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Research

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Deducing insulin producing cells in goat adipose tissue derived mesenchymal stem cells

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Abstract

Background: Mesenchymal stem cells (MSCs) is a new herald for regenerative medicine for control of incurable diseases in human and animals. Diabetes occurs when the blood glucose is high due to lack of insulin hormone secreted by the pancreatic cells. The global diabetes in 2019 is estimated 463 million people and rising to 578 million by 2030.

Methods: Here we differentiated goat adipose tissue derived MSCs into insulin producing cells. To achieve this, the goat MSCs were cultured in serum-free DMEM/F12 medium containing glucose, nicotinamide, activin-A, exendin-4, pentagastrin, retinoic acid and mercaptoethanol for three weeks. The *in vitro* differentiation ADSCs into insulin-producing cells was confirmed by detecting the pancreatic endoderm specific markers i.e. Igf-1, Sst, Ngn3, Pdx-1, Isl-1, c-Kit, Thy-1, and Glut-2 in differentiating cells.

Results: There was a significant increase in insulin specific gene expression with respect to duration of differentiation. Pancreatic insulin-producing cells were further characterized by immunolocalization of Pdx-1, insulin, and Islets-1 specific protein. The release of insulin in response to a glucose challenge was also evaluated.

Conclusions: The study provides new opportunities for deciphering the basic mechanism of *in vitro* genesis of pancreatic cells and basic properties, availability, and abundance of ADSCs render them well-suited for applications in regenerative medicine.

Keywords: Adipose tissue, Goat, Glucose challenge, Insulin producing cells, Mesenchymal stem cells.

Background

The therapeutic potential of stem cells is gaining much more attention in the field of regenerative medicine from the last decades in humans as well as in farm animals. Both embryonic stem cell and mesenchymal stem cells (MSCs) are being widely used in the treatment of defects and various diseases. Since accessibility, an abundance of cells, easy handling, and availability are much more efficient for MSCs in comparison to embryonic stem cells; make these cells a more attractive therapeutic tool for regenerative medicine. MSCs are non-hematopoietic cells, mesodermal origin, and have vast multiplication potency, quick cell doubling time, and cells could be expanded in culture for more than 60 doublings[1]. These cells do not elicit immuneresponse[2] and do not show any tumour formation after transplantation. The use of adult stem cells can avoid the ethical dilemma surrounding embryonic stem cells in humans and animals[3]. Disease in animal-like rabbit articular defects[4], tendon regeneration in horses[5], tendon repair in dogs wound healing in caprine[6], osteosarcoma in the dog[7], bone regeneration in goat[8] and other diseases have been treated with adult stem cells. Several studies have reported that MSCs are readily the available source of insulin-producing cells[9],[10],[11].

As the importance and use of adult stem cells is increasing in the field of therapeutic potentials and tissue engineering they have been isolated from different tissues like bone marrow[12], adipose tissue[13], umbilical cord blood[14], dental pulp[15], lung, liver, spleen[16], testis[17], pancreas[18] and even brain[19]. Fetal tissue is a rich source of MSCs and it has been obtained from different cell types like placenta, amniotic fluid, umbilical cord, and cord blood[20]. Adipose tissue-derived stem cells are gaining tremendous attention as a prime source of cell therapy by virtue of their easy availability, enormous expandability, ease of isolation (that is to say with minimum patient discomfort)[21]. Isolation, culture, and characterization of adipose tissue-derived mesenchymal stem cells have been reported in small ruminants[22]. A large number of instances regarding the use of ADMSCs for the treatment of various diseases in humans and rodents were previously reported[23], [24], [25], [26]. But very few reports about transplantation of ADMSCs for treatment of various livestock diseases were available[27]. When it comes to deducing insulin-producing cells from ADMSCs a very few studies are available in species like humans and rodents[28], [29]. However, derivation of insulin-producing cells from ADMSCs is not reported so far in any livestock species. Among the domestic animals, goat plays a significant role in the socio-economic development of our country and is small in size with a short gestation period, the goat is convenient for current

biological investigation. With this background we herewith envisaged deducing insulin-producing cells from goat ADMSCs *in vitro* for the first time to the best of our knowledge.

Methods

Reagent

All chemicals such as pentagastrin, activin, retinoic acid, exendin, trypsin, collagenase type 1,bovine serum albumin (BSA), antibiotics (gentamycin, penicillin and streptomycin), glucose, Dexamethasone, B-glycerol phosphate, ascorbic acid,BMP-4, ITX, and Dulbecco's modified Eagle's medium (DMEM) used for the culture of ADSCs and for differentiation were from Sigma Chemical Company, St. Louis, MO. FBS was from Hyclone (Logan, Utah, USA). For the characterization of ADSCs by examining the surface marker CD44, CD90 and for characterization of pancreatic islets-like cells primary antibody Islets-1, Insulin, Pdx-1 is purchased from Santa Cruz Biotechnology USA. Specific secondary antibodies, which included goat anti mouse IgM-FITC conjugate and goat anti-mouse IgG-FITC conjugate, were purchased from Santa Cruz, USA. Some other secondary antibodies like goat anti-rabbit IgG-FITC conjugate was purchased from Santa Cruz, Inc. USA.

Isolation and Culture of adipose Tissue derived mesenchymal stem cells

Adipose tissue was collected from the abdominal region of a slaughtered goat in a nearby slaughterhouse and transferred in a transport medium containing chilled PBS with antibiotic solution. In the laboratory, adipose tissue was transferred in an HBSS medium containing gentamycin 1% (V/V) and minced into very small pieces (1-2 mm), the minced tissues incubated in a digestion medium containing DMEM F/12, 1% type1 collagenase enzyme (CAT No. C5894), 1% trypsin, and 1% gentamycin (V/V) for 4 h under standard culture condition (37.5 ˚C, 5% CO2, 90% air) with regular shaking at an interval of 20 min. Dissociated tissues were then filtered in 41 µm filter paper (Millipore Company, Billerica, USA, Cat no. NY4102500) to remove undigested fat tissue. In the filtered cell, suspension add the same volume of complete medium (DMEM F/12 containing 10% FBS) and incubated for 5 min under standard culture condition. The cell suspension was centrifuged at 1000 rpm for10 min and the pellets were seeded in 25 cm^2 culture flask at a density of 2 $x10^5$ cells/cm^2 in growing medium under standard culture condition. The cells were trypsinized at 80% confluence, centrifuge at 1000 rpm for 10 min, and re-seeded at density 2 x 10^5 cells/cm2 in a 25 cm^2 culture flask.

RT-PCR and immunostaining of gADSC markers

Total RNA was isolated from differentiated and control gADSCs cell using RNAqueous® kit (Ambion, USA). Subsequently, RT-PCR of 3 µg RNA sample was performed using 'Revertaid first-strand cDNA synthesis kit' (Thermo scientific, USA). Further cDNA was amplified for 36 cycles (94˚C for 30 sec, annealing at 58˚C for 30sec, extension at 72˚C for 30 sec) using specific primers. Amplified PCR products were confirmed on 2% Agarose gel electrophoresis containing 0.5 µg ethidium bromide. The gel was examined on a UV transilluminator and take photos by the gel documentation system. The specific surface marker that is CD44 and CD90 was examined by Immunofluorescence staining at 3rd passage gADSCs of differentiated and control cell was fixed in 4% paraformaldehyde permeabilized with 1% triton x and incubated with blocking buffer (4% bovine serum albumin, Sigma, USA) for 30 min at room temperature. Cells were incubated with primary antibody (1:100) to CD44 and CD90 (Santa Cruz Biotechnology, USA) over night at 4˚C. Three times washing with DPBS and cells were incubated with FITC-conjugated secondary antibody (goat-anti mouse IgG or IgM, 1:500 dilution) for 2h at room temperature. Fluorescence was examined and photographed under a fluorescence microscope (Diaphot, Nikon, Tokyo, Japan).

Flow cytometric detection of surface antigens

At the 3rd passage, gADSCs were seeded at a density of 1×10^5 cells in a 25 cm^2 flask and grown in DMEM/10% FBS medium. Cells were trypsinized, subsequently washed 2 times with chilled PBS, and separate the cells having 10 x 10^5 cells in each flow tubes with 1 ml PBS. Add 1µl of CD90, CD73, CD105, and CD34 (Company details) antibody in a separate tube, incubate for 1 h on ice. Unstained gADSCs were used as control. The gADSCs were analyzed by Flow Cytometer (BD FACS Caliber) and then analyzed by FACS Caliber Becton Dickinson flow cytometer and Cell Quest Pro computer software.

Adipogenic differentiation of gADSCs and confirmation

For adipogenic differentiation cells at passage 3rd were seeded in a culture dish and incubated with DMEM F/12 containing 3% FBS, 1 µM Dexamethasone, ITX (1X), and 1%V/V gentamycin for 28 days, parallel control was incubated with only complete medium(DMEM containing 10% FBS). After 28 days differentiated cells were fixed with5% formalin at 37˚C, washed with PBS and 70% ethanol and stained by Oil Red O (Sigma Aldrich, USA).

Chondrogenic differentiation of gADSCs and confirmation

The cells at the passage 3rd were incubated in DMEM F/12 containing 100 nM dexamethasone, 1.25 µg/ml BSA, 10 ng/ml BMP-4, ITX (1x) and 1% (v/v) gentamycin for three weeks and change medium an interval of five days. After 21 days chondrogenic differentiation was confirmed by Safranin Red staining.

Osteogenic differentiation of gADSCs and confirmation

To induce osteogenic differentiation, cells at passage 3rd were taken in the multiwell dish and cultured in DMEM F/12 medium containing 20 mM β glycerol phosphate, 10 mM dexamethasone, and 50 µM ascorbic acid (Sigma Aldrich) for 20days, change medium after three days interval. After 20 days cells were fixed in acetone for 30 min at 37˚C. Cells were stained with alizarin red S staining (Sigma Aldrich) for 10 min and remove nonspecific stain by washing with PBS.

Differentiation of gADSCs into pancreatic islets like cells

In vitro differentiation of gADSCs into pancreatic cells were done with cells at passage4th or 5th. Cells were seeded at density 4 x 10^5 cells/well of 6 well culture plates. Differentiation medium is having serum-free DMEM/F12 with 17.5 mM glucose in the presence of nicotinamide 10 mM, activin-A 2 nM, exendin-4 2 nM, pentagastrin 10 nM, retinoic acid 10 µM and β-mercaptoethanol20 µM. The differentiation medium changed every three days interval up to 28days. The morphological changes of cells were seen under the phase-contrast microscope (Diaphot, Nikon, Tokyo, Japan).

RT PCR and immunocytochemistry

After 28 days of differentiation, cells were harvested for RNA isolation. RNA isolation was done using an RNAqueous kit (Ambion, USA). RNA samples were quantified spectrophotometrically by using a Nano drop instrument at 260/280. Sample having 1.8-2 value are used for cDNA synthesis, 2 µg of RNA was subjected to reverse transcription by using Revertaid^{tm} first-strand cDNA synthesis kit. The polymerase chain reaction was performed on a thermal cycler (Biorad, USA), specific genes were amplified at initial denaturation 94˚C for 5 min then again denatured at 94˚C for 30 sec, annealing at 55-60˚C for 30 sec, extension at72˚C for 30 sec for 34 cycles and a final extension at 72˚C for 5 min. The amplification

product is confirmed by gel electrophoresis using 1.5% Agarose gel containing 0.5 µg ethidium bromide in a tris-EDTA buffer along with 100bp ladder (MBI, Fermentas). The gel was examined under a UV transilluminator and photographed by a gel documentation system (BIO-RAD). For characterization of Islets like cells expression of Pdx-1, Islets-1, and insulin was examined by Immunofluorescence. After 28 days gADSCs differentiated cells were seeded in four well petri dish, cells were fixed by 4% formaldehyde after washing permeabilized by 1% Triton-X further cells were incubated with blocking solution (4% bovine serum albumin) for 30 min at room temperature. The cells were then incubated with primary antibody (1:100) to Pdx-1, Islets-1, and insulin (Santa Cruz, Biotechnology, USA) for overnight at 4˚C. After repeated washing cells were incubated with the FITC-labelled secondary antibody (1:500) goat anti-mouse IgG or IgM for 2 h at room temperature. Fluorescence was examined and photographed under a fluorescence microscope (Diaphot, Nikon, Tokyo, Japan).

Gene name	Sense primer	Antisense primer	Annealing temp.	Product length	Accession no.
Igf-1	GATGTACTGTGCGCCTCTCA	TTGTTTCCTGCACTCCCTCT	58	129	JX570672.1
sst	TTGTTTCCTGCACTCCCTCT	GAAATTCTTGCAGCCAGCTT	60	198	JX570673.1
Pdx-1	TGGAGCTGGAGAAGGAGTT	TGGAGCTGGAGAAGGAGTT	58	98	JF728303.1
Ngn3	GCAATAGGATGCATAACCTCAAC	CGTCAGCGCCCAGATATAAT	58	131	KC154005.1
Isl-1	CGGCAATCAAATTCACGATCAG	CGTACAGCTCTCGTCCAAATAC	56	112	NM_001099130.1
C-kit	TCCTGATTGACCTTCCCTTG	GCCACAAACGTCAAATCCTT	60	139	D45168.1
Thy-1	GCCACAAACGTCAAATCCTT	TCCCCAAGAAAAGGAAGGAT	60	118	NM_001034765.1
Glut-2	CAGAAGTCCTGCAGAGAAGAAA	TTCCAGTACGTTGCGGTAAG	60	108	HQ585494.1

Ethical approval and informed consent. Ethical approval was taken from Institute ethics committee, ICAR-National Dairy Research Institute (NDRI), Karnal, India during the study and all methods were performed in accordance with the relevant guidelines and regulations.

Results

Isolation of goat ADSCs

Isolated gADSCs shows the fibroblast-like phenotypic characteristic and they are plastic adherent (Fig. 1A). Primary cultured and 1st passage cells have a heterogeneous population of cells but as they are growing by passage they show the homogenous population of cells so this is the reason behind doing flow cytometric analysis with or after 3rd passage cells. Adhered cells become full extended after 24 hr of seeding and they reached 80% percent of confluency by 5thdays (Fig. 1A). The cells were regularly trypsinized by 4th days and seeded at a density of 1 × 10^5 cells. Directed differentiation of gADSCs into Adipogenic lineages at passage 3rd was performed and confirmed after 28 days of differentiated cells stained by Oil Red O (Fig. 1Ba). The gADSCs were directed differentiated into Chondrogenic lineage and confirmed after 21 days of chondrogenic differentiation by Safranin Red staining (Fig. 1Bb). Induction of osteogenic differentiation of gADSCs at passage 3rd was observed Osteogenic lineages and confirmation by stained with alizarin red S staining (Fig. 1Bc).

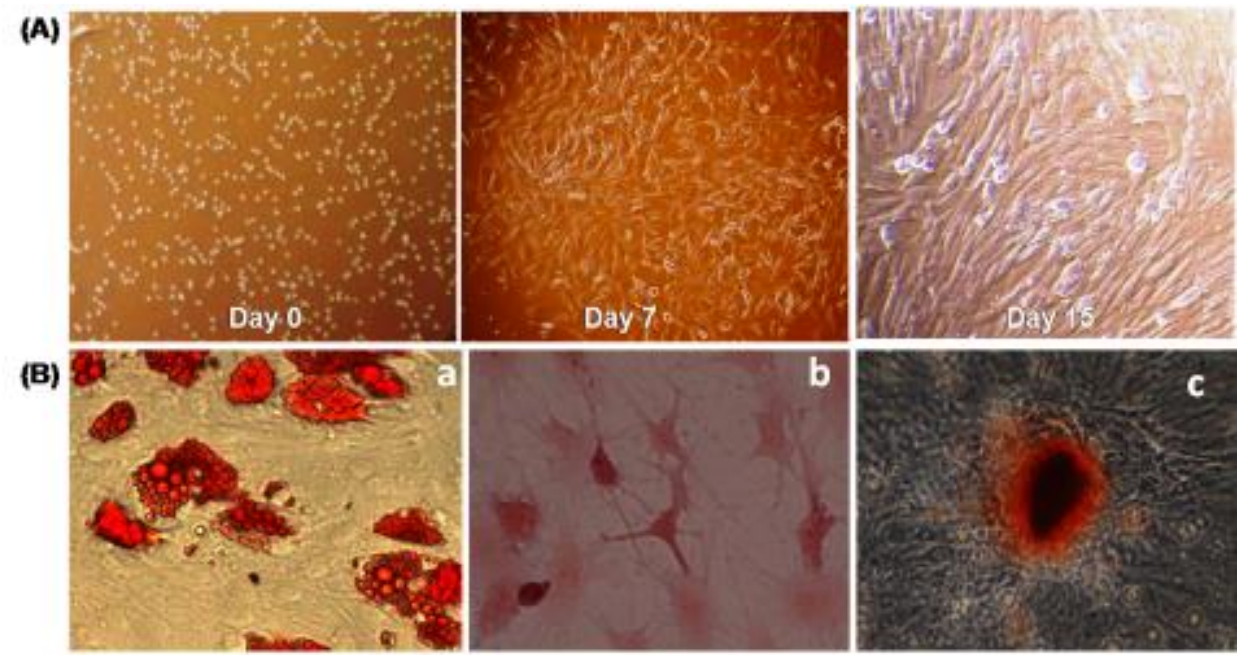

Figure 1. Isolation, culture, characterization and differentiation of gADMSCs. (A) gADMSCs of Day 0, Day 7, Day 10 and day 15. **(B)** Representative images showed the differentiation of gADMSCs into Adipocytes (a), Chondrocytes (b), Osteocytes cells (c). Magnification 40 X.

Characterization of gADSCs

Characterization ofgADSCs were performed with RT-PCR of CD73, CD90, CD105, CD44 taken as a positivemarker and CD34 as a negative marker, data normalized with GAPDH as an internalcontrol (Fig. 2B). The gADSCs were also characterized by immunohistochemistryof CD90, CD73, CD105, and CD44 as positive markers. Cells showed the cell surfaceexpression of proteins (Fig. 2 A). Flow cytometric analysis 3rd passagegADSCs cell showed the presence of mesenchymal stem cell marker CD90 (98%)highly expressed, CD105 (90%), CD73 (97%) used as positive marker and CD34 (0.4%)as a negative marker (Fig. 2C).

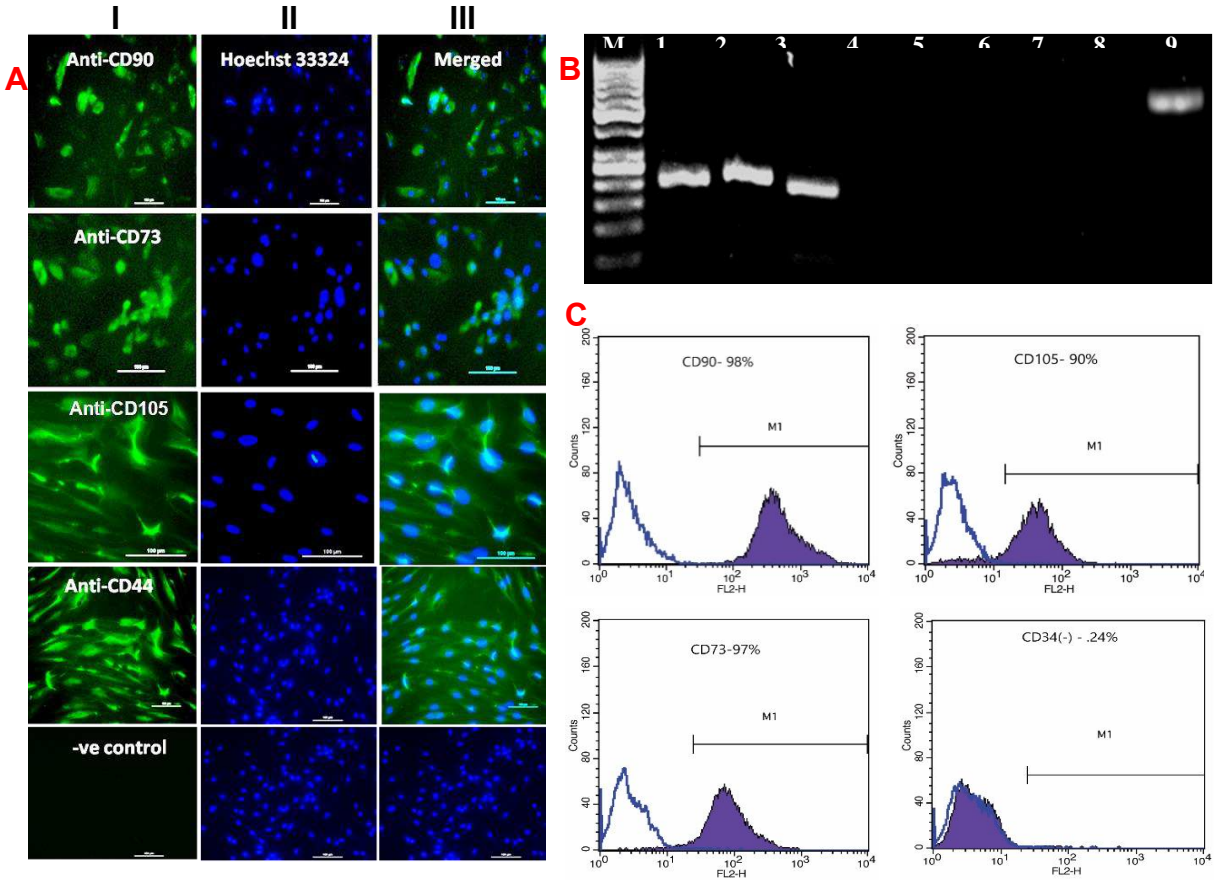

Figure 2. Characterization of gADMSCs. (A) Detection of mesenchymal specific markers in gADMSCs. (Column I): Immunolocalization of a mesenchymal specific protein in respective gADMSCs. (Column II): Nuclear chromatin staining of gADMSCs with Hoechst 33342 dye. (Column III): The merged image of column I and column II. Primary antibody was absent in negative control. Scale bar 100 µM. (B) RT-PCR analysis of mesenchymal specific markers in gADMSCs. Detection of positive markers in lane1 (CD73), lane 2 (CD90) and lane 3 (CD105) and no expression of negative markers lane 4 (CD34), lane 5 (CD45), and lane 6(CD79a) was observed. Fibroblast cells (lane 7) and RT (−) (lane 8) served as a negative control. GAPDH acts as an internal positive standard. (C) Detection of mesenchymal stem cell-specific protein by flow cytometer.

Differentiation of gADSCs into pancreatic islets-like cells

In this study, goat ADSCs were successfully differentiated into pancreatic islet like cells on day 7th, 14th, 21st, and 28th in *in vitro* condition (Fig. 3).

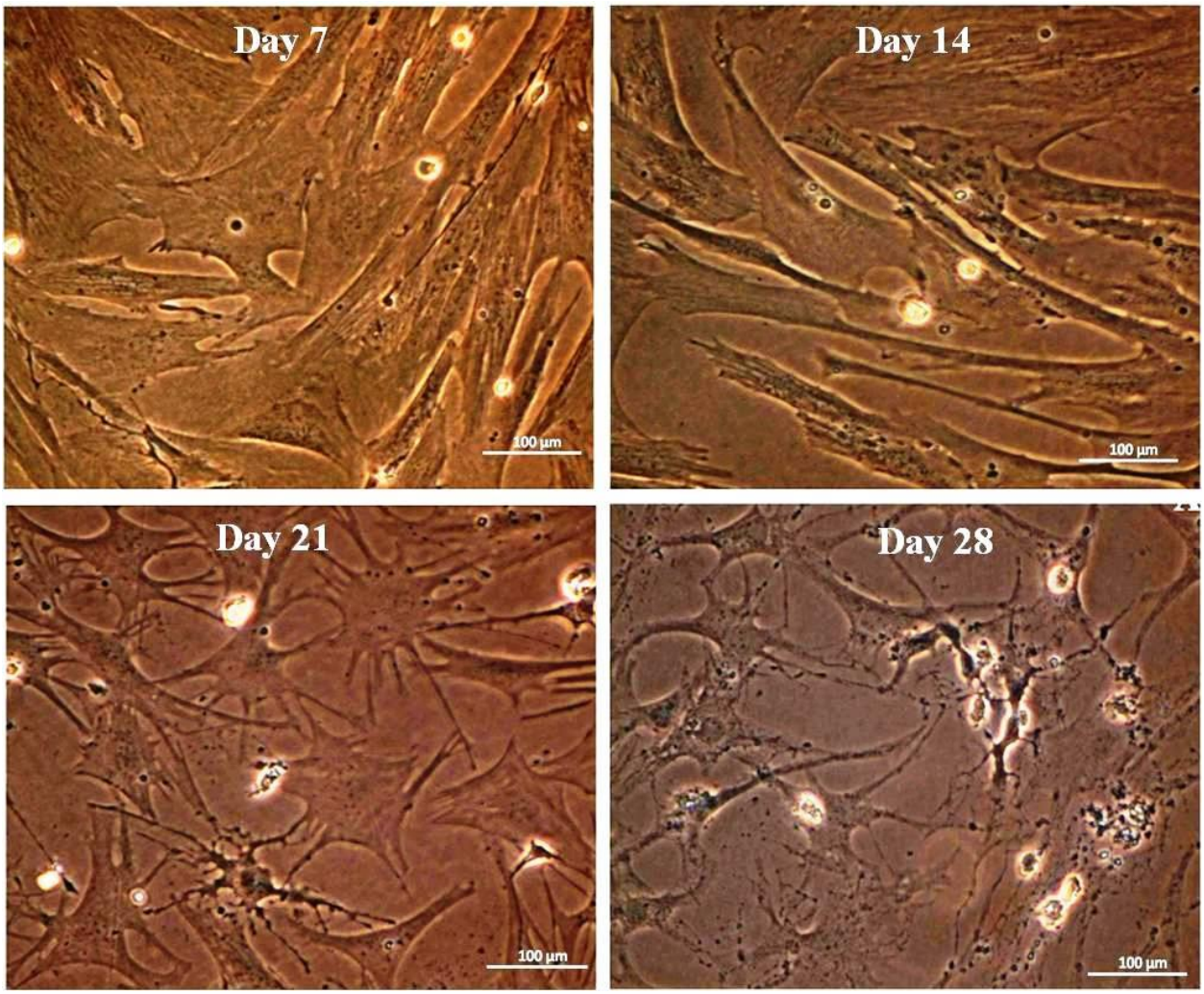

Figure 3. Differentiation of gADMCs into pancreatic islets like cells. The representative image showed the differentiation of gADSCs of 7, 14, 21 and 28 days after culture in conditioned medium.

Morphological changes have been clearly seen after the 5th of differentiation. Although the differentiated cells become thin and extended they do not attain the morphology similar to pancreatic cells. Differentiated pancreatic islet-like cells were readily expressed of Islets-1, Pdx-1 and Insulin proteins. Differentiated cells were confirmed by immunostaining with Islets-

1, Pdx-1 and Insulin specific primary antibody (Fig. 4). Negative control experiment with mouse IgG isotype revealed no specific staining of cells (Fig.4).

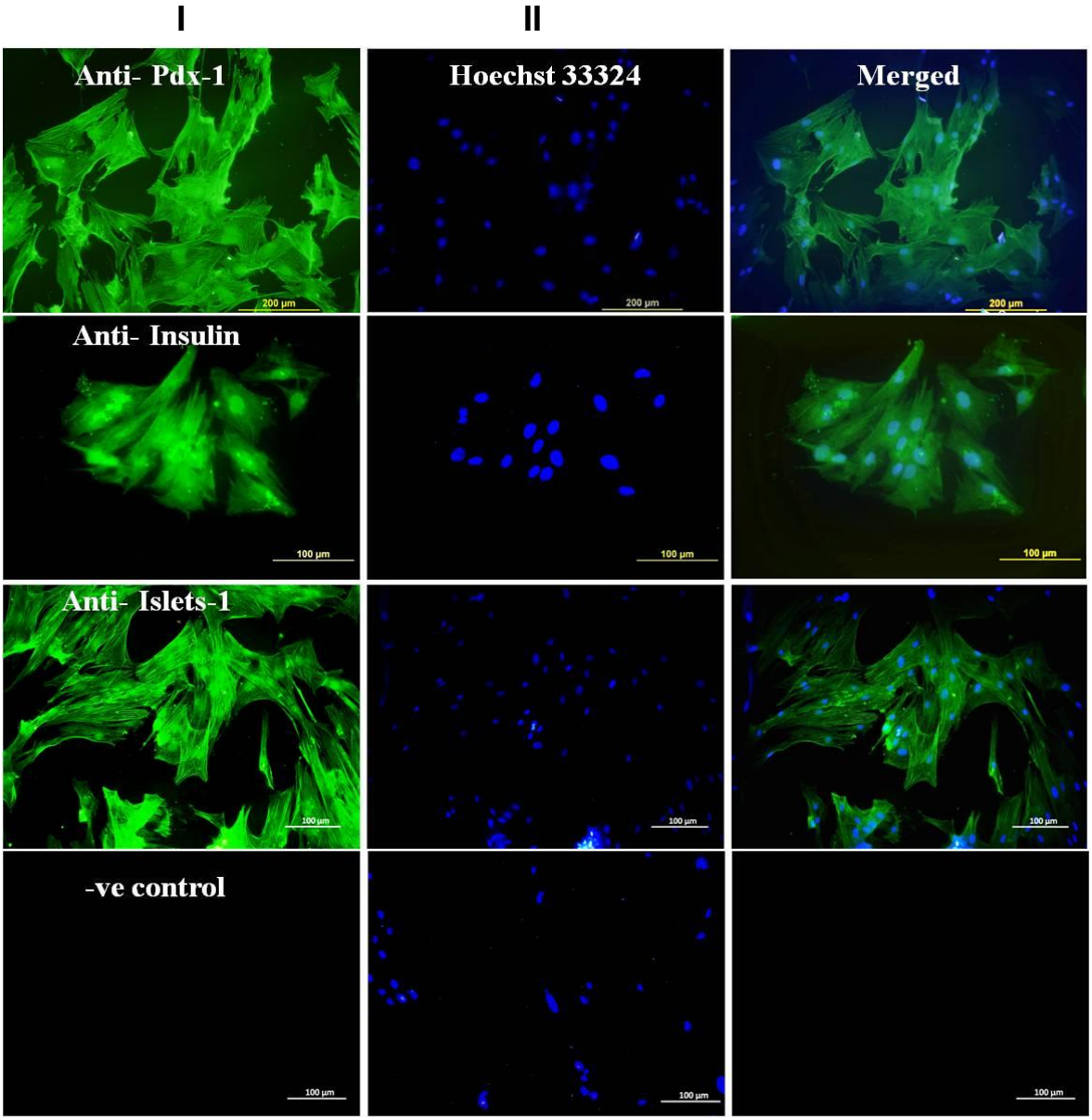

Figure 4. Detection of pancreatic cells specific markers in differentiating cells. (Column I): Immunolocalization of pancreatic islet like cells specific markers in differentiating gADMSCs. (Column II): Nuclear chromatin staining of gADMSCs with Hoechst 33342 dye. (Column III): Merged image of column II and column III. Primary antibody was absent in negative control. Scale bar 100 µM.

The experiment was repeated three times with reproducible results. The qPCR results showed that the differentiated gADSCs was readily expressed Ngn3, Pdx1, Isl1, and Glut2; pancreatic lineage-specific genes (Fig. 5A). Relative expression of Pdx1, Ngn3, Isl1 and Glut2 expression

is significantly increased when analyzed at three different time points after in-vitro differentiated cultured condition (Fig. 5A). Several studies in mice and human ES cell differentiation suggest that Pdx1 expressing progenitor cells is essential for beta-cell differentiation. Early expression of Pdx1 will turn on the Neurogenin3 and other beta cell-specific genes[30]. Then glucose stimulation leads to insulin secretion in the conditioned medium was performed using ELISA based Insulin quantification in these differentiated pancreatic islets-like cells and found insulin secretion highly significant on day 14th after differentiation of gADSCs (Fig. 5B).

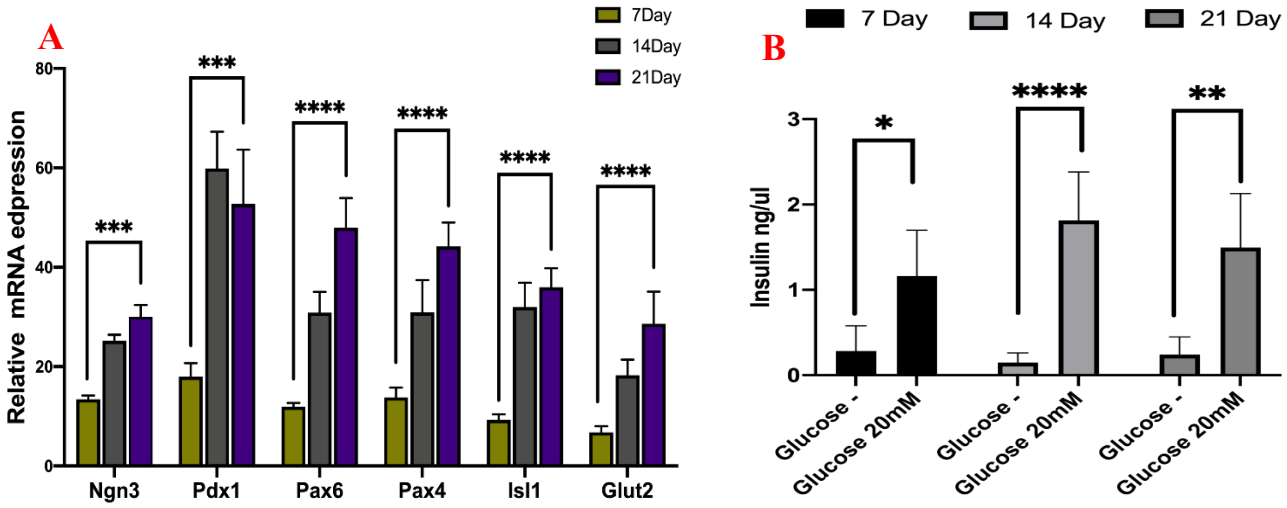

Figure 5. Gene expression study of pancreatic islets like cells after differentiation of gADMCs. (A) Relative expression of genes like Ngn3, Pdx1, Pax6, Pax4, Isl1, and Glut2 on day 7, 14and 21. (B) Glucose stimulation leads to insulin secretion in the conditioned medium from differentiated culture cells used for the ELISA based Insulin quantification. n=3 mean±SEM ∗ p<.05.

Discussions

Mesenchymal stem cell, the next generation of regenerative medicine in human as well as in animals; are obtained from several sources without associated with ethical concern, by using different strategies like mechanical digestion, enzymatic digestion, mononuclear cell fractionation, and centrifugation[31] Friedenstein[32] were the first to describe and characterized the multipotent mesenchymal stem cells from the bone marrow of mice. The present study demonstrates that gADMSCs are successfully isolated from goat adipose tissue by trypsin/collagenase enzymatic digestion method. They must express classical MSC marker such as CD90, CD73, CD44, CD105, CD44, CD166 etc., and lack haematopoietic and endothelial markers such as CD34, CD14, CD45, etc.[33]. These markers are associated with

proper adhesion, proliferation, differentiation, migration, and niche establishment[34]. The expression level of these markers in particularCD105, CD73, and CD90, above 90% showed the homogeneous population and characterization of isolated gADMSCs. CD90/Thy-1 is a cell-surface anchored glycoprotein expressed on human mesenchymal stem cells (MSCs) and have a role in cell-cell and cell-matrix interactions as well as cell motility[35] (CD105 (Endoglin), is a component of the receptor complex of transforming growth factor-beta (TGF-ß) which involved in cell proliferation, differentiation and migration[36].In the present study, all the positive and negative surface markers were expressed in gADMSCs.

Mesenchymal stem cells have the property to differentiate into different types of cells by culturing under a conditioned medium. Differentiation into adipocytes, chondrocytes, and osteocytes is set as basic criteria for characterization of mesenchymal stem cells by the International Society for Cellular Therapy. The differentiation of the potential of the 3rd and 6th passage of gADMSC sunder proper conditioned medium into adipocytes, chondrocytes, and osteocytes was the same and results were confirmed by respective staining and RT-PCR profiling of specific markers. It has already been reported that they must be differentiated into this mesodermal lineage and it is a set as a basic criterion to describe MSCs[37]. Exact composite of mainly three compound Dexamethasone[38], Ascorbic acid[39] and β-glycerophosphate[40] in a conditioned medium leads to osteogenic differentiation. Dexamethasone is essential for osteoblast maturation and differentiation, it induced Runx2, an essential transcription factor required for osteogenic differentiation, expression by β-catenin-mediated transcriptional activation.

The gADMSCs are able to adopt a pancreatic endoderm phenotype in vitro condition. In vitro differentiation achieved through a culture of gADMSCs under a defined conditioned medium. For successful and efficient differentiation, we have used condition medium (DMEM-F12 supplemented with exendin-4, pentagastrin, activin-A, retinoic acid, beta-mercaptoethanol, nicotinamide, and glucose). The high-glucose, serum-free medium restricted cell proliferation, and enhanced differentiation. Previous reports have demonstrated the potential of activin A to induce undifferentiated human ES cells to undergo endoderm differentiation[41]. Mercaptoethanol mainly protected MSCs from the stress induced by serum-free culture conditions during differentiation[42]. Differentiated gADMSCs into pancreatic islets like cells was confirmed by immunostaining with islets, PDX, and insulin specific primary antibody, and cells are expressed ISL-1, PDX, and Insulin marker. The transcription factor Isl-1 is crucial for

the development of pancreatic endocrine cells. Disruption of Isl-1 expression is associated with the absence of dorsal mesenchymal and a marked reduction of Pdx-1 gene expression in the dorsal epithelium in mice[43]. Induction of Isl-1 expression in humans may represent a critical event that allows the adoption of a pancreatic endocrine phenotype. The LIM homeodomain factor Isl-1 is required for the generation of all endocrine cells. Animals lacking Isl-1 have no endocrine cells, indicating the function of Isl-1 in the generation of endocrine progenitor cells. The expression of Pdx1 is associated with pancreas development, as both exocrine and endocrine components of the pancreas develop from Pdx1 positive cells. ThePdx1 is expressed in the pancreatic endoderm and is essential for its early development and later becomes restricted to a beta-cell fate. In adult animals, Pdx1 regulates insulin gene expression[44]. The role of Pdx1 was demonstrated by showing that mutant mice do not develop any pancreatic tissues[45]. Islet-1 (Isl-1), neurogenin-3 (ngn-3), Nkx2.2and Beta2/NeuroD are other transcription factors involved in the proliferation and specification of early endocrine progenitors[43]. Two members of the Pax gene family, Pax4 and Pax6, are essential for proper differentiation of endocrine cells. Pax6 the expression is detected throughout pancreas development and is presented in all endocrine cells. In contrast, Pax4 is required for the development of cells restricted to the beta- and delta-cell-lineages. Mice lacking Pax4 fail to develop any beta-cells and become diabetic[46], while the alpha-cell population is absent inPax6 mutant mice. It should be noted that there was a relatively long interval between the activation of insulin and PDX-1. This implies that PDX-1 can promote insulin expression.

GLUT2 (glucose transporter 2) is another important gene that regulates glucose-dependent insulin secretion in β cells and is especially expressed in pancreatic β cells and liver cells. For differentiation, serum-free medium and many supplemental components such as activin A, TGF-beta, B27, N-2, exendin-4, nicotinamide and HGF was used. High glucose (17 mM glucose) DMEM/F12, which increases the division of beta cells was used as the basal medium[39]. Activin A is an important molecule that is part of the TGF- β (transforming growth factor-beta) family and plays a crucial role in hormone synthesis, cell count management, and maturation to adult cells[47]. Using serum-free medium supplemented with factors like exendin-4, pentagastrin, activin-A, HGF, etc. are known for their beneficial effects on the differentiation of precursor cells into insulin-producing cells[48]. Nicotinamide could preserve islet cells' viability and function while Exendin-4 and activin were used to promote islet differentiation and maturation or to increase insulin content of the various cell

lines[49].We also induced the activation of pancreatic transcription factors Ipf-1, Isl-1, Ngn-3, as well as the islet proteins insulin, glucagon, and somatostatin were confirmed by q-RTPCR.

Conclusions

Adult stem cell based therapy is just a new herald in Regenerative Medicine implied in humans as well as in animals. This study reported successful isolation of gADMSCs by the enzymatic digestion method and characterized as per the International Society of Cellular Therapy as the expression of positive and negative marker genes and in vitro differentiation into adipocytes, chondrocytes, and osteocytes. Then the gADMSCs were in-vitro differentiated into pancreatic islet-like cells by giving a suitable conditioned medium. Transient increase in expression of early pancreatic endoderm differentiation genes and other factors eventually lead to differentiation under *in vitro* condition and further characterized by different marker genes. To the best of our knowledge, this is the first report on livestock species.

Ethics approval and consent to participate

Collection of biological samples were performed according to the guidelines approved by the Institutional Animal ethics committee, NDRI, Karnal. All animal procedures followed the regulations and guidelines established by this committee and minimized the suffering of animals.

Consent for publication

All authors gave their consent to publish the research work

Availability of data and material

All the data in this manuscript can be requested from the corresponding author on rational demand.

Competing interests

The authors declare no competing interests

Funding

Funding is not applicable

Authors' contributions

A. D. Carried out the research work, Methodology. H. N. M. Conceptualization, Writing-Original draft preparation. D. K. S. Methodology, helping research. S. S. Data curation, helping research. S. K. Investigation, Visualization. A. K. D. Supervision, PPT Preparation. D. B. Reviewing, Validation. D. M. Conceptualization, Reviewing and Editing.

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Figures

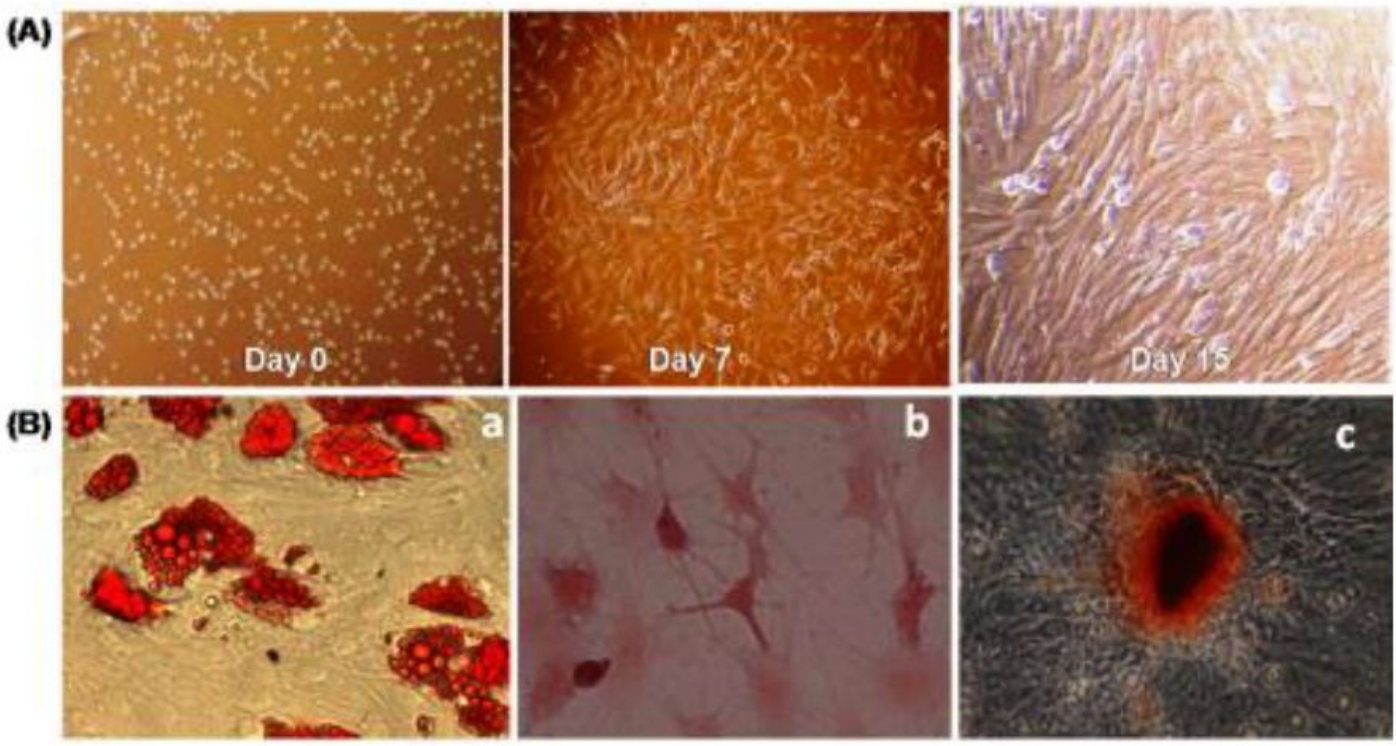

Figure 1

Isolation, culture, characterization and differentiation of gADMSCs. (A) gADMSCs of Day 0, Day 7, Day 10 and day 15. (B) Representative images showed the differentiation of gADMSCs into Adipocytes (a), Chondrocytes (b), Osteocytes cells (c). Magnification 40 X.

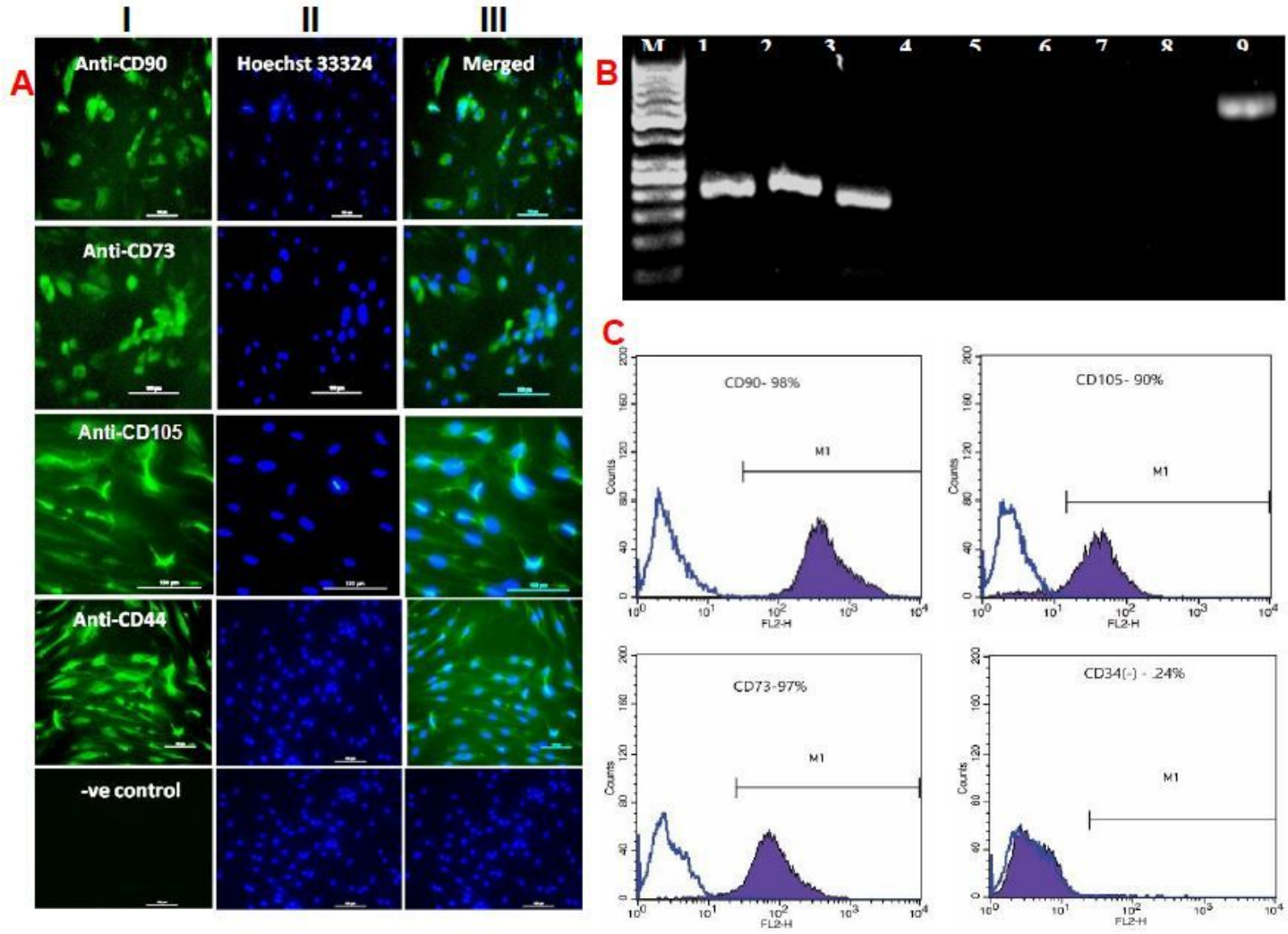

Figure 2

Characterization of gADMSCs. (A) Detection of mesenchymal specific markers in gADMSCs. (Column I): Immunolocalization of a mesenchymal specific protein in respective gADMSCs. (Column II): Nuclear chromatin staining of gADMSCs with Hoechst 33342 dye. (Column III): The merged image of column I and column II. Primary antibody was absent in negative control. Scale bar 100 μM. (B) RT-PCR analysis of mesenchymal specific markers in gADMSCs. Detection of positive markers in lane1 (CD73), lane 2 (CD90) and lane 3 (CD105) and no expression of negative markers lane 4 (CD34), lane 5 (CD45), and lane 6(CD79a) was observed. Fibroblast cells (lane 7) and RT (−) (lane 8) served as a negative control. GAPDH acts as an internal positive standard. (C) Detection of mesenchymal stem cell-specific protein by flow cytometer.

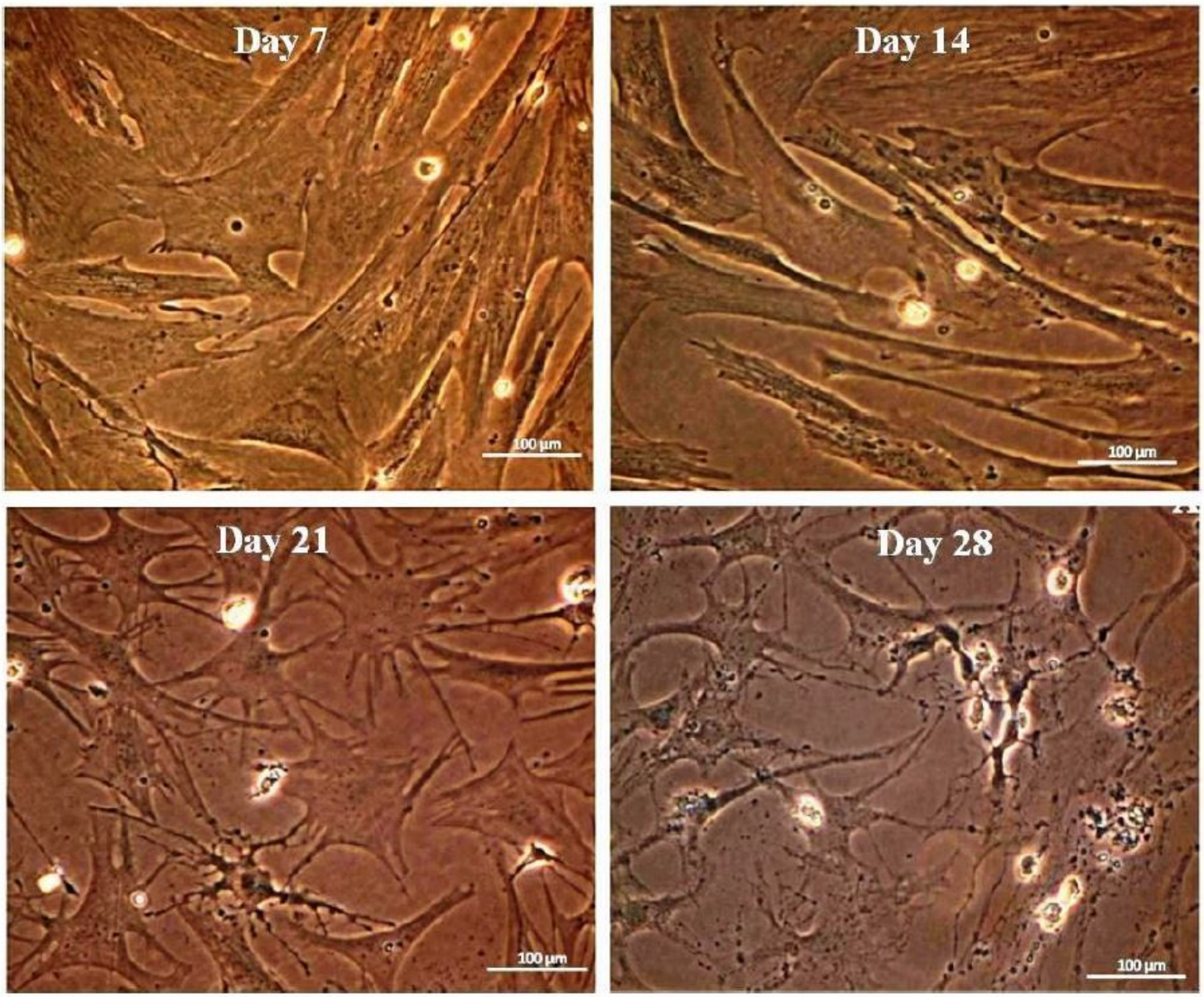

Figure 3

Differentiation of gADMCs into pancreatic islets like cells. The representative image showed the differentiation of gADSCs of 7, 14, 21 and 28 days after culture in conditioned medium.

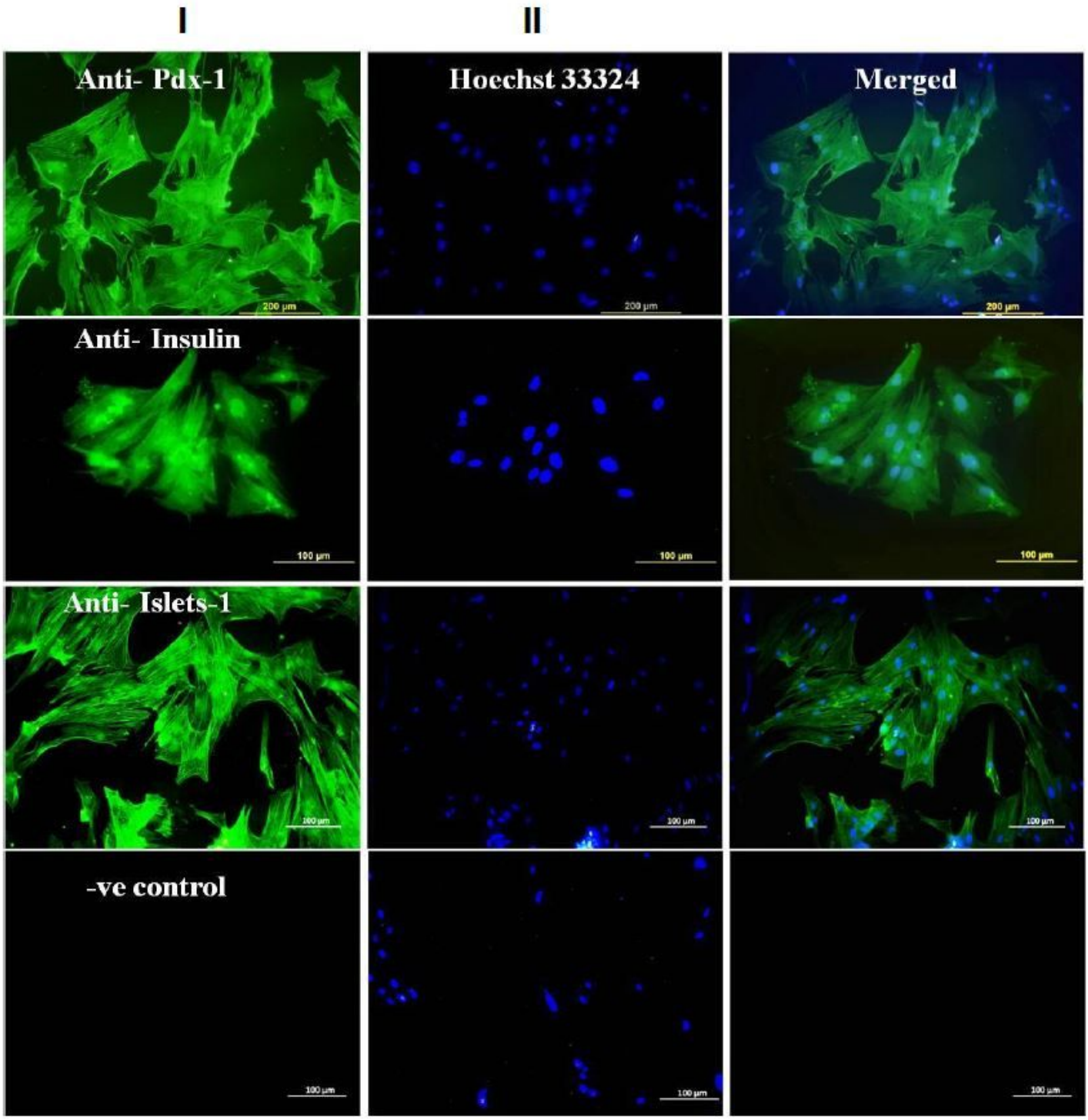

Figure 4

Detection of pancreatic cells specific markers in differentiating cells. (Column I): Immunolocalization of pancreatic islet like cells specific markers in differentiating gADMSCs. (Column II): Nuclear chromatin staining of gADMSCs with Hoechst 33342 dye. (Column III): Merged image of column II and column III. Primary antibody was absent in negative control. Scale bar 100 μM.

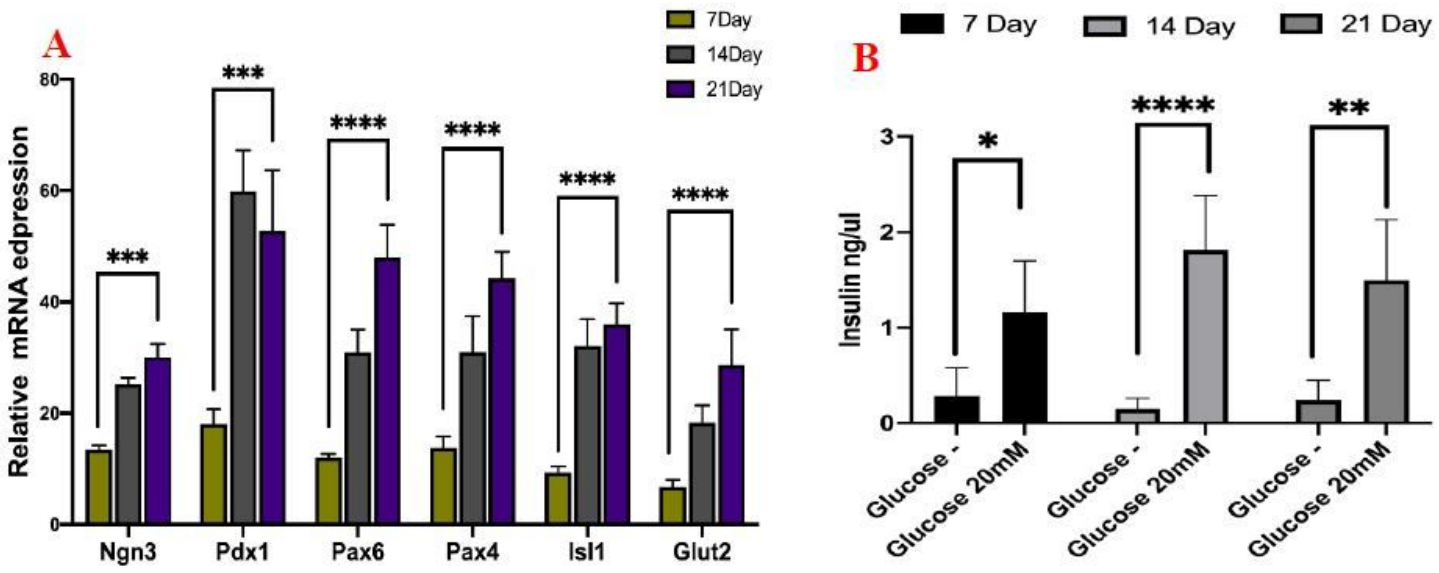

Figure 5

Gene expression study of pancreatic islets like cells after differentiation of gADMCs. (A) Relative expression of genes like Ngn3, Pdx1, Pax6, Pax4, Isl1, and Glut2 on day 7, 14and 21. (B) Glucose stimulation leads to insulin secretion in the conditioned medium from differentiated culture cells used for the ELISA based Insulin quantification. n=3 mean±SEM p<.05.