SHEDs into Islet like cell aggregates: A new horizon for treating insulin dependent diabetes mellitus

ABSTRACT:

Background and Objectives: Stem cells from human exfoliated deciduous teeth (SHEDs) have been demonstrated as a novel population of adult stem cells capable of multi-differentiation potential.

Methods: Study samples comprise of 30 extracted exfoliating primary teeth collected from children aged 6 to 14 years. After attaining the required cell passage, flowcytometric analysis and trilineage differentiation was done to characterize SHEDs. Further SHEDs were differentiated into Islet like cell aggregates (ICAs) using Serum free media A,B&C. Differentiated ICAs were characterized by RT-PCR, immunocytochemistry, DTZ stain and insulin assay.

Results: Flowcytometric analysis of SHEDs showed expression of positive markers CD73, CD90 while no expression of negative markers CD34, CD45 and HLA-DR. Isolated SHEDs had the potential to differentiate into tri-lineages and ICAs. RT-PCR analysis of derived ICAs showed up-regulated expression of GAPDH, insulin, Glut2, PDX1 and PAX6. Immunofluorescence analysis gave expression of Ngn3, Isl-1, C-peptide, Glut2 and PDX1. DTZ stained positive on derived ICAs.Insulin secretion of SHED derived ICAs were measured 26 ± 6 MIU/L at basal glucose level, 128±3 MIU/L and 240 ±9 MIU/L at stimulated glucose level which gave a statistically significant difference in mean value of insulin secreted in different concentration of glucose **($p<0.001$).** The net insulin secretion value of SHED derived ICAs at different glucose concentration was less when compared with Min 6 cells used as positive control.

Interpretation and Conclusion: Stem cells from Human Exfoliated Deciduous teeth are mesenchymal stem cells which has the unique potential to differentiate into islet like cell aggregates and serve as a promising source of insulin which under standardized protocols and experimentations can be used for stem cell based therapy for insulin dependent diabetes mellitus.

Key words: Mesenchymal Stem Cells, Stem cells from exfoliated deciduous teeth (SHEDs), Islet like cell aggregates (ICAs), Insulin, Type 1 diabetes mellitus.

INTRODUCTION:

Stem cells are clonogenic cells capable of both self-renewal and multilineage differentiation with varying degrees of potency and plasticity.[1] Modern concept of medicine emphasizes prevention and reversal of disease and thus broader strategies and multidisciplinary approaches have become the need of the hour. The discovery of stem cells was a giant breakthrough in the field of science and opened the door for a whole era of experimentations, with their potential use in the cell based therapy of various disorders. Which gave a fascinating pavement for tissue engineering focused to change the face of human disease and alleviate suffering.

Postnatal stem cells have been isolated from a variety of sources which includes bone marrow, brain, skin, hair follicles, skeletal muscle and especially dental tissues[2] such as the pulp of the Primary(SHEDs)[1] and Permanent teeth(DPSCs)[3] , Periodontal Ligament(PDLSCs)[4] , Periapical Follicle(DFSCs)[5] and Apical Papillae(SCAPs)[6] which place the dentists at the forefront of engaging their patients in potentially life-saving therapies derived from a patient's own stem cells present in the teeth. Moreover dental stem cells are considered to be an appealing source for mesenchymal stem cells, since they are non-controversial, readily accessible, have a large donor pool, and pose no risk of discomfort for the donor.[7]

Stem cells from human exfoliated deciduous teeth (SHEDs) represent a population of postnatal stem cells capable of extensive proliferation and multipotential differentiation which was verified and confirmed in the previous studies. An exfoliated deciduous tooth is similar in some ways to an umbilical cord, containing stem cells that may offer a unique postnatal stem cell source for potential clinical applications.[8]

Diabetes is characterized by abnormally high levels glucose in the bloodstream. This excess of glucose is responsible for most of the complications of diabetes, which include blindness, kidney failure, heart disease, stroke etc. Type 1 diabetes or juvenile-onset diabetes, typically affects children and young adults. It develops when the body's immune system targets islet cells as foreign bodies and destroys them. As a result, the beta cells of islet of Langerhans in the pancreas, which normally produce insulin, are destroyed. Transplantation of insulin-producing islet cells isolated from a donor pancreas could be a cure for type 1 diabetes. However, a critical shortage of sufficient donor organs and the side-effects of immunosuppressive therapy limit its therapeutic usage, prompting a search for alternative sources of islet cells.[9]

Evolution of stem cell studies claims that stem cells when injected near the pancreas develops into pancreatic cells and regenerates the pancreas's ability to produce natural insulin. However for human trials a wide depth of knowledge in the literature is required from in vitro and in vivo animal studies to benchmark the success of cell based therapy for type 1 diabetes.

The aim of this study is to demonstrate that stem cells from human exfoliated deciduous teeth could be differentiated into pancreatic cell lineage and offer an easily accessible and non-controversial source of human tissue that could be used for autologous stem cell therapy in type 1 diabetes mellitus.

MATERIALS AND METHODS:

The study was conducted in the Department of Pedodontics and Preventive Dentistry, Rajarajeswari Dental College and Hospital in collaboration with Manipal Institute of Regenerative Medicine, (Stempeutics) Bangalore. Institutional ethical committee approval was obtained from the Ethical Committee, Rajarajeswari Dental College and Hospital. A written informed consent was obtained from the parents or guardian of the children included in the study.

Sample collection:

Thirty primary teeth from healthy children aged between 6 to 14 years were extracted under aseptic condition and rinsed with normal saline gently to remove blood and other debris. The samples collected from each individual were then separately transferred to 15ml conical base centrifuge tubes (BD Falcon) containing Dulbecco's Phosphate Buffer Saline (DPBS) solution(Invitrogen).

Isolation of SHEDs:

In the laboratory, the samples were processed inside the Bio-laminar flow chamber. First, the samples were washed with PBS + 1% antimycotic twice. Then teeth were placed inside a sterile surgical glove and broken into pieces with an osteotome wrapped with aluminium folds, so that the pulpal tissues can be easily removed. The pulpal tissues were then removed with broaches or tweezers and placed in 50mm x 12mm petri dishes. Followed by which, mincing was done using 2mg/ml collagenase blend (Sigma) and the tissues were cut in to smaller pieces using Surgical Scalpel Blade no. 21 to increase the surface area of action to the enzyme. The tissue was incubated in the incubator (Heracel Thermo) at 37°C for 60 minutes. After incubation, the culture medium, Dulbecco's Modified Eagles Medium- Knock out (DMEM-KO) with 10% Fetal Bovine Serum (Hyclone), 100µM ascorbic acid and 2mM L-Glutamax supplemented with 100U/ml penicillin and 100U/ml streptomycin, was added. The samples were transferred to BD falcon tubes and then centrifuged (Eppendorf Centrifuge Machine 5415R, Germany) at 1800 rpm for 5 minutes. The supernatants were discarded and the tissue pellets were collected in 6 well culture plate which appropriately containing 1ml of culture medium, DMEM-KO. The cells were finally incubated in the incubator (Heracel Thermo) in a humidified atmosphere at 95% air and 5% CO_2 at 37°C. The plates were then reviewed after 48 to 72 hours to check for growth and attachment of cells.

Cell Passaging :

Media was changed every 48 hours. Several representative microphotographs were taken each time during observation with inverted microscope at 4x and 10x magnification. Once the cells become 80% confluent, the spent media was removed and cells were washed by DPBS and detached by adding 0.25% trypsin. After neutralization, the cells were transferred into a centrifuge tube and centrifuged at 1800 rpm for 5 minutes. Then cells were reseeded in larger containers passage to passage progressively using petri dish and 35mm^2 flask.

Characterization of SHEDs

i) Flowcytometry

Flowcytometric analysis was used to determine the surface phenotypic profile of the isolated cells. Ten microliter of tagged primary antibody were added on appropriate number of cells. Isotopes of Immunoglobulin G2 (IgG2) and Immunoglobulin G1 (IgG1) were used as control groups. Cells were stained for 1hour on ice. Then 500µl of FACS buffer were mixed thoroughly by pipetting and then transferred to flowcytometry tubes. The samples were runned in the flowcytometry machine. The cells were stained using fluoresceinisothiocyanate (FITC) – conjugated CD34 and PE (R- phycoerythrin)-conjugated CD45, CD90, CD73 and HLA-DR antibodies. The process was repeated in 10 samples for each marker. BD CellQuest^{TM} Pro Version 5.2.1 software (BD Bioscience,USA) was used to analyze the flow cytometric results.

Table 1: Types of markers studied to characterize SHEDs.

Positive markers	CD 73, CD 90
Negative markers	CD 34, CD 45, HLA-DR

ii) Tri-lineage differentiation

Cell lineage induction was performed when cultures had reached 90% confluency. SHEDs were cultured in complete media supplemented with the respective induction media in 35 mm^2 dish (Table – 4,5). Media was replenished every 3 days. After 7 to 21 days, cells were fixed to do Von Kossa staining for osteocytes, Oil red O for adipocytes and Alcian Blue Stain Analysis for chondrocytes. The differentiation induction was repeated in 3 samples for each lineage.

Table 2: Media composition for osteocytes differentiation.

Reagents	Concentration
Basal media (DMEM-KO)	
FBS	10%
Pen – strep	200 mM
Dexamethasone	0.5%
Ascorbic acid	50 µgm/ml
B-glycerophosphate	10 Mm

Table 3: Media composition for adipocytes differentiation.

Reagents	Concentration
Basal media (DMEM-KO)	
FBS	10%
Pen – strep	0.5%
Glutamin	200 Mm
Dexamethasone	1 µM
Insulin	1 µgm/ml
Indomethacin	100 µM
IBMX	0.5 mM

STEMPRO Chondrogenesis Differentiation Kit (Invitrogen) was used for chondrocytes differentiation.

Differentiation of SHEDs into ICAs :

After checking for the tri-lineage differentiation, stem cells differentiation into islets like cell aggregate was initiated. Stem cells from human exfoliated deciduous teeth at passage 3 or 4 are resuspended in serum free media A (SFM-A) and plated in a 50mm x 12mm petri dish ($1x10^6$cells/cm^2). The cells were cultured in this medium for 2 days. On the third day the medium was changed to SFM-B and on the fifth day finally shifted to SFM-C. The cells were fed with fresh SFM-C every 2 days for another 5 days.

Table 4: Media composition for serum free media A (SFM-A).

Reagents	Concentration
Basal media (DMEM-KO)	
BSA Cohn fraction V	1%
Insulin-transferrin-selenium(ITS)	1 x
Activin A	4 nM
Sodium butyrate	1 mM
2-mercaptoethanol	50 µM

Table 5: Media composition for serum free media B (SFM-B).

Reagents	Concentration
Basal media (DMEM-KO)	
BSA Cohn fraction V	1%
Insulin-transferrin-selenium(ITS)	1 x
Taurine	0.3 mM

Table 6: Media composition for serum free media C (SFM-C).

Reagents	Concentration
Basal media (DMEM-KO)	
BSA Cohn fraction V	1.5%
Insulin-transferrin-selenium(ITS)	1 x
Taurine	3 mM
Glucagon like peptide (GLP-1)	100 nM
Nicotinamide	1 mM
Non-essential amino acids (NEAAs)	1 x

Characterization of ICAs:

i) Reverse transcriptase polymerase chain reaction (RT-PCR)

RT-PCR is used to qualitatively detect gene expression through creation of complementary DNA transcripts from RNA. Commercially available RNA from human pancreas (Clontech , USA) was used as the positive control. The primers (Sigma-Aldrich) used were GAPDH, insulin, Pdx1, PAX6, SOX17 and glut2. PCR Analysis was done on 3^{rd} and 10^{th} day of islet differentiation.

Table 7: Pancreatic genes used for reverse transcriptase polymerase chain reaction with their sequences.

Pancreatic genes	Sequence
GADPH	Forward (5'- TGGTATCGTGCAAGGACTCATGAC-3') Reverse (5'- ATGCCAGTCAGCTTCCCGTTCAGC-3')
Insulin	Forward (5'- AGCCTTTGTGAACCAACACC-3') Reverse (5'- GCTGGTAGAGGGAGCAGATG-3')
Pdx1	Forward (5'-GTCCTGGAGGAGCCCAAC-3') Reverse (5'- GCAGTCCTGCTCAGGCTC-3')
PAX6	Forward (5'-ATGAACAGTCAGCCAATGGG-3') Reverse (5'-CACACCAGGGGAAATGAGTC-3')
SOX17	Forward (5'-CGCACGGAATTTGAACAGTA-3') Reverse (5'-GGATCAGGGACCTGTCACAC-3')
Glut2	Forward (5'-GGTTTGTAACTTATGCCTAAG- 3') Reverse (5'-GCCTAGTTATGCATTGCAG-3').

ii) Immunocytochemistry

Undifferentiated SHEDs (Control) and Differentiated ICAs were fixed for 20 min in 4% para - formaldehyde and treated with 0.1% Triton X-100 as cell membrane penetration agents. Cells were blocked at room temperature in 0.5% BSA solution for 0.5 hour and incubated with primary antibodies overnight at 4^0C. Subsequently, cells were washed with PBS and incubated with FITC-conjugated secondary antibodies, Ngn3, Isl-1, C-peptide, Glut-2 and PDX1 at room temperature for 1.5 hours. Slides were counterstained with 4',6-diamidino-2-phenylindole (DAPI) for 5 min, and fluorescent images were captured.

iii) Diphenylthiocarbazone staining: (DTZ)

Islet-specific DTZ stain, a zinc-chelating agent selectively marks the pancreatic beta-cells because of its high zinc content. ICAs were maintained with 10 ml DTZ stain for 1 hour at 37^0 C and observed under microscope. DTZ staining was primarily used for cell counting and cell sorting for further procedure.

iv) Insulin release assay

Hundred live ICAs with the diameter of approximately 150 micro meter were seeded in each well of a 6 well plate and incubated with 200 mL Krebs-Ringer bicarbonate buffer (pH 7.4) supplemented with 10 mmol/L Krebs' ringer bicarbonate HEPES medium and basal glucose level (5.5 mmol/L) at 37^0C for 1 hour. The mixture of ICAs and buffer was centrifuged, and the obtained supernatant was stored at 80^0C until analysis. Subsequently, the ICAs were reseeded in 6 well plate supplemented with 10mmol/L Krebs' ringer bicarbonate HEPES medium and stimulated glucose level (11 mmol/L and 16.5 mmol/L) at 37^0C for 1 hour followed which

centrifugation was done. The supernatant were subjected to human insulin enzyme-linked immunosorbent assay (Mercodia,Sweden) at room temperature to measure the insulin secretion. Similar protocol was conducted on Min 6 cells (Clontech,USA) which was used as positive control to compare with SHED derived ICAs.

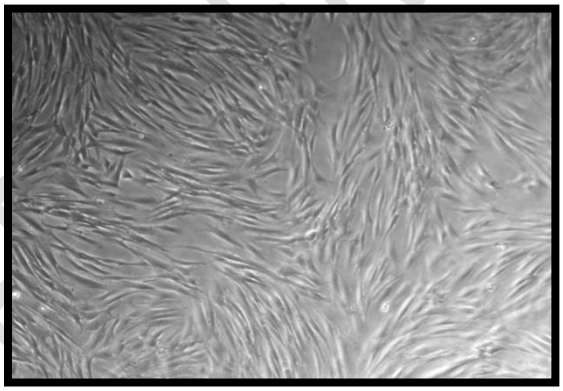
RESULTS:

A) Isolation and Characterization of SHEDs:

i) Cell Culture

Dental pulp cells were observed to grow with colony formation at primary culture which mainly composed of fibroblastic cells. A number of small clear cells were also observed on the fibroblastic cells. The cultures tended to achieve confluence in 10 days. Confluent cultures of cell Passage 4 were selected, which composed of multiple bundles of fibroblastic cells, each running in a particular direction, a typical feature of human bone marrow-derived Mesenchymal Stem Cell. Morphologically, stem cells are spindle shaped cells with central large nucleus, multiple cytoplasmic processes usually project from the outer surface.

Figure 1: Inverted microscopic(4X) view of SHEDs at passage number 4.



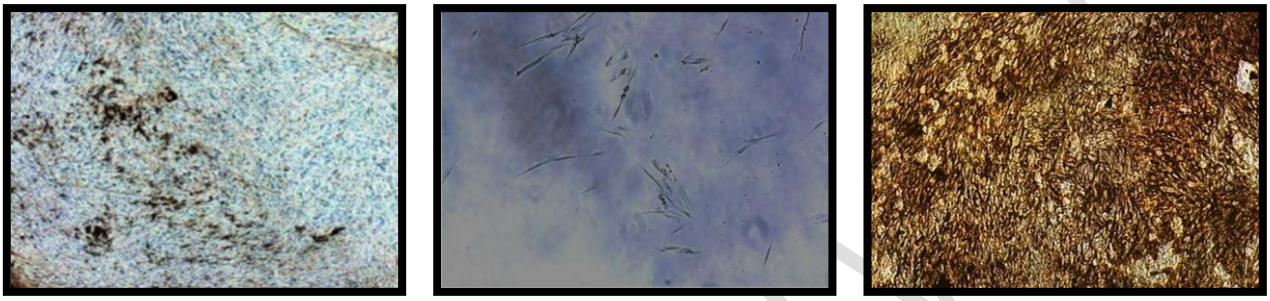
ii) Flowcytometry

Flowcytometric analysis of SHEDs obtained from BD Cell Quest ^{TM} Pro Version 5.2.1 software showed high expression of positive mesenchymal marker CD73 (96.69 %) and CD90 (97.70 %) and negative expression for haematopoietic markers [CD34 (1.76%) and CD45 (0.88%)] and HLA-DR (0.58%).

iii) Tri-lineages Differentiation

Isolated SHEDs successfully differentialed into osteocytes, chondrocytes and adipocytes when induced with their respective reagents as explained in the previous study.

Figure -2: Tri-lineage differentiation



Osteocytic differentiation

Chondrocytic differentiation

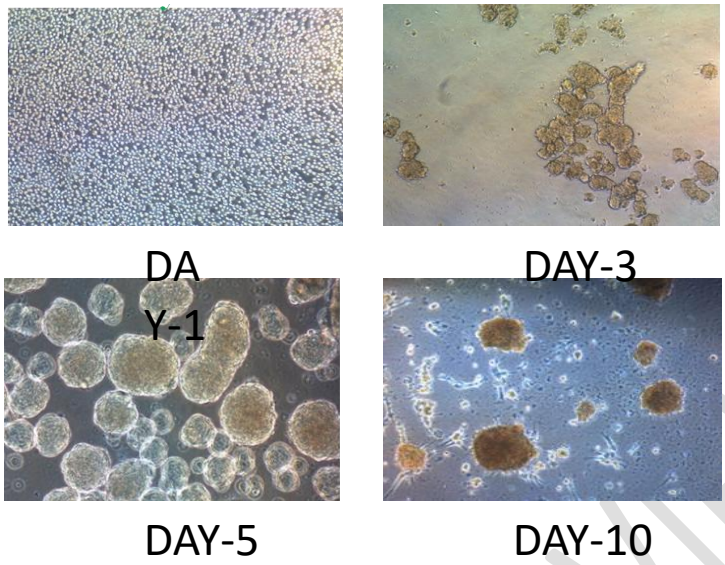
Adipocytic differentiation

B) Differentiation and Characterization of ICAs:

i) Cell Culture

Computer aided inverted microscopic images were obtained at different time interval, once the normal meschenchymal Stem cells growth medium of SHEDs was replaced with islet induction medium (serum free medium) for 10 days the adherent cells started to aggregate into spherical islet like cells.

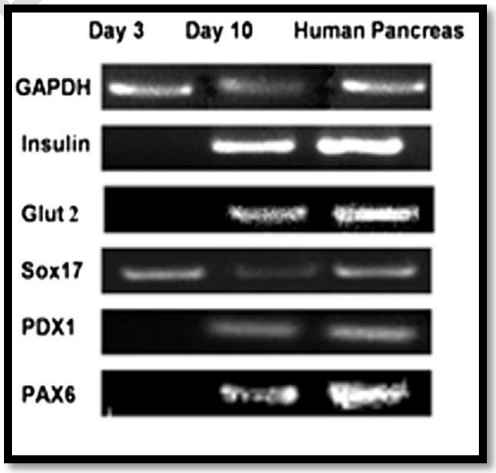
Figure 3: showing aggregation of spherical islet like cells



ii) Reverse transcriptase polymerase chain reaction

Human pancreas transcript factors was taken as a positive control. Day 10 ICAs derived from SHED showed up-regulated expression of markers GAPDH, insulin, Glut2, PDX1, PAX6 and down-regulated expression of marker SOX 17 when compared with their day 3 counterparts. This reverse transcriptase polymerase chain reaction analysis gave a result that day 10 derived ICAs possess the gene expression of human pancreas**.**

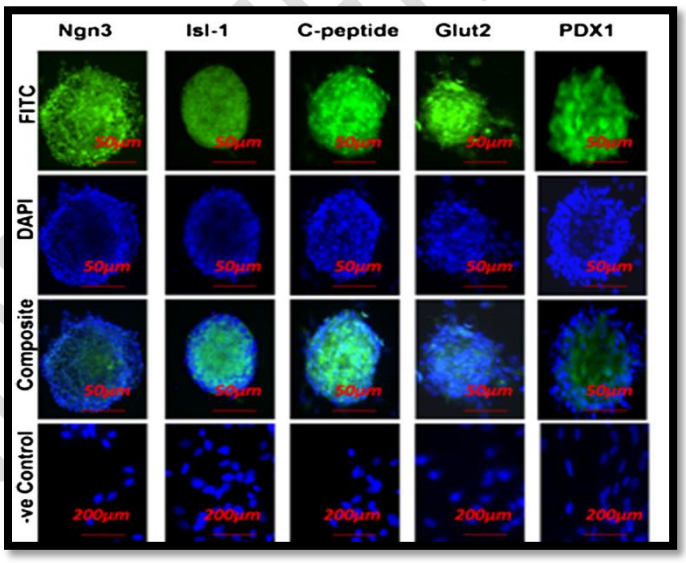
Figure 4: Results of reverse transcriptase polymerase chain reaction show comparable expression of GAPDH, insulin, Glut2, PAX6, SOX17 and Pdx1 markers in ICAs derived with counterpart in human pancreas used as positive control.



iii) Immunocytochemistry

Immunofluorescence analysis was performed on control (undifferentiated) SHEDs and on day 10 ICAs for the expression of the Ngn3, Isl-1, C-peptide, Glut2 and PDX1. The immunofluorescence experiments confirmed expression of the above-mentioned pancreatic markers on day 10 ICAs derived from SHEDs. whereas there was no expression of the markers in the control (undifferentiated) SHEDs**.**

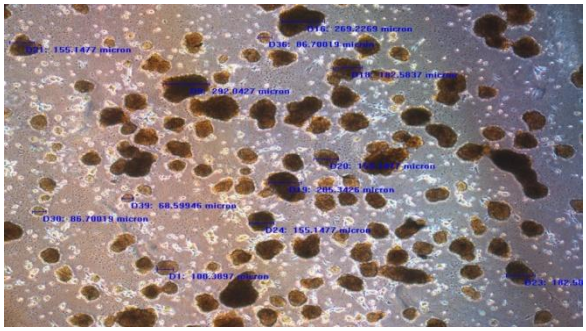
Figure 5: Immunofluorescence analysis shows expression of Ngn3, Isl-1, C-peptide, Glut2 and PDX1 on day 10 ICCs derived from SHEDs and no expression from undifferentiated SHEDs kept as negative control.



iv) Diphenylthiocarbazone staining (DTZ)

Islet specific diphenylthiocarbazone stained the day 10 derived islet like cell aggregates in reddish brown colour and claimed positive. Derived ICAs were of acceptable size with a diameter of 168 +/- 55.8 micro meter**.**

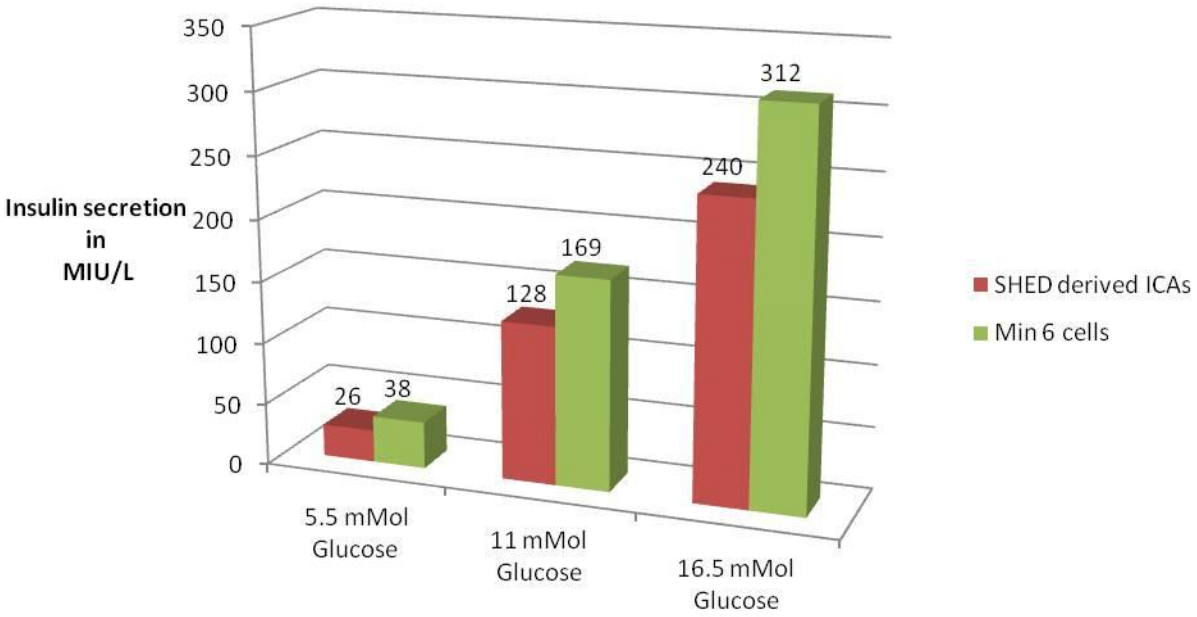
Figure 6: SHED derived Islet clusters stained positive for DTZ stain.



v) Insulin release assay: ELISA

Insulin secretion of SHED derived islets like aggregates were measured 26 ± 6 MIU/L at basal glucose level (5.5 mmol glucose), 128±3 MIU/L and 240 ±9 MIU/L at stimulated glucose level (11 mmol glucose and 16.5 mmol glucose respectively). Insulin secretion of Min 6 cells were measured 38±4 MIU/L at basal glucose level (5.5 mmol glucose), 169±3 MIU/L and 312 ±3 MIU/L at stimulated glucose level (11 mmol glucose and 16.5 mmol glucose respectively). The difference in mean value of insulin secreted at different concentration of glucose for both ICAs and Min 6 cells gave a statistically significant value **(p<0.001).** The net insulin secretion by Min 6 cells were higher when compared with that of SHED derived ICAs.

Figure 7: Represents insulin secretion of SHED derived ICAs and Min 6 cells at basal and stimulated glucose level.



DISCUSSION:

The source of Mesenchymal stem cells (MSCs), which reside within the stromal compartment of bone marrow were first identified in the pioneering studies of Friedenstein and Petrakova[10], which gave a head start for research on other sources of postnatal stem cells. Stem cells from human exfoliated deciduous teeth (SHEDs) have become one of the most efficient, feasible and non-invasive source of postnatal stem cells which made it an attractive alternative in the field of tissue engineering.[1]

In the present study**,** SHEDs were successfully isolated and identified. we used an osteotome to retrieve pulp from the tooth by splitting the tooth into pieces which allows to easily access the pulp tissues with a broach or Luer's forceps. Pulp retrieval using a diamond disc was not employed as it can cause heat and severe mechanical stress to the pulpal tissues.[11]

The International Society of Cellular Therapy (ISCT) has strongly encouraged the scientific community to adopt a set of criteria that standardizes the identification of mesenchymal stem cells (MSCs). The **first criteria** is that the MSCs must be adhered to base of container when maintained in culture. In **second criteria,** the MSCs must be positive for the surface markers CD73, CD 90 and CD105; negative for CD34, CD45 and HLA-DR when analysed by flowcytometry. **Final criteria** is that mesenchymal stem cells must have the potential to differentiate into osteocytes, adipocytes and chondrocytes under standard in vitro differentiation conditions.[12]

The present study fulfilled all the three criteria put forth by ISCT. **Trypinization** was done to detach the stem cells from the base of the containers for cell passaging similar to the studies conducted by Peneva M et al[13] and Govindasamy et al[7] which illustrates their potency and plasticity. **Flowcytometric analysis of SHED** expressed positive markers CD73 (96.69 %) and CD90 (97.70 %). The cultivated SHED cells are not hematopoietic, because they did not express negative markers CD34 (1.76%), CD45 (0.88%) and HLA-DR (0.58%), the results were in accordance with the study conducted by Govindasamy et al.[7] A phenotype analytical study demonstrated that SHEDs also showed positive expression for CD 44, CD 117 and CD 116.[14] CD105 is dominantly associated with endothelial cells although it has been used in immunomagnetic selection for human MSCs.[15] A study carried out on dental pulp stem cells reported that the phenotypic expression of CD105 was found to be increased with increase in passage number.[16] **In the present study** cell passage 3 or 4 were used for islet differentiation and considering the limitations, CD 105 was not employed for phenotypic analysis.

The final criteria which was evaluated in our study was the tri-lineage differentiation potential of SHEDs. The samples in our study were found to be successfully differentiated into osteocyte, chondrocyte and adipocyte lineage. Tri-lineage may seem to be surprising because these tissues are not resident components of the dental pulp cavity or its surrounding tissues which explains the ectomesenchymal origin of the dental pulp.

In present study stem cells from human exfoliated deciduous teeth (SHEDs) were used for islet differentiation by inducing serum free media. This method was in accordance with study conducted by Kerkis et al 2006[17] and Govindasamy et al 2010[7] which illustrated that stem cells isolated from permanent teeth were more restricted in their potential and were mainly committed to a neuro-ectoderm lineage. Furthermore, they lose their plasticity over increased numbers of passages. So stem cells from human exfoliated deciduous teeth possess increased pluripotency and greater plasticity which makes it ideally suited for pancreatic lineage differentiation.[18]

In the literature insulin producing pancreatic islet cells were differentiated from a variety of sources which includes embryonic stem cells[19], bone marrow stem cells[20], hepatic stem cells[21], adult human skin fibroblast[22] and placental stem cells[23]. However they have their own ethical limitations and scarcity of sources, which claims stem cells from dental origin as leading frontier in islet differentiation.

In the present study, pancreatic differentiation has been confirmed at the transcription level by up-regulation of GADPH, insulin, Glut2, PDX1 and PAX6 gene expression. In addition, immunocytochemistry analysis verified expression of early pancreatic genes such as Ngn3 and pancreatic specific genes such as Isl-1, C-peptide, Glut2 and PDX1 in ICAs derived from SHEDs with reference to undifferentiated SHEDs as control. DTZ-stained positive for ICAs derived from SHEDs which serves as a reliable evidence for pancreatic differentation. Superiorly insulin secretion by ICAs at different glucose concentration gave a highly statistically significant result **($p<0.001$)** which added the strength of the study. Their efficiency were less when compared with Min 6 cells, which was used as control. As per the literature Min 6 cells has the ability to responds similar to human islet cells.[24]

Bone marrow meschenchymal stem cells (BM-MSCs), which possess pluripotent differentiation capabilities, are standard candidate for stem cell therapy in diabetic islet cell replacement benchmarked in literature. Conversely, other studies have failed to support the ability of BM-MSCs to differentiate into islet cells.[25] A study reported that BM-MSCs differentiate into immature islets *in vitro,* and these islets mature under *in vivo* conditions upon transplantation.[26] whereas SHEDs can be inevitably differentiated into mature islet cells in vitro.

The most common limitation of Stem cells transplantations was pathogen transmission and the need of immunosuppression, so autologous stem cell source is the best option. Dental pulp stem cells especially from deciduous teeth will be better fitting tool due to easy surgical access, the very low morbidity of the anatomical site after the collection of the pulp. However there is no scientific proof in the literature for practical implementations of SHED derived islet cells in the treatment of insulin dependent diabetes mellitus.

The present study clearly documents the unique potential of stem cells derived from deciduous teeth to differentiate into insulin-producing cells (ICAs). It demonstrated a statistically significant insulin secretion at different glucose concentration, thus offering yet another non-pancreatic, non-invasive source of cells for islet generation that can be used for autologous transplantation for the treatment of children with type 1 diabetes mellitus.

CONCLUSION:

Dental pulp of exfoliated teeth thereby represents alternative and easily accessible source of tissue-specific stem cells which are histocompatible with patient–specific tissues. Exfoliated deciduous teeth which is often discarded instead when cryopreserved in a stem cell bank will be useful for research and clinical applications. Pancreatic cell lineage obtained from SHEDs have proven to be a worthy candidate for stem cell based therapy for type 1 diabetes but however additional animal trials are required to bench mark its success in humans.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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