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Research article

Subcutaneous transplantation of bone marrow derived stem cells in macroencapsulation device for treating diabetic rats; clinically transplantable site

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ABSTRACT

Background/aim: Diabetes mellitus (DM) is a serious, chronic and epidemic disease. Its effective therapy with exogenous insulin places an overwhelming burden on the patient's lifestyle. Moreover, pancreatic islet transplantation is limited by the scarceness of donors and the need for chronic immunosuppression. Cell-based therapy is considered an alternative source of insulin-producing cells (IPCs); encapsulating such cellular grafts in immunoisolating devices would protect the graft from immune attack without the need for immunosuppression. Herein, we investigate the ability of TheraCyte capsule as an immunoisolating device to promote the maturation of differentiated rat bone marrow derived mesenchymal stem cells (BM-MSCs), transplanted subcutaneously to treat diabetic rats in comparison with intratesticular transplantation.

Main methods: Rat BM-MSC were differentiated into IPCs, and either encapsulated in TheraCyte capsules for subcutaneous transplantation or transplanted intratesticular into diabetic rats. Serum insulin, C-peptide & blood glucose levels of transplanted animals were monitored. Retrieved cells were further characterized by immunofluorescence staining and gene expression analysis.

Key findings: Differentiated rat BM-MSC were able to produce insulin in vitro, ameliorate hyperglycemia in vivo and survive for 6 months post transplantation. Transplanted cells induced higher levels of insulin and C-peptide, lower levels of blood glucose in the cured animals of both experimental groups. Gene expression revealed a further in vivo maturation of the implanted cells.

Significance: These data suggest that TheraCyte encapsulation of allogeneic differentiated stem cells are capable of reversing hyperglycemia, which holds a great promise as a new cell based, clinically applicable therapies for diabetes.

1. Introduction

We have provided evidence that mesenchymal (stromal) stem cells derived from bone marrow (BM-MSCs) or adipose tissue (AT-MSCs), can be differentiated to form insulin-producing cells (IPCs) [1, 2]. We have also demonstrated that the transplanted cells underwent further differentiation *in vivo* [3]. Subcutaneous tissue offers several advantages for cell transplantation. Its access entails a minimal invasive procedure. Furthermore, it allows the monitoring of the grafted cells. Herein, we compare two transplantation modalities in rats; subcutaneously within an encapsulation device and interstitially in the testis.

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2. Method

This study was approved by the Medical Research Ethics Committee of Mansoura University, Egypt.

2.1. The experimental animals

The experimental animals were delivered from the Animal Research Facility of Urology and Nephrology Center, Mansoura University and housed in individual cages, kept in a 12-hours light-dark cycle and supplied with food and water *ad libitum*. The experiment was performed under the protocol approved by the guidelines of the Institutional Animal Care and Use Committee.

2.2. Isolation and expansion of rat BM-MSCs

Rat bone marrow stem cells were isolated from the long bones of adult Sprague Dawley (SD) rats according to the method previously described [4]. Cells were cultured at a density of 1×10^6/mL in 25 cm^2 cell culture flasks coated with 0.1% gelatin in water solution (STEMCELL Technologies, Vancouver, Canada) and fed with DMEM containing 10% fetal bovine serum (FBS) (Sigma-Aldrich, USA), GlutaMAX 100X (1 ml/L) (Gibco, USA) and penicillin-streptomycin solution (100 U/mL) (Sigma-Aldrich). The non-adherent populations were discarded; adherent spindle-shaped mesenchymal stem cells (MSCs) were fed every 2–3 days with DMEM supplemented with 10 % FBS. Cells were detached at 80% confluence, with 0.25% trypsin solution (Sigma-Aldrich, USA) containing 1 mmol/L EDTA (Sigma-Aldrich). This step was repeated for three passages.

2.3. Characterization of the isolated rat BM-MSCs

2.3.1. Phenotyping

MSCs at passage 3, were stained with antibodies specific for CD14, CD45, CD44 (FITC) or CD34, CD29, CD106 phycoerythrin (PE) (Becton, Dickinson, USA). The labelled cells were analyzed using an argon ion laser at a wavelength of 488 nm (FACSCalibur, Becton, Dickinson, United States). A total of ten thousand events were obtained and analyzed using Cell Quest software (Becton, Dickinson). Control staining using the appropriate isotype-matched monoclonal antibodies was included.

2.3.2. Multilineage differentiation potential

BM-MSCs were induced to differentiate into adipocytes, chondrocytes, and osteocytes using a previously described differentiation protocol [5]. Oil Red O was used to stain adipocytes, Alcian blue was used to stain chondrocytes, and Alizarin-Red was used to stain osteocytes.

2.4. Differentiation of rat BM-MSCs into IPCs

Differentiation was carried out according to a protocol reported previously by Tayaramma et al. [6]. Initially, the cells were cultured in serum free low glucose DMEM supplemented with 55 nM Trichostatin-A (TSA, Sigma-Aldrich, USA) for 3 days. Then, the cells were cultured for an additional 7 days in high glucose (25 mM) medium containing DMEM: DMED/F12 (Sigma-Aldrich) at a ratio of 1:1. This mixture was supplemented with 10% FBS (Sigma-Aldrich), 10 nM Glucagon like peptide-1 (GLP-1, Sigma-Aldrich), 10 nM Nicotinamide (Sigma-Aldrich).

2.5. Characterization of differentiated BM-MSCs

2.5.1. Immunolabelling

Anti-insulin Antibody (1:200) (mouse monoclonal, Novus Biologicals, Littleton, CO, USA), anti-c-peptide antibody (1:100) (rabbit polyclonal; Cell Signaling Technology) were used as the primary antibodies. Anti-mouse immunoglobulin G (IgG; H + L) Alexa Fluor 488 conjugate (1:200) and anti-rabbit (IgG; H + L) Alexa Fluor 555 conjugate (1:100) (Cell Signaling Technology) were utilized as the secondary antibodies.

2.5.2. Immunocytochemistry

Differentiated rat BM-MSc were cultured on 2-chamber slides (Nunc, Rochester, NY, USA), and fixed with 4% paraformaldehyde for 10 min at room temperature. Cells were then permeabilized by methanol (100%) for 10 min and blocked with goat serum (5%) at RT for one hour, followed by overnight incubation at 4 °C with the primary antibodies. Thereafter, the cells were washed with PBS and incubated for 2 h at RT with the secondary antibodies. Nuclei were counterstained by DAPI (Invitrogen, UK). For negative controls; the primary antibody was omitted. Leica TCS SP8 microscope (LeicaMicrosystems, Mannheim, Germany) was used for capturing confocal images.

2.5.3. In vitro insulin and c-peptide release in response to increasing glucose concentrations

Differentiated rat BM-MSCs were cultured in triplicates at a density of 10×10^4/well in a 12 well plate. Cells were incubated in glucose-free Krebs-Ringer bicarbonate buffer (KRB) for 30 min to remove residual insulin. The cells were then incubated for 1 h in KRB containing glucose concentrations of 5.5, 12, or 25 mM in separate wells. At the end of the incubation period, the supernatant was collected for determination of rat insulin and c-peptide using rat Insulin and c-peptide enzyme-linked immunosorbent assay (ELISA) Kits (Bioseps, Chongqing China). Finally, the protein content of each sample was determined by the Bradford method using a spectrophotometer (Azzota Corporation, Claymont, DE, USA). Results were expressed as ng/μg protein/hr.

2.6. The in vivo experiments

A total of 38 adult inbred SD rats were used. Six animals served as normal non-diabetic controls. Diabetes was chemically induced in the remainder (32 animals) using a single intraperitoneal injection of streptozotocin (STZ, Sigma, USA) in a dose of 45 mg/kg body weight [7]. Animals with 2 consecutive blood glucose readings of more than 300 mg/dl were considered diabetic. Out of these, 6 animals were untreated to serve as diabetic control. For transplantation, 5×10^6 differentiated cells were used for each animal. Cells were loaded into an encapsulation device (TheraCyte™ capsule, Irvine, CA, USA) and transplanted under the dorsal skin of 12 rats (group A). In group B (14 animals), cells were engrafted into the testicular interstitium using a wide-bore cannula. All these procedures were carried out under general anesthesia.

2.7. Follow-up

The animals were followed up for a period of 6 months. Fasting blood sugar (FBS), rat insulin and c-peptide were monthly determined. A glucose tolerance curve was carried out at 24 weeks. At the end of the observation period, the cell-bearing devices were explanted and the cell-bearing testes were surgically removed. One week later, the fasting blood sugar were determined. Then the animals were euthanized and their pancreata were removed. Samples were sent for histopathologic examination.

2.8. Gene expression by real-time PCR

Total RNA was extracted from undifferentiated cells, *in vitro* differentiated cells and *In vivo* transplanted cells inside TheraCyte capsules or testes using RNeasy plus Mini Kit protocol (Qiagen, Germany). After measuring the concentration of yielded RNA by Nanodrop spectrophotometer (Thermofisher, USA) cDNA was generated from 3 μg of total RNA using RT^2 First Strand Kit (Qiagen, Germany). Gene expression of endocrine genes (insulin, GCG, and SST) and transcription factors (PDX-1, Ngn3, Pax-4, and NeuroD1) was evaluated in the cells using real time PCR relative to that of undifferentiated cells Table 1. In brief, amplifications were performed in a total of 20 μL reaction volume that contains

Table 1. List of Rat gene-specific primers for RT-qPCR. DX1 (Pancreatic and Duodenal Homeobox 1); SST (Somatostatin); Ngn3 (Neurogenin 3); Pax4 (Paired Box 4); NeuroD1 (neuronal differentiation 1), GAPDH (glyceraldehyde-3-phosphate dehydrogenase).

Gene	Forward primer	Reverse primer	Accession number
Insulin	CACCTTTGTGGTCCTCACCT	GACGGGACTTGGGTGTGTAG	NM_019129.3
Glucagon	CATTCACAGGGCACATTCAC	CGGTTCCTCTTGGTGTTCAT	NM_012707.2
PDX1	TGCCACCATGAATAGTGAGG	CAGGGGGATTAGCACTGAAC	NM_022852.3
SST	GCTACTGGAGTCGTCTCTGC	CTCAGACAGCCGAGCTTGAG	NM_012659.2
Ngn3	CAGCCTCATTGGAGGAGTTC	GGCCCTTCACAAGAAGTCTG	NM_021700.1
Pax4	ATGGCGCAGACAAGAGAAGT	GGACTGCTGTGCAGAGATGA	XM_006236233.2
Neurod1	AGCCCCCTAACTGATTGCAC	TCGGTGGATGGTTCGTGTTT	NM_019218.2
GAPDH	TGCCACTCAGAAGACTGTGG	TGGTACATGACAAGGTGCGG	NM_017008.4

10 μL 2X SYBR Green master mix (Qiagen, Germany), 100 ng of cDNA template, 10 pmol of each primer and to a final volume by nuclease-free water. The real time PCR was done using CFX96 thermal cycler (Bio-Rad, USA) which was programmed as follows: initial denaturation at 95 °C for 3 min followed by 40 cycles of denaturation at 95 °C for 20 s and annealing & extension at 60 °C for 1 min. Undifferentiated S.D rat BM-MSCs were included as a negative control as well as GAPDH gene was evaluated as an internal control and for normalization of calculations. A mathematical model introduced by Pfaffl [8] was used for the relative quantification of target genes.

2.9. Immunofluorescent and histological studies

The harvested capsules and testes were processed according to the manufacturer's instructions (10% formalin, alcohol gradients, xylene gradients, and finally paraffin embedding). Sections were taken at a thickness of 3 μm and mounted on positive-charged coated slides (Citoglas, Citotest Labware Manufacturing Co., Haimen, China). The slides were then deparaffinized using xylene and a decreasing ethanol gradient. Antigens were unmasked by boiling of the slides in 10 mM sodium citrate buffer (pH 6.0) for 30 min and a sub boiling temperature was maintained for 10 min. The sections were blocked with 5% normal goat serum and then incubated overnight with the primary antibody at 4 °C. Thereafter, the slides were washed 3 times in PBS and incubated with the secondary antibody for 2 h at RT. Nuclei were using 4′,6-diamidino-2-phenylindole. Image J software 1.51h (developed by NIH) was used to determine the proportion of insulin-producing cells within the graft. To this end, 10 fields were randomly selected for cell counting which was carried out by 2 independent histopathologists. In all the above studies, confocal digital images were captured using a Leica TCS SP8 microscope (Leica Microsystems, Mannheim, Germany). For immunolabeling of the native pancreas, the primary antibody was mouse monoclonal anti-insulin (Novus Biologicals) and the secondary antibody was the Power-Stain Version 1.0 Poly HRP DAB Kit for mouse (Genemed Biotechnologies). For determination of the nature of material inside TheraCyte capsules,

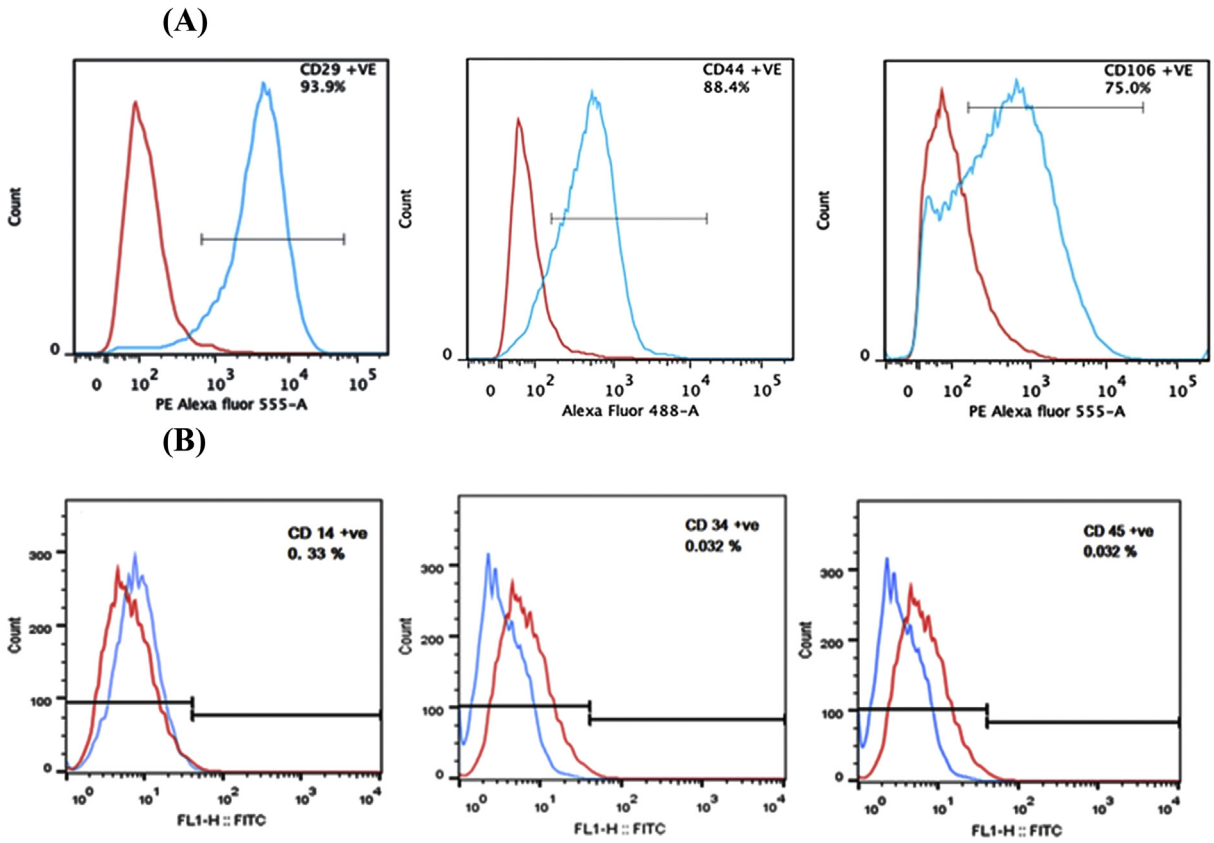

Figure 1. Flow cytomtery of BM-MSCs revealed that the cells (blue) expressed high levels of CD29, CD44 and CD106 (panel A), but negligible levels of CD14, CD34, and CD45 (Panel B) in comparison with their isotype control (red).

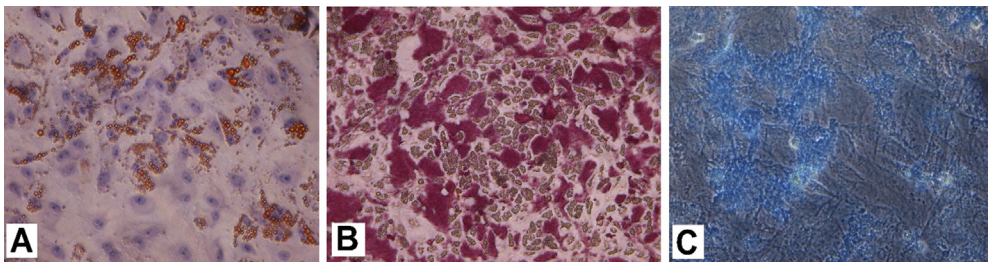

Figure 2. Multilineage differentiation of rat BM-MSCs. (A) Adipogenesis was detected using Oil-Red-O staining. (B) Osteogenesis was detected using alizarin-red staining. (C) Chondrogenesis was detected using Alcian blue.

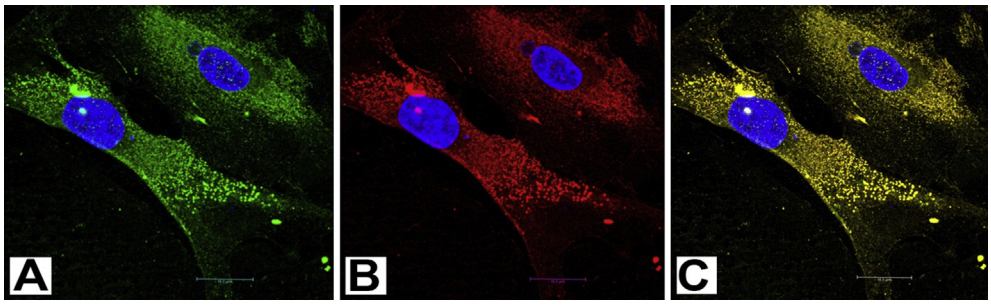

Figure 3. Immunocytochemistry of rat BM-MSCs at the end of in vitro differentiation. (A) Cells with insulin-positive granules (green). (B) Cells with c-peptide-positive granules (red). (C) Coexpression of insulin and c-peptide within the same cells (yellow). Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (blue).

engrafted capsules with pericapsular tissues were immunostained for Masson trichrome and phosphotungstic acid hematoxylin.

2.10. Statistical analysis

Results are expressed as means ± standard error of the mean (SEM). For multiple comparisons, the one-way analysis of variance (ANOVA) was used followed by LSD post hoc analysis using Statistical Package for the Social Sciences, v.16.0 (SPSS, Inc., IL, USA). A P-value of $P \leq 0.05$ was considered to be significant.

3. Results

3.1. Characteristic features of rat BM-MSCs

The isolated cells adhered to the plastic of the culture flasks and exhibited a spindle-shaped morphology. Phenotypically, the cells were strongly positive for the rat MSC surface markers CD29, CD44 and CD106 and were negative for the haematopoietic stem cell surface markers CD14, CD34 and CD45 Figure 1. In addition, the cells could be differentiated to form adipocytes, chondrocytes, and osteocytes when the appropriate growth factors were used Figure 2.

3.2. Functional evaluation of differentiated rat BM-MSCs

Differentiated cells were positive for insulin and c-peptide. Insulin and c-peptide were co-expressed within the same cells Figure 3. In addition, there was a stepwise increase in insulin and c-peptide release as a function of increasing glucose concentrations. Figure 4.

3.3. Results of the in vivo experiments

3.3.1. General outcomes

In group A (Capsule), 8 out of 12 animals were cured (66.6%). In group B (Testis), 8 animals out of 14 were cured (57%). Uncured animals from both groups died within 2 months after transplantation.

3.3.2. Fasting blood sugar *Figure 5*

FBS levels of normal (non-diabetic) rats ranged from 89 to 96 mg/dl. After chemical induction of diabetes, the FBS increased in all animals to

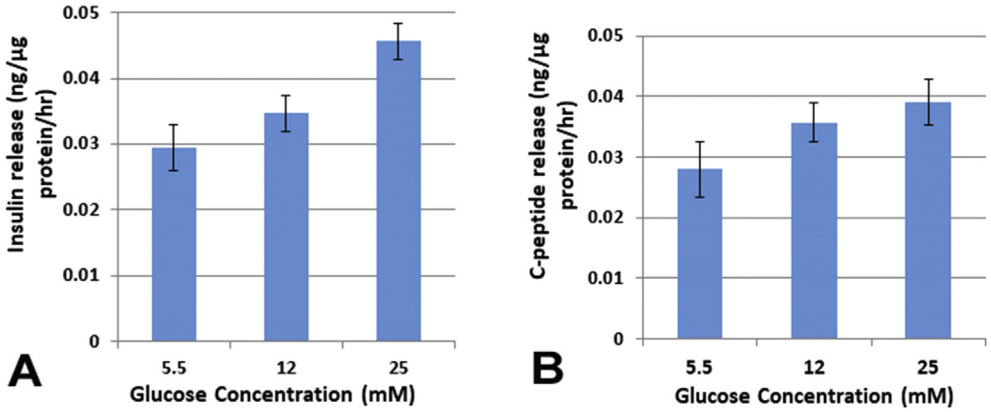

Figure 4. In vitro rat insulin and c-peptide release in response to glucose challenge. There was a stepwise increase in the release of insulin (A) and c-peptide (B) in response to increasing glucose concentrations ($P < 0.05$). These findings indicate that differentiated rat BM-MSCs are glucose sensitive and insulin responsive.

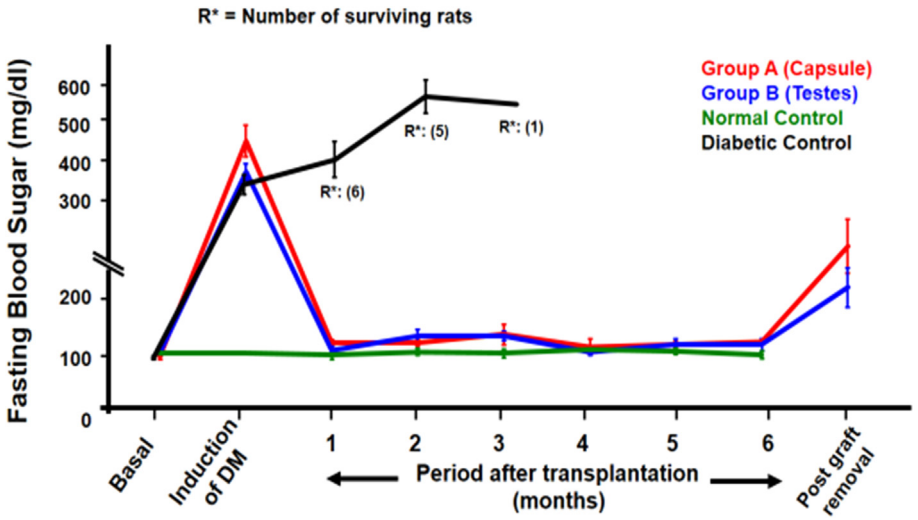

Figure 5. The blood glucose levels of cured animals were normalized in both experimental groups within one month after implantation and maintained throughout the observation period. The diabetic status resumed following removal of the engrafted MSCs. Mean ± SEM.

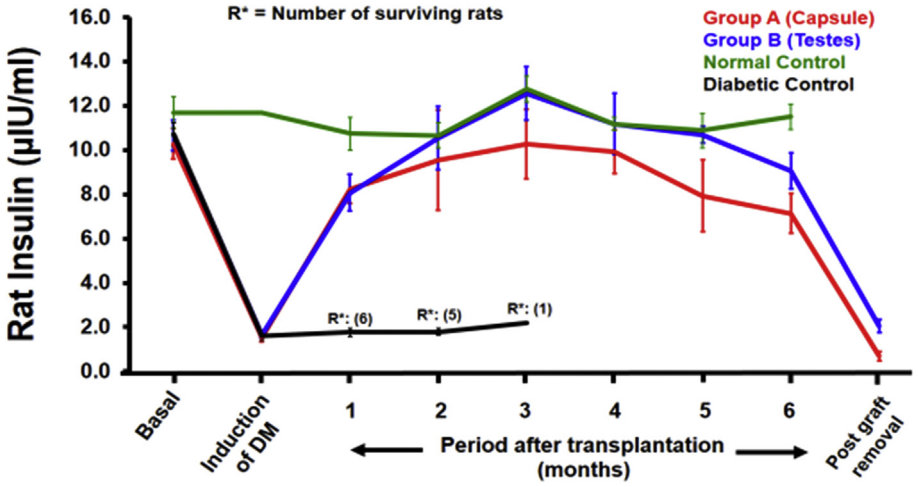

Figure 6. Rat insulin levels. Following the induction of diabetes, rat insulin was negligible. Four weeks after transplantation, rat insulin became measurable in the cured animals. Insulin levels were sustained among the cured animals for 6 months. Mean ± SEM.

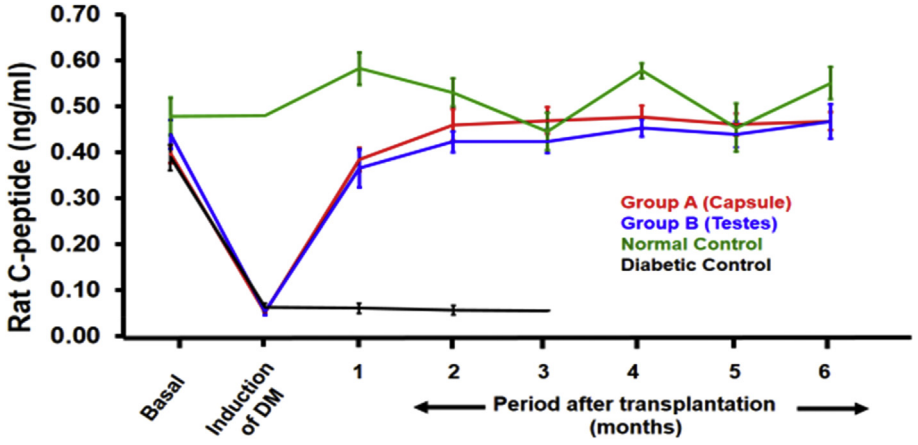

Figure 7. Following the induction of diabetes, rat c-peptide levels were negligible. While after transplantation, rat c-peptide levels became measurable in all cured animals. Mean ± SEM.

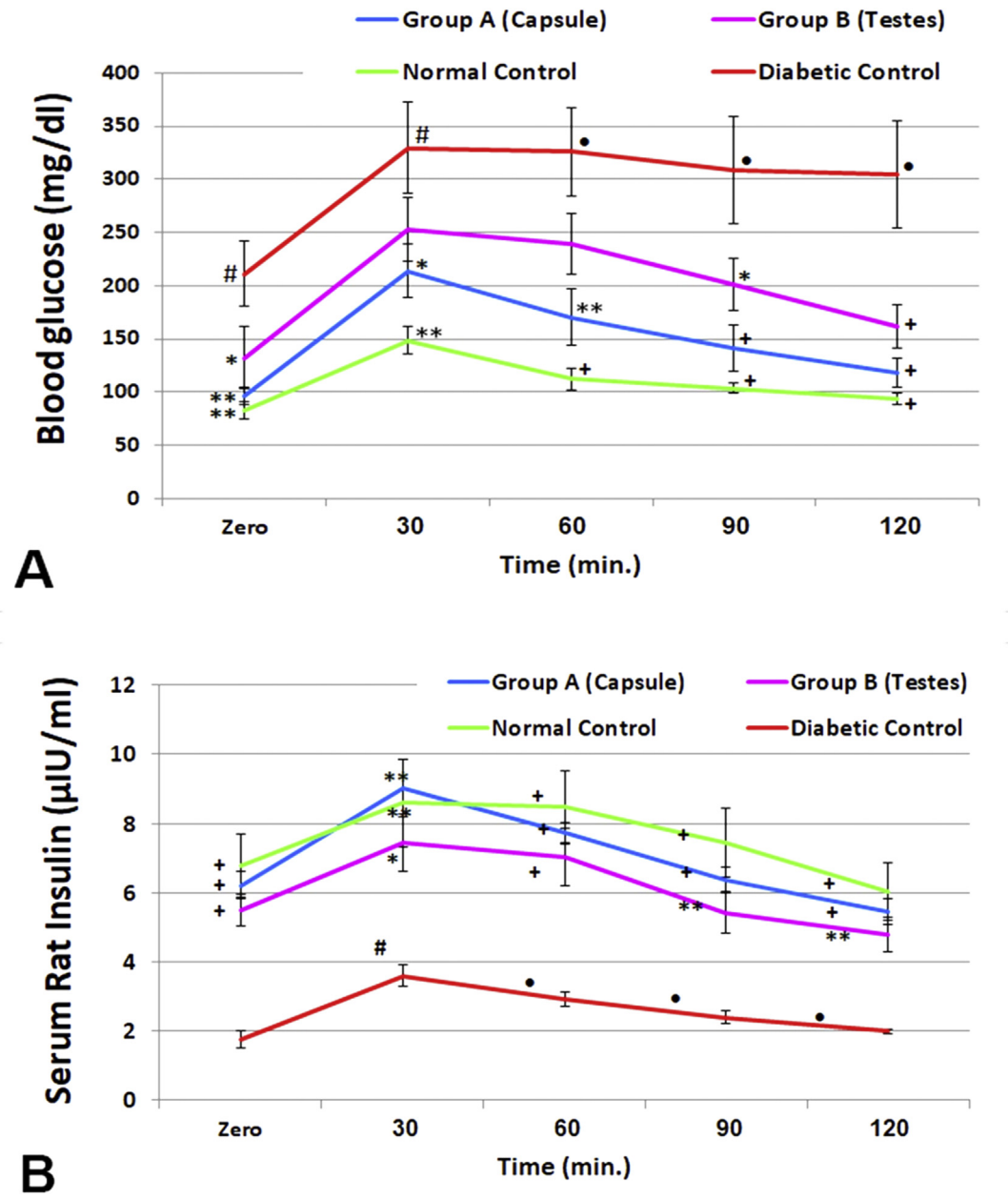

Figure 8. The oral glucose tolerance tests of diabetic SD rats transplanted with differentiated rat BM-MSCs in testis or loaded in TheraCyte capsule; in compare with diabetic control and normal control groups. Blood glucose levels (A), serum rat insulin (B). Data were expressed as Mean ± SEM. *P < 0.05 significant difference compared with diabetic control. **P < 0.01 highly significant difference compared with diabetic control. + P < 0.001 very highly significant difference compared with diabetic control. #P < 0.01 significant difference compared with normal control. • P < 0.001 highly significant difference compared with normal control.

reach values ranging between 306 and 528 mg/dl. Among the cured animals, FBS was normalized within one month after transplantation. FBS values of the cured animals ranged from 101 to 119 mg/dl in group A and from 95 to 117 mg/dl in group B. These rats remained euglycemic throughout the observation period. Following removal of the capsules or the grafted testes FBS levels increased promptly to values ranging from 136 to 500 mg/dl in group A and ranging from 135 to 325 mg/dl in group B.

3.3.3. Serum rat insulin and c-peptide

After transplantation, the serum insulin levels of cured rats increased to reach near normal levels in both groups Figure 6. These values remained measurable and stable throughout the follow-up period. Rat serum c-peptide showed a similar pattern Figure 7.

3.3.4. Glucose tolerance curve Figure 8

After the oral administration of glucose, blood sugar levels increased sharply to reach a maximum after 30 min. Thereafter, there was a gradual decline to reach normal levels after120 min in case of cured animals; there was a parallel change in serum rat insulin levels.

3.4. Relative gene expression by real-time PCR Figure 9

At the end of the *in vitro* differentiation period, relevant pancreatic endocrine genes were expressed in the differentiated rat BM-MSCs as well as transcription factors. Relative expression of these genes was more up-regulated after transplantation of the differentiated cells. Insulin gene expression was expressed by 28-fold in the *in vitro* differentiated cells, 141-fold in group A and 191-fold in group B relative to that of

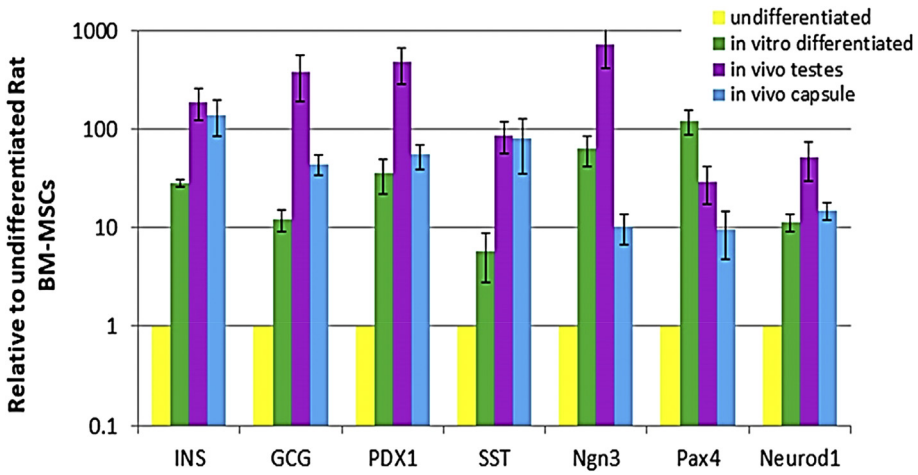

Figure 9. Relative gene expression by real-time PCR. At the end of differentiation and 6 months after graft removal, the relevant pancreatic endocrine genes were expressed by all samples. The expression of all genes had been increased after transplantation except for Pax4. Insulin gene was increased by 5–6 folds (more than the *in vitro* differentiated cells) after transplantation in capsule and testes groups respectively.

undifferentiated cells. Another endocrine gene; glucagon gene expression was expressed by 12-fold in the *in vitro* differentiated cells, 44-fold in group A and 378-fold in group B. Somatostatin gene expression was found to be 6-fold in the *in vitro* differentiated cells and was up-regulated to 81-fold in group A and 87-fold in group B. Again most of the transcriptional genes expression was also peaked after transplantation; PDX-1 gene was expressed by 35-fold in the *in vitro* differentiated cells, 55-fold in group A and 479-fold in group B; Neurogenin 3 (Ngn3) gene was 63-fold in the differentiated cells, 10-fold in group A and 721-fold in group B; PAX4 gene was 122-fold in the differentiated cells, 10-fold in group A and 29-fold in group B. Finally, NEUROD1 gene was expressed 11-fold in the differentiated cells, 15-fold in group A and 52-fold in group B when compared to the undifferentiated BM-MSCs.

3.5. Immunofluorescence and histological studies

At the end of the observation period, Immunofluorescence staining of the BM-MSCs-bearing testes or capsules revealed that the engrafted cells were positive for insulin and c-peptide. The coexpression of insulin and c-peptide within the cytoplasm of these cells was confirmed Figure 10 A-F.

3.6. Additional histological study of the retrieved capsules

Massons's trichrome stain revealed heavy pericapsular deposition of fibrous tissue Figure 11. Immunolabeling of the native pancreata of the transplanted rats revealed that islets were few in number and atrophic with near absent insulin expression Figure 12.

4. Discussion

Clinically, allogeneic pancreatic islets transplantation has been considered as a potential treatment for type 1 diabetic patients. These islets are usually infused intrahepatically [9]. Most of the transplanted islets are lost by instant blood-mediated inflammatory reaction [10]. Other sites for clinical islets transplantation were tried, including omental pouch [11], pre-peritoneal space [12], intramuscular [13, 14], bone marrow cavity [15, 16], and kidney sub-capsule [17]. However, these sites involve invasive procedures and their efficiency was not verified or reproduced [18].

We have provided evidence that our utilized cells are mesenchymal in origin by phenotyping and by their ability for trilineage differentiation. Differentiation was carried out according to a protocol designed by

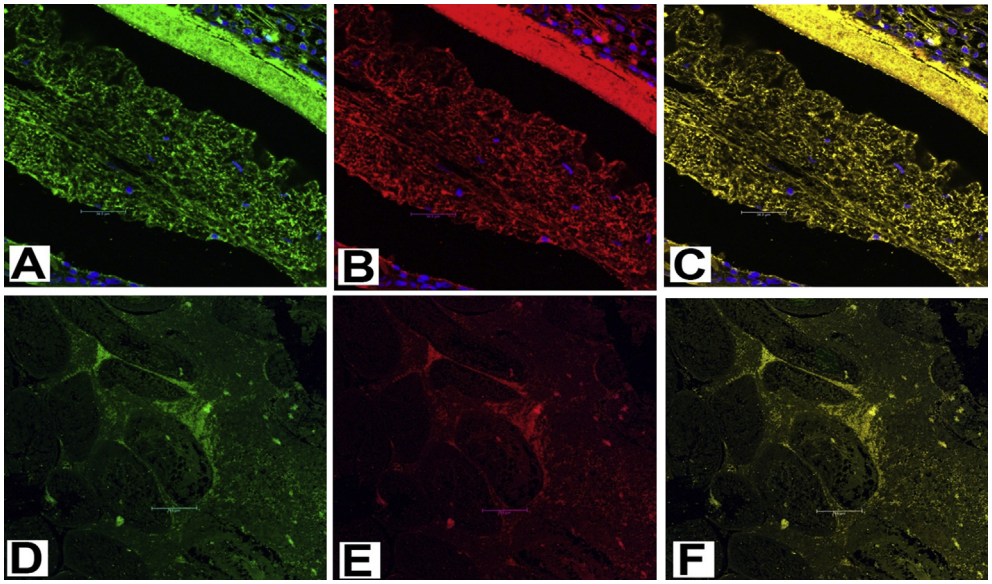

Figure 10. Histology harvested capsules (A–C) and testes bearing BM-MSCs 6 months after transplantation. (A) Immunofluorescence study was positive for intracytoplasmic insulin (green), (B) for C-peptide (red), and (C) electronic merging (yellow) confirmed the coexpression of insulin and c-peptide in same cells. (D) Staining of testes showed aggregates and sheets of polygonal cells in between the seminiferous tubules that were Positive immunofluorescence staining for insulin (green), (E) for C-peptide (red), and (F) electronic merging (yellow).

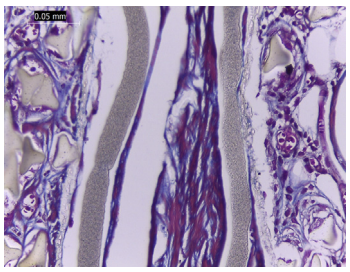

Figure 11. TheraCyte capsule retrieved 6 months post-transplantation. Masson's trichrome revealed heavy pericapsular deposition of fibrous tissue.

Tayaramma et al. [6]. We have chosen such a protocol in view of its simplicity and short period.

For clinical transplantation of islets or differentiated stem cells from different sources, a minimally invasive procedure is necessary. Transplantation into the subcutaneous space offers such a possibility. In addition, the transplanted cells could be monitored or retrieved if necessary. The transplanted cells may need an encapsulation within an immunoisolating device to prevent a possible allogeneic rejection. In our experiment, we have utilized a commercially available encapsulation device: "TheraCyte capsule". This device is designed with an outer rough membrane to induce neovascularization and an inner semipermeable membrane to protect against alloimmunity while allowing free exchange of glucose and insulin [19]. The efficiency of such a device to control chemically-induced diabetes in rats was tested. Results of encapsulation were compared against a background of a well-studied site for experimental islet transplantation (the testes) [4, 20].

During the observation period, the results among the two groups were essentially similar without statistical difference in terms of their ability to control chemically-induced diabetes in rats. In addition, the results of glucose tolerance curves where blood glucose and insulin were measured at the same time points provided evidence that our cells are glucose-sensitive insulin producing. At the end of the observation period, the retrieved capsules and all the testes were subjected to immunofluorescence studies as well as determination of relative gene expression. Immunofluorescent studies confirmed the presence of insulin positive cells and c-peptide positive cells, both cell types were expressed within the same cells. An additional study, whereby the retrieved capsules and the pericapsular tissue were stained with the Masson's trichrome and revealed heavy deposition of fibrous tissue. This may represent an important negative aspect of the encapsulation at least using the current material [21, 22].

All the relevant pancreatic endocrine genes were expressed. Improvement in fasting blood glucose in both groups was the result of insulin release from the transplanted cells since the histology of the native pancreata revealed very few islets with low insulin expression.

In this study, some success was achieved with encapsulation. However, there are several problems that have to be solved. In order to have success in the long-term. It is logical to transplant the encapsulation device in the subcutaneous tissue. However, the subcutaneous tissue is poorly vascularized and hence a source for immediate oxygenation is needed, early vascularization must be induced and late fibrosis must be prevented [23, 24]. The used material should be not only biodegradable and biocompatible but unable to induce foreign body reaction as well. This observation, clearly points out the drawbacks of the current available macroencapsulation devices. Kevin D'Amor presented the results of the first in-human clinical evaluation of embryonic stem cell-derived islet replacement for type 1 diabetes (Clinical Trials. gov.: NCT02239354). It seems that they have also encountered problems with encapsulation. It was reported that the trial was paused to improve the Encaptra device (ViaCyte, Inc. San Diego, CA, USA) [25]. In a second attempt, they will encapsulate their cells into an open device, to allow ingrowth of new blood vessels, and administer anti-inflammatory agents as well as a standard immunosuppressive regimen. Since our insulin-producing cells are derived from MSCs, an encapsulation device may not be necessary. It may be also necessary to encapsulate allogeneic-derived cells in view of their immunomodulatory capacity, this has to be verified. To this end, an open system may be used such as scaffolds. Pepper et al. [26], suggested that scaffolds may be transplanted in two stages. In the first stage plastic tubes are inserted subcutaneously to induce vascularization, in the second stage the cell-bearing scaffold is transplanted in an already vascularized bed. Experimental trials for all these alternatives are currently under investigation in our lab.

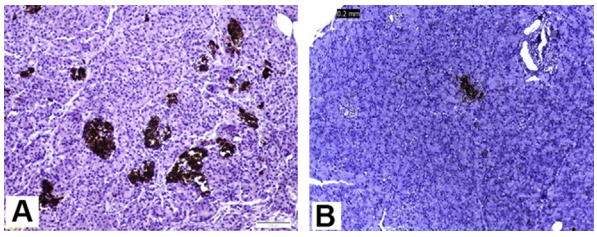

Figure 12. Immunostaining of rat pancreas (×200). (A) The islets from pancreas harvested from normal untreated rat stained positive for insulin. (B) The islets from a pancreas of cured rats retrieved 6 months post-transplantion were very few in number with low insulin expression.

5. Conclusion

Although our study demonstrated that there was no significant difference between the results of both experimental groups, these findings indicate the potential use of the encapsulation technology to promote survival and further maturation of differentiated stem cells for future treatment of diabetic patients. Subcutaneous transplantation of such immunoisolating device provides a large transplant volume capacity, minimally invasive procedures and ability to retrieve the graft if necessary.

Efforts must be taken on behalf of material scientists to come up with a material which does not provoke pericapsular fibrosis before a clinical trial is contemplated.

Declarations

Author contribution statement

M. Gabr, M. El-Far and A.F. Refaie: Analyzed and interpreted the data; Wrote the paper.

S.M. Elhalawani, M.M. Zakaria: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

M.M. Zakaria and S.M. Khater: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

M.A. Ghoneim: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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