics* 2011, 8, 2350−2357

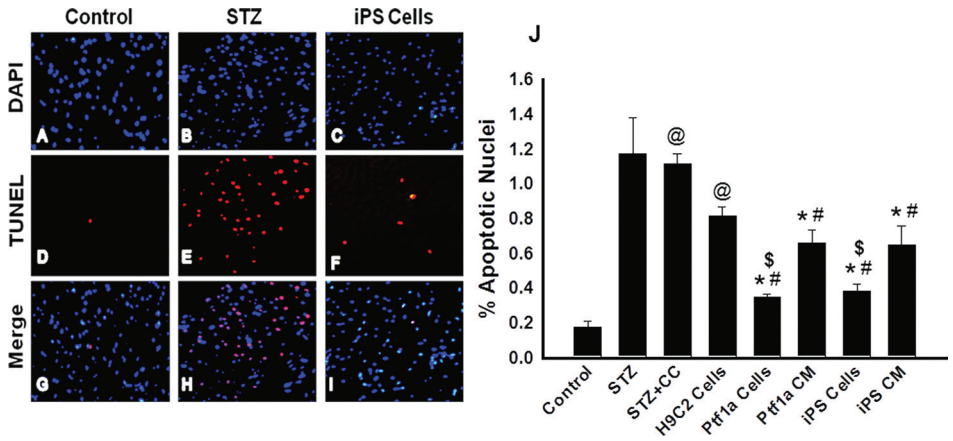


Figure 1. Effects of transplanted Ptf1a and iPS cells and their CM on apoptosis in the STZ-induced diabetic rat heart. Representative photomicrographs of control, STZ, and iPS cell hearts demonstrating total nuclei stained with DAPI in blue (A–C), apoptotic nuclei stained with TUNEL in red (D–F), and merged nuclei in pink (G–I). Magnification, 40×. (J) Histogram shows quantitative % apoptotic nuclei per total nuclei from control and treated groups. $^{\#}p < 0.001$ vs STZ, $^{*}p < 0.05$ vs STZ + CC, $^{\$}p < 0.05$ vs H9c2 cells, and $^{@}p > 0.05$ vs STZ. Data set is from $n = 6$ animals per group.

using Image J software by measuring 10 microscopic fields from 6 different rats per experimental group, at 20× magnification. Vascular fibrosis was also quantitated using the same Masson's trichrome stained sections. Initially, a qualitative analysis was done to assess the range of vascular fibrosis. Subsequently representative photomicrographs of 10 vessels per section for 6 animals/experimental group were analyzed. Total vessel area and fibrotic areas were measured using image J software. Percent vascular fibrosis was calculated as vascular fibrosis/total vessel area × 100.

Assessment of Cardiac Function. At D28 post STZ injections and cell or CM transplantation, two-dimensional (2D) echocardiography was performed on the rats as previously reported.[23] The rats were anesthetized with 2% isoflurane through nose cone continuous inhalation while placed on a heated platform in the supine position. Internal diastolic and systolic left ventricular diameters (LVIDd and LVIDs, respectively) were quantified using short-axis M-mode images on a Philips Sonos 4500 or 5500 echocardiograph machine. Fractional shortening (FS) was calculated using obtained LVIDd and LVIDs values (FS % = (LVIDd − LVIDs)/LVIDd × 100.

Statistical Analysis. Statistical analysis was completed using Student's *t* test, one-way analysis of variance (ANOVA), Bonferroni, and Tukey tests. Statistical significance was assigned when $p < 0.05$.

RESULTS

Ptf1a/iPS Cells and Their CM Inhibit Apoptosis in SIDC. To assess whether Ptf1a/iPS cells and their CM inhibit apoptosis in the SIDC hearts, TUNEL staining was performed (Figure 1A–I) at D28. Our data suggests that there was a significant increase in TUNEL-positive nuclei in the STZ treated group compared with control (STZ, 1.17 ± 0.21%, vs control, 0.18 ± 0.03%, mean ± SEM, $p < 0.001$, Figure 1J). Moreover, rat hearts transplanted with Ptf1a/iPS cells demonstrated significantly reduced apoptosis compared with STZ, STZ + CC, and H9c2 cell groups (Ptf1a cells, 0.35 ± 0.02%, and iPS cells, 0.39 ± 0.04%, vs STZ, 1.17 ± 0.21%, $p < 0.001$, STZ + CC, 1.11 ± 0.06%, $p < 0.05$, and H9c2 cells, 0.82 ± 0.05%, $p < 0.05$; Figure 1J).

To corroborate our TUNEL apoptotic data, we performed an additional activity assay for caspase-3, an apoptotic hallmark. As with the TUNEL data, hearts from the SIDC groups had a significant increase in caspase-3 activity compared with controls (STZ, 0.22 ± 0.01, vs control, 0.12 ± 0.01, $p < 0.001$, Figure 2).

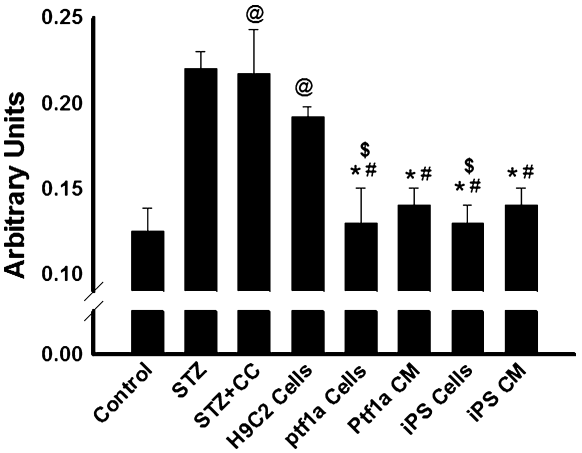


Figure 2. Effects of caspase-3 activity in Ptf1a and iPS cell and their CM treated hearts. Histogram shows quantitative analysis of caspase-3 activity in hearts transplanted with and without Ptf1a/iPS cells and CM or H9c2 cells. Data set is from $n = 6$ animals per group. $^{\#}p < 0.001$ vs STZ, $^{*}p < 0.05$ vs STZ + CC, $^{\$}p < 0.05$ vs H9c2 cells. Data set is from $n = 6$ animals per group.

Furthermore, Ptf1a/iPS cells and their CM transplanted hearts exhibited reduced capsase-3 activity compared with STZ group (Ptf1a cells, 0.13 ± 0.02, Ptf1a CM, 0.14 ± 0.01, iPS cells, 0.13 ± 0.01, and iPS CM, 0.14 ± 0.01, vs STZ, 0.22 ± 0.01, $p < 0.05$, and STZ + CC, 0.217 ± 0.03, $p < 0.05$; Figure 2). Additionally, the H9c2 cell group did not show a statistically significant decrease in either TUNEL staining or caspase-3 activity as compared with STZ treated groups. Moreover, there was no statistically significant difference in the inhibition of apoptosis as determined by TUNEL staining and caspase-3 activity between hearts transplanted with cells (Ptf1a and iPS) or their CM (Figures 1 and 2). Figure 3 shows apoptotic nuclei stained with TUNEL, colabeled with active caspase-3 antibody and

dx.doi.org/10.1021/mp2004675|*Mol. Pharmaceutics* 2011, 8, 2350–2357

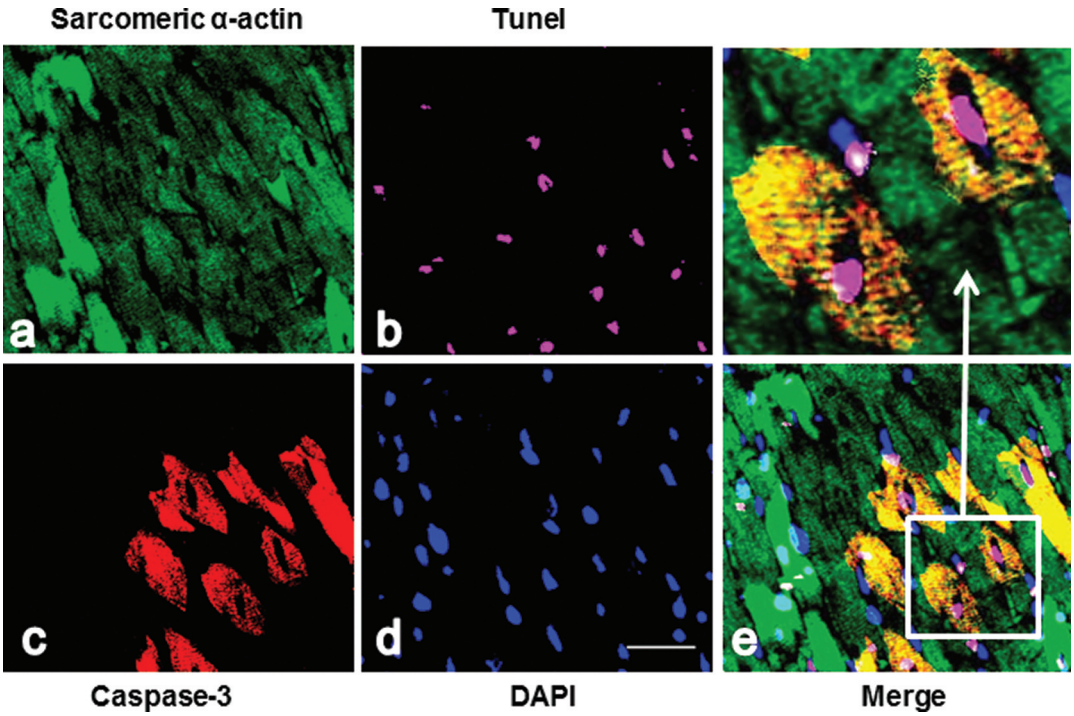


Figure 3. Representative photomicrographs of apoptosis present in cardiac myocytes shows sarcomeric a-actin staining in green (a), TUNEL staining in pink (b), caspase-3 in red (c), and DAPI in blue (d); a merged image is shown (e). Scale bar: 100 μm.

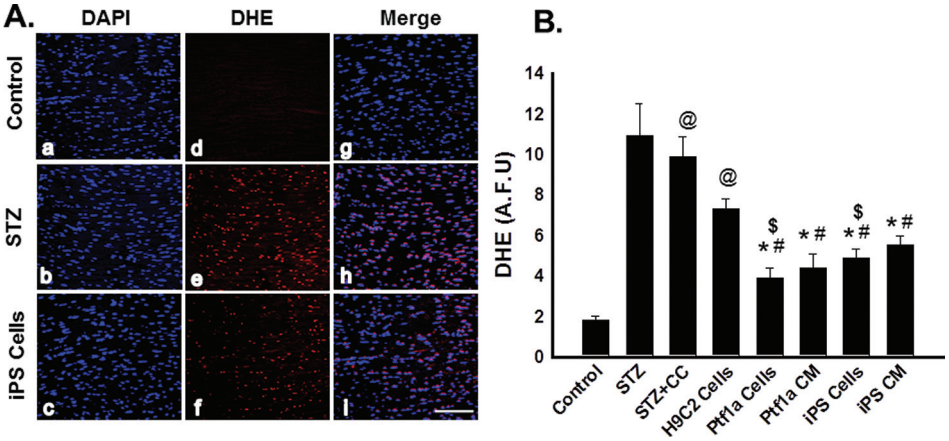


Figure 4. Effect of transplanted Ptf1a, iPS cells, and their CM on superoxide production in the SIDC rat heart. (A)[39]Representative photomicrographs of DHE stained rat heart show total nuclei stained with DAPI in blue (a–c), DHE positive cells stained in red (d–f), and merged nuclei appeared in pink (g–i). Scale bar: 100 μm. (B) Histogram shows quantitative corrected fluorescence integrated density from control and treated groups. $^{\#}p < 0.001$ vs STZ, $^{*}p < 0.05$ vs STZ + CC, $^{\$}p < 0.05$ vs H9c2 cells, and $^{@}p > 0.05$ vs STZ. Data set is from $n = 6$ animals per group.

cardiac specific sarcomeric α-actin antibody, suggesting that apoptosis is present in cardiac myocytes in SIDC hearts.

Ptf1a/iPS Cells and Their CM Inhibit Superoxide Production in the SIC Heart. Our data shows a significant increase in superoxide production in STZ, STZ + CC and STZ + H9c2 cell groups when compared to normal controls ($p < 0.001$, Figure 4). Additionally, transplantation of Ptf1a/iPS cells and their CM demonstrated a reduced superoxide production as compared with STZ and STZ + CC groups (Ptf1a cells, 3.9 ± 0.47, Ptf1a CM, 4.41 ± 0.7, iPS cells, 4.9 ± 0.4, and iPS CM, 5.51 ± 0.47, vs STZ, 10.94 ± 1.59, $p < 0.001$, and STZ + CC, 9.88 ± 0.97, $p < 0.05$ (Figure 4). H9c2 and STZ + CC treated groups demonstrated no significant reduction in superoxide production as compared to STZ, suggesting that growth factors present in the cell culture system as well as factors released from nonpluripotent cell line, H9c2 cells, are not protective in nature (Figure 4).

Ptf1a/iPS Cells and Their CM Inhibit Fibrosis in the DCM Heart. To determine the effects of cardiac fibrosis in hearts transplanted with Ptf1a/iPS cells and their CM as well as H9c2 cells, we performed Masson's trichrome staining and quantified interstitial and vascular fibrosis (Figure 5A and Figure 6A). Interstitial fibrosis in the STZ-treated group was significantly increased compared to control (STZ, 0.09 ± 0.02, vs control, 0.00 ± 0.00, mean ± SEM, $p < 0.001$, Figure 5B). Following transplantation of Ptf1a/iPS cells and their CM, interstitial fibrosis was significantly reduced compared with STZ and STZ + CC groups (Ptf1a cells, 0.03 ± 0.01, Ptf1a CM, 0.05 ± 0.01, iPS cells, 0.01 ± 0.00, and iPS CM, 0.03 ± 0.01, vs

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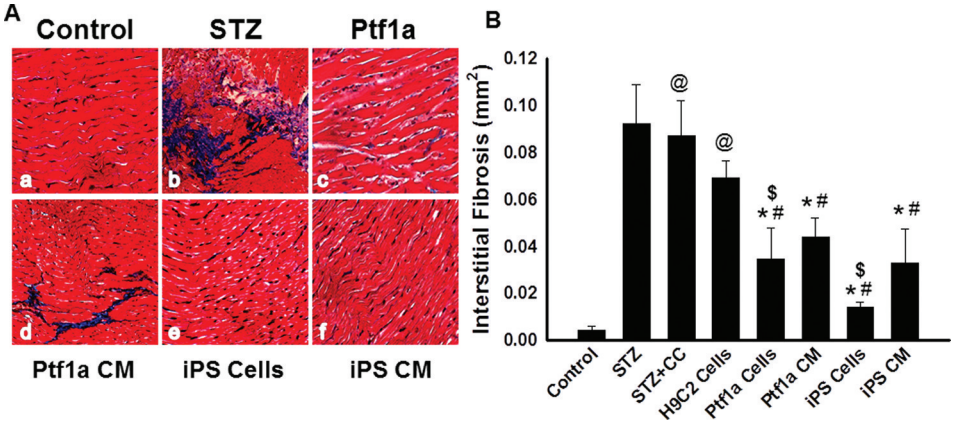

Figure 5. Effects of transplanted Ptf1a/iPS cells and CM or H9c2 cells on interstitial fibrosis. (A) Representative photomicrographs from sections stained with Masson's trichrome 28 days following cell and CM transplantation. Magnification, 40×. (B) Histogram data demonstrates quantitative interstitial fibrotic area in DCM rat hearts transplanted with CC, H9c2, Ptf1a, iPS cells, and Ptf1a and iPS CM. $^{\#}p < 0.001$ vs STZ, $^{*}p < 0.05$ vs STZ + CC, $^{\$}p < 0.05$ vs H9c2 cells, and $^{@}p > 0.05$ vs STZ. Data set is from $n = 6$ animals per group.

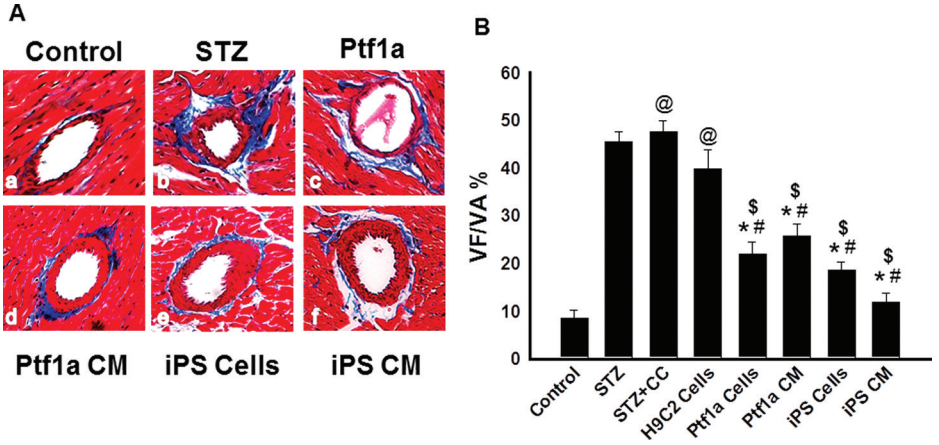

Figure 6. Effects of transplanted Ptf1a and iPS cells and their CM on vascular fibrosis in the DCM rat heart. (A) Representative photomicrographs of vessels stained with Masson's trichrome from the control, STZ, Ptf1a, Ptfla CM, iPS, and iPS CM experimental groups. (B) Histogram representing the quantitative analysis of percent vessel fibrosis per vessel area in all experimental groups. $^{\#}p < 0.001$ vs STZ, $^{*}p < 0.001$ vs STZ + CC, $^{\$}p < 0.05$ vs H9c2 cells, and $^{@}p > 0.05$ vs STZ. Data set is from $n = 6$ animals per group. VF = vessel fibrosis, VA = vessel area.

STZ, 0.09 ± 0.02, and STZ + CC, 0.09 ± 0.01, $p < 0.05$, Figure 5B).

Next, our quantitative data demonstrate that vascular fibrosis in the STZ treated animals was significantly increased compared with controls (Figure 6B, $p < 0.001$). Furthermore, Ptf1a/iPS cell groups demonstrated a significant ($p < 0.05$) reduction in vascular fibrosis. Similar effects were observed with CM obtained from iPS and Ptf1a cells. H9c2 cell treated hearts did not show an improvement in interstitial or vascular fibrosis when compared to STZ or STZ + CC groups. Consistent with our previous apoptotic findings, our fibrosis data, both interstitial and vascular, suggests that Ptf1a and iPS cells and their CM inhibit fibrosis in the STZ-induced cardiomyopathy rat heart (Figure 5 and 6).

Echocardiography. Cardiac function was assessed by measuring fractional shortening transthoracically by M-mode echocardiography. Fractional shortening was significantly reduced in the STZ treated groups compared to control (STZ, 29.19 ± 0.80%, vs control, 48.92 ± 2.45%, mean ± SEM, $p < 0.001$, Figure 7). Fractional shortening was significantly increased in Ptf1a/iPS cell and CM treated groups compared to STZ and STZ + CC groups (Ptf1a cells, 39.37 ± 0.90%, Ptf1a CM, 35.95 ± 1.16%, iPS cells, 40.29 ± 1.41%, and iPS CM,

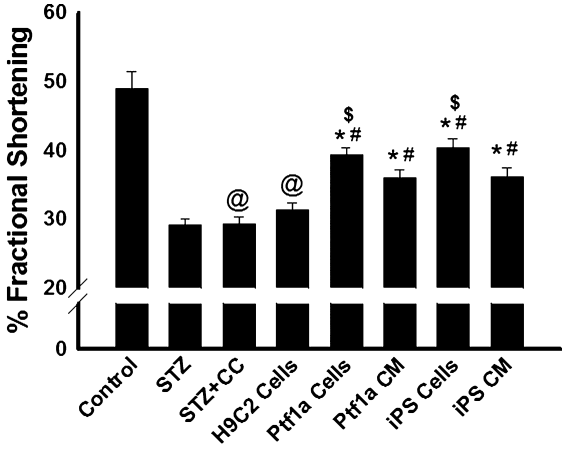

Figure 7. Effects of transplanted H9c2, Ptf1a, iPS cells, and Ptf1a and iPS CM on cardiac function. Echocardiography was performed at D28 following cell transplantation, and fractional shortening was determined. Histogram data shows average fractional shortening at D28 for all experimental groups. $^{\#}p < 0.001$ vs STZ, $^{*}p < 0.001$ vs STZ + CC, $^{\$}p < 0.05$ vs H9c2 cells, and $^{@}p > 0.05$ vs STZ.

dx.doi.org/10.1021/mp2004675|*Mol. Pharmaceutics* 2011, 8, 2350–2357

36.13 ± 1.29%, vs STZ, 29.19 ± 0.80%, and STZ + CC, 39.43 ± 1.0, $p < 0.001$, Figure 7). However, there was no statistical significance observed in fractional shortening between groups treated with Ptf1a/iPS cells or their CM. Additionally, the H9c2 cell treated group did not show improvement in cardiac function when compared to nontreated groups (Figure 7).

DISCUSSION

Diabetes is a leading cause of death worldwide with the majority of these deaths attributable to CVD.[36,41] Although great progress has been made toward the development of therapeutic options for the treatment of DCM, novel and effective strategies are still needed.[1,21,38] Stem cells and their CM have recently gained significant attention for their potential to repair and regenerate the injured myocardium. Pathogenesis for the progression of diabetes is complex.[2,12,32] However, it has been reported that apoptosis, necrosis, and fibrosis are among the major players in DCM.[1,3,5,19] Apoptosis is a programmed cell death regulated by the gene program in the cell.[10] Once apoptosis in the heart occurs, cardiac cells are eliminated and replaced by proliferating cardiac fibroblasts, which gives rise to cardiac fibrosis and creates stiffening of the heart muscle along with decreased cardiac function.[29]

Accordingly, we have examined whether apoptosis and fibrosis are present in the SIDC rat heart model we have generated. Our data confirms the presence of increased circulating blood glucose levels, cardiac apoptosis, and fibrosis compared with the control animals. Therefore we have generated viable STZ-induced diabetic cardiomyopathy rat models with characteristics which are similar to those reported by others in DCM.[5,14,22]

The major goal of this study was to determine the effects of transplanted iPS/Ptf1a cells and their CM on cardiac apoptosis and fibrosis in the SIDC rat model. Upon transplantation of Ptf1a/iPS cells or their CM, the number of cardiac apoptotic nuclei and caspase-3 activity was significantly decreased. We have for the first time shown that Ptf1a/iPS cells and their CM inhibit apoptosis in the DCM heart. Previous studies have shown that transplanted ES and mesenchymal stem cells significantly inhibit apoptosis in the infarcted heart.[17] Additionally, ES and mesenchymal stem cell CM have been shown to inhibit apoptosis *in vitro* and in *in vivo* models of injured myocardium.[31,43] However, this study was not designed to examine detailed mechanism of inhibited apoptosis. Therefore, further studies are needed.

There are reports that stem cells and their CM inhibit fibrosis in pathological cardiac conditions including cardiomyopathies and myocardial infarction.[8,31] However, no study defines the effects of Ptf1a/iPS cells and CM on fibrosis in SIDC. Accordingly, in our present study, interstitial and vascular fibrosis was quantified. As stated previously, rats receiving only STZ had significantly enhanced interstitial and vascular fibrosis compared to normal control animals. However, following transplantation of Ptf1a/iPS cells or CM, interstitial and vascular fibrosis were significantly inhibited. Endothelial to mesenchymal transition has been postulated to contribute to larger numbers of fibroblasts and an increase in their secreted fibrotic matrix proteins as reported by Widyantoro et al.[40] It is hypothesized that factors secreted from stem cells may actually prevent the transition from endothelial to mesenchymal cell type. Stem cells or their conditioned media may act in reducing endocardial to mesenchymal transition, reducing numbers of fibroblasts and hence fibrosis.[44] A few other possible mechanisms are cell fusion, mitochondrial transfer, and activation of endogenous cardiac progenitor cells.[28,34,35] Although our data suggests that transplanted Ptf1a ES and iPs cells and their CM inhibit fibrosis in SIDC, further studies will be required to elucidate mechanisms by which this occurs. In our SIDC rat model, the presence of apoptosis and interstitial and vascular fibrosis was not statistically significant when compared with STZ + CC and STZ + H9c2 cells, suggesting that growth factors present in the cell culture medium as well as nonpluripotent cell line, H9c2 cells are different and are unable to provide cell protective effects as observed in pluripotent cell lines. Moreover, we also suggest that attenuation of apoptosis and fibrosis in SIDC following cell or CM transplantation is mediated by autocrine or paracrine factors secreted by the transplanted stem cells. An additional possibility that warrants further study is that these autocrine or paracrine factors have activated endogenous cardiac progenitor cells, contributing to cardiac regeneration.

Significantly increased apoptosis and fibrosis contributing to poor cardiac function have been well documented in the setting of SIDC.[5,14,22] Previous studies have reported improved cardiac function following ES, mesenchymal stem, and iPS cell transplantation in infarcted hearts.[2,16,27,28,30] Moreover, CM generated from mesenchymal stem cells and ES cells containing released paracrine factors were shown to increase fractional shortening in the infarcted heart.[37] Our functional data within the present study demonstrating improved cardiac function following Ptf1a/iPS cell and CM transplantation further corroborates these previous findings.

In this study, we have shown for the first time that Ptf1a cells and their CM inhibit apoptosis and fibrosis and enhance cardiac repair in the STZ-induced diabetic rat model of DCM. Additionally, iPS cells and their CM were equally effective in inhibiting adverse remodeling and improving fractional shortening in the DCM heart. Cytoprotective mechanisms of Ptf1a/iPS cells and CM in the inhibition of apoptosis and fibrosis in the setting of DCM have not been delineated yet. Therefore, in the present study, we provide initial beneficial effects of transplanted ES or iPS cells in the DCM heart as well as opening new avenues to understand their mechanisms.

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ACKNOWLEDGMENTS

The authors are grateful to Latifa Abdelli for her technical assistance in performing an additional two groups during revisions.

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