Regenerative Therapy 26 (2024) 251–259

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Original Article

# The generation of islet-like insulin-producing cells from Wharton's jelly-derived mesenchymal stem cells on the PES/fish gelatin scaffold

Fatemeh Soleimanifar [a], Nazli Aghapur [b], Zeinab Rezaei-Kiasari [c], Hosein Mahboudi [a], Mohammad Kaabi [b], Reyhaneh Nassiri Mansour [d], Mousa Kehtari [e], Mohammadfoad Abazari [f], Seyed Ehsan Enderami [g, *], Hadi Hassannia [h, **]

[a] Department of Medical Biotechnology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran
[b] Stem Cell Research Technology, Tehran, Iran
[c] Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[d] Department of Regenerative Medicine, School of Advanced Technologies in Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[e] Department of Animal Biology, School of Biology, Faculty of Science, University of Tehran, Tehran, Iran
[f] Research Center for Clinical Virology, Tehran University of Medical Sciences, Tehran, Iran
[g] Diabetes Research Center, Mazandaran University of Medical Sciences, Sari, Iran
[h] Amol Faculty of Paramedical Sciences, Mazandaran University of Medical Sciences, Sari, Iran

ARTICLE INFO

*Article history:*
Received 21 March 2024
Received in revised form
12 May 2024
Accepted 30 May 2024

*Keywords:*
Insulin-producing cells
Wharton's jelly-derived mesenchymal stem cells
Fish gelatin
Scaffold
Polyethersulfone
3D culture

ABSTRACT

Diabetes Mellitus (DM) disrupts the body's capability to control blood glucose statuses. Type 1 diabetes mellitus (T1DM) arises from inadequate insulin production and is treated with insulin replacement therapy. Stem cell therapy is a hopeful treatment for T1DM that involves using adult stem cells to generate insulin-producing cells (IPCs). Mesenchymal stem cells (MSCs) are particularly advantageous for generating IPCs. The islet cells require interactions with the extracellular matrix for survival, which is lacking in conventional 2D culture systems. Natural or synthetic polymers create a supportive 3D microenvironment in tissue engineering. We aim to construct superior differentiation conditions employing polyethersulfone (PES)/Fish gelatin scaffolds to differentiate Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs) to IPCs. In this study, the PES/fish gelatin scaffold (3D) was manufactured by electrospinning, and then its biocompatibility and non-toxicity were investigated by MTT assay. After that, scaffold-supportive effects on WJ-MSCs differentiation to IPCs were studied at the gene and protein levels. After exposure to the differentiation media, 2D and 3D (PES/Fish gelatin) cultured cells were slowly aggregated and developed spherical-shaped clusters. The viability of cells was found to be comparable in both 2D and 3D cultures. The gene expression analysis showed that efficiency of differentiation was more elevated in 3D culture. Additionally, ELISA results indicated that C-peptide and insulin release were more significant in 3D than in 2D culture. In conclusion, the PES/fish gelatin scaffold is highly promising for pancreatic tissue engineering because it supports the viability, growth, and differentiation of WJ-MSCs into IPCs.

© 2024 The Author(s). Published by Elsevier BV on behalf of The Japanese Society for Regenerative Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

## 1. Introduction

Diabetes mellitus (DM) is a persistent metabolic condition that disrupts the body's power to regulate blood glucose levels. Elevated blood glucose levels define the situation due to impairments in the secretion of insulin, its action, or both. Insulin, a hormone synthesized by the pancreas, regulates blood glucose levels. In DM, insufficient insulin production or ineffective utilization by the body leads to heightened glucose levels in the bloodstream. This medical

* Corresponding author. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Mazandaran University of Medical Sciences, P.O.Box: 4816859354, Sari, Iran.
** Corresponding author. Amol Faculty of Paramedical Sciences, Mazandaran University of Medical Sciences, P.O.Box: 2097754151, Sari, Iran.
*E-mail addresses:* ehsan.enderami@gmail.com (S.E. Enderami), hadi3977@yahoo.com (H. Hassannia).
Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2024.05.019
2352-3204/© 2024 The Author(s). Published by Elsevier BV on behalf of The Japanese Society for Regenerative Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

issue can give rise to various challenges associated with metabolizing carbohydrates, proteins, and fats, which may result in adverse consequences on the heart, kidneys, eyes, and nerves [1].

DM is considered a prevalent cause of mortality and morbidity on a global scale [2]. The number of adults affected globally is 537 million, which is anticipated to rise to 783 million by 2045. In 2021, the global prevalence was 10.5%, while in Southeast Asia and India; it was 8.8% and 9.6%, respectively. By 2045, rates are expected to increase to 12.5%, 11.5%, and 10.9%, respectively [3].

Diabetic patients can be divided into broadly categorized into two main groups, namely Type 1 and 2 Diabetes Mellitus. Type 1 diabetes mellitus (T1DM) arises due to the body's inadequate insulin production. “Juvenile diabetes” or “insulin-dependent diabetes mellitus” (IDDM) are phrases that were previously used to describe this medical condition [4].

The etiology of T1DM has yet to be entirely understood. Still, the pathogenesis of the disease arises due to an autoimmune invasion of the pancreatic β cells, leading to the cessation of insulin synthesis and elevation of blood glucose levels and ketosis. Therefore, the administration of insulin is vital for effectively managing the condition. Optimizing glucose control is crucial in effective management strategies to minimize temporary and long-term complications [5].

The initial treatment for T1DM is insulin replacement therapy, which entails the administration of exogenous insulin injections multiple times per day. This therapeutic strategy exposes T1DM patients to the risk of experiencing severe hypoglycemic episodes, insulin resistance, and mild obesity. Furthermore, various factors, including the acceptance, availability, storage, and devotion to insulin, lead to the complexity of diabetes management [6,7]. The management of T1DM requires addressing both insulin production and secretion regulation by glucose in patients. Stem cell therapy is a promising treatment for T1DM. Recent advancements suggest it could be a breakthrough in treating the disease [8].

Two main classifications of stem cells are present: adult stem cells (ASCs) and embryonic stem cells (ESCs) [9]. ASCs possess multipotency. Also, these cells exhibit the capacity to experience self-renewal and differentiation into diverse cell lines that constitute the tissues in which they reside. Adult stem cells can be located in different body regions, including the hematopoietic, liver, skin, cornea, and skeletal muscle [10]. Mesenchymal stem cells (MSCs) are a category of ASCs derived from multifarious tissues throughout the human body, such as adipose, Wharton's jelly (WJ), umbilical cord, dental pulp, and bone marrow [11]. WJ-MSCs have demonstrated significant promise in the field of cell therapy and are extensively employed in scientific investigations due to their regenerative potential and low immunogenicity [12].

Scientists have been studying stem cells, which can differentiate into insulin-producing cells (IPCs) [13,14]. It is possible to generate IPCs in vitro using ESCs, induced pluripotent stem cells (iPSCs), or MSCs. However, ethical concerns exist in the use of ESCs, whereas the reprogramming of iPSCs has been linked to the formation of teratomas. Additionally, the potential risks of employing pluripotency-induced viral transgenes in clinical settings raise concerns about their safety. MSCs exhibit characteristics that make them particularly advantageous. These include their immune-privileged nature and remarkable plasticity, which contribute to their suitability as a reliable and secure option for generating IPCs. Numerous prior studies have demonstrated the potential of MSCs to differentiate into IPCs and their clinical efficacy [15].

Islet cells require interactions with the extracellular matrix for survival, proliferation, and insulin secretion [16]. So, the most effective way to culture MSCs is in an environment that closely resembles the human body [17]. However, these interactions are lacking in conventional 2D culture systems [18,19]. To create a supportive 3D microenvironment in tissue engineering, natural or synthetic polymers are used to facilitate cell adhesion, differentiation, proliferation, migration, and responsiveness [14,20,21].

This investigation aims to examine the potential of differentiation of Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs) to IPCs utilizing a scaffold composed of natural fish gelatin polymers with synthetic polyethersulfone (PES) polymers (PES/fish gelatin) in comparison to traditional 2D culture methods.

## 2. Materials and methods

### 2.1. Cell isolation and culture

After conveying permission from a wholesome volunteer mother who had a full-term delivery, the umbilical cord was accumulated instantly at Imam Hospital in Sari, Iran. The cord, which had been kept in a solution of normal saline, including antibiotics, was transported to the Mazandaran University of Medical Sciences laboratory. Under aseptic conditions, all the steps were carried out. The WJ-MSCs were isolated, according to Wang et al. [22], with minor changes. Firstly, the umbilical cord was rinsed with PBS, and then Wharton's jelly tissues were cut into smaller pieces after the removal of blood vessels. These pieces were then positioned on tissue culture plates, including DMEM/F12 (Nita fanavaran, Iran) with 10% FBS (GIBCO, UK), 1% penicillin and streptomycin as well as amphotericin B. The cultured cells were left to expand at 37 °C and 5% CO2. The medium was replaced every further day. Once the cells had grown to proper confluency (average 80% confluency), they underwent sub-culturing at consistent intervals of 3–4 days using trypsin-EDTA. WJ-MSCs were used exclusively between passages 3 to 5.

### 2.2. Characterization of cultured cells

MSCs were extracted from WJ and cultivated in DMEM/F12 containing 10% FBS. Isolated cells were characterized at passage three using flow cytometry. For this purpose, the trypsin-EDTA was used to detach cells, and about $5 \times 10^5$ cells were then suspended in DMEM and separated into multiple tubes. After centrifuging the tubes for 5 min at 1200 RPM, the resulting pellets were resuspended in 1 ml of human serum and maintained at 4 °C for 30 min before undergoing another centrifugation step. The last pellet was incubated with specific antibodies, such as CD105, CD31 and CD34 phycoerythrin (PE) conjugated antibodies, along with CD73, CD90, and CD45 (All antibodies are from BioLegend, San Diego, CA, USA) fluorescent isothiocyanate (FITC)-conjugated mouse anti-human antibodies, in goat's serum 3% (v/v) in human serum albumin/phosphate-buffered saline (PBS) on ice for 1 h. After washing with PBS and another centrifugation step, the pellets were suspended again in PBS and investigated with flowcytometry and FlowJo software.

### 2.3. Scaffold fabrication

The PES solution was prepared following the procedure outlined by Shabani et al. [23]. Subsequently, at a high temperature, 10 ml of water was used to dissolve 1.2 g of fish gelatin. The consequent solutions were merged and packed into a pair of 10 ml plastic syringes positioned at a set length of 18 cm through the collector. The electrospinning process was performed with a flow rate of 1 ml per hour. The needle and the collector were kept at a potential difference of 23 kv to ensure consistency. In order to obtain uniform nanofiber diameter, the humidity during this process was maintained at 60% RH at RT.

### 2.4. Seeding cells on scaffolds

To sterilize the nanofibrous scaffolds, we employed a multi-step process: At the first, ultraviolet radiation (UV) was applied to the scaffolds for a duration of 1 h. Then, the scaffolds were immersed in 70% ethanol for 20 min to ensure the elimination of any remaining microorganisms on their surface. At the end, residual ethanol was removed from the scaffolds by washing them thrice with PBS. The scaffolds were immersed in DMEM/F12 supplemented with 10% FBS and left overnight prior to cell seeding. Once 70–80% confluency was attained by the WJ-MSCs, 1 ml of 0.25% trypsin with 0.1% EDTA was used to detach the cells. This process was carefully timed to ensure the cells were detached at optimal growth. Once detached, the scaffold was seeded with 105 cells and incubated in DMEM/F12 containing 10% FBS. Within 2 h of seeding, the cells had successfully attached to the scaffold surface. Finally, new DMEM/F12 was surcharged to promote cell growth and proliferation during further incubation.

### 2.5. Morphological characterization of scaffold by SEM

Using a scanning electron microscope (SEM), the morphology of cultivated and differentiated cells on implanted scaffolds and the arrangement of nanofibers on unseeded scaffolds were examined. The PBS was used to wash away the glutaraldehyde buffer from the scaffolds. To remove moisture, we gradually immersed the scaffolds in ethanol with increasing concentration (50%, 75%, and 100%) for 10 min each. Subsequently, the scaffolds were left to dry in the air and covered with a layer of gold. Gold coating of scaffolds was done with plasma sputtering apparatus before being analyzed through an electron scanning microscope. The gold covering can be applied directly to non-seeded nanofibrous scaffolds without pre-treatment [14].

### 2.6. MTT assay

Evaluation of the metabolic activity and viability of WJ-MSCs on PES/Fish gelatin nanofibers was conducted by employing the 3-[4, 5-Dimethyl-thiazolyl-2]-2,5-diphenyltetrazolium bromide (MTT) test. The test involved seeding a sterile scaffold with $1 \times 10^3$ cells per $cm^2$ in a cell culture plate and incubating it at 37 °C with 5% CO2. The assay was conducted on the first, third, fifth, and seventh days after seeding to assess the progress of cell growth. On specific cultural days, 20 μl MTT solution (5 mg/ml serum-free DMEM) was added per well and incubated at 37 °C with 5% CO2 for 4 h. After removing the supernatant, 200 μl of dimethyl sulfoxide solution (DMSO) was added per well, which was then incubated at room temperature for 20 min. After the formazan crystal was fully dissolved, the ELISA reader was used to measure the adsorption at 570 nm.

### 2.7. Differentiation into IPCs in 2D and 3D culture

In this study, $1 \times 10^4$ WJ-MSCs from passage four were cultured in cell culture plates and utilized four groups, two control and two experimental groups each containing both 2D and 3D cultures using PES/Fish gelatin. The experiment was conducted in two stages.

For the first 10 days, medium A was used to seed the WJ-MSCs, consisting of DMEM enriched with 2% FBS (Gibco), 10 ng/ml basic fibroblast growth factor (bFGF; Merck), 10 mmol/ml epidermal growth factor (EGF), 10 mmol/ml Nicotinamide (Sigma), 2% B27 (Gibco), 500 μl Insulin Transferrin-Selenium (ITS), 500 μl L-Glutamine (Gibco), 500 μl Non-Essential amino acids (NEAA; Gibco), and 10 μmol/ml Retinoic acid (Sigma).

For the next 8 days, medium B was used to cultivate the WJ-MSCs, which consisted of serum-free DMEM with 2% B27, 10 ng/ml EGF, 10 mmol/ml nicotinamide, 50 ng/ml Activin-A (Sigma), 500 μl Non-Essential amino acids (NEAA), 500 μl L-Glutamine, and 10 μmol/ml Extendin-4 (Sigma). The differentiation process was monitored by changing the medium every two days.

### 2.8. Real time polymerase chain reaction

At first, $5 \times 10^6$ cells were collected for each experimental and control group, and RNA was extracted using the kit (Yekta Tajhiz Azma, Iran). The cDNA was synthesized utilizing a cDNA synthesis kit (Yekta Tajhiz Azma, Iran) following the instructions provided by the manufacturer. Gene expression level quantification was done employing real-time polymerase chain reaction (PCR) with SYBR Green master mix (Arian Gene Gostar, Iran) according to the manufacturer's guidelines. The analysis focused on four specific genes. Table 1 contains a comprehensive list of primers. For the RT-PCR reaction, the instrument utilized was the ABI StepOne-plus. The volume of the sample was 15 μl. GAPDH was employed as the reference gene for data normalization, while the data analysis was carried out using the relative quantification method ($2^{-\Delta\Delta Ct}$).

### 2.9. C-peptide and glucagon immunocytochemical staining

The cells differentiated in 2D and 3D, along with the control, was thoroughly rinsed with PBS. The sample was subjected to a 20 min treatment at 4 °C utilizing a 4% (w/v) paraformaldehyde solution within the wells of the cell culture plate. They were washed thrice with PBS, then treated with 0.4 % triton X-100 for permeabilization at room temperature for 10 min, and washed again thrice with PBS. Following the procedure, the cells were exposed to a blocking buffer that included normal goat serum in PBS. This was done for duration of 45 min at room temperature. Primary antibodies, which included mouse monoclonal anti-glucagon and rabbit monoclonal anti-C-peptide (Abcam), were incubated with the cells overnight after emptying the blocking buffer. Both antibodies were diluted 2/200 in 0.2 % bovine serum albumin (BSA)/PBS at 4 °C. The cells underwent three rounds of washing with 0.1% Tween for duration of 5 min in each round the following day. Following incubation with 1% BSA for 30 min at room temperature, the supernatant was aspirated, and the secondary fluorescein isothiocyanate-labeled antibodies (Abcam) were added for 1 h at room temperature in an unlit place. Finally, the cells were washed with 0.1% tween, and DAPI (0.1 μg/ml) was added for 2 min to facilitate visualization of cell nuclei. The Nikon fluorescent microscope was utilized to visualize the cells.

### 2.10. Measurement of insulin and C-peptide secretion

Following a differentiation span of 18 days, two separate groupings of cells, namely the control and the experiment, were exposed to varying glucose concentrations (5.5, 15, and 25 mM) at a temperature of 37 °C for a period of 1 h. The aim was to evaluate the levels of insulin and C-peptide release. The experiment started with cell incubation in a glucose concentration of 5.5 mM. Next, the collected medium was processed. Subsequently, the cells were rinsed with PBS and introduced to 15 mM glucose for another hour, after which the medium was collected once again. Finally, the cells were introduced to 25 mM glucose. The collected medium was kept at −20 °C until additional usage. ELISA kit (Mercodia, Sweden) was used to measure insulin and C-peptide release levels in the supernatant, and an ELISA plate reader read the absorbance at 450 nm.

**Table 1**
Primer sequences for Real-Time PCR.

| Human genes | Primer sequences | Product size (bp) |
|---|---|---|
| Pdx1 | Forward: 5′-AAGCTCACGCGTGGAAAGG-3′<br>Reverse: 5′-CGGCCGTGAGATGTACTTGTT-3′ | 146 |
| Glucagon (Gcg) | Forward: 5′-GCAACGTTCCCTTCAAGACAC-3′<br>Reverse: 5′-ACTGGTTAATGTGCCCTGTG-3′ | 124 |
| Insulin | Forward:5′-TCTACCTAGTGTGCGGGGAA-3′<br>Reverse: 5′-TCCACCTGCCCCACCTG-3′ | 85 |
| Glut2 | Forward: 5′-TCACTGCTGTCTCTGTATTCC-3′<br>Reverse: 5′-TGCTCACATAACTCATCCAAG-3′ | 147 |
| GAPDH | Forward: 5′-GGTGGTCTCCTCTGACTTCAACA-3′<br>Reverse: 5′-GTTGCTGTAGCAAATTCGTTGT-3′ | 126 |

### 2.11. Statistics

The accuracy was ensured by repeating the experiments three times. The collected data was statistically analyzed using one-way ANOVA and *t*-test and reported as a mean ± SD. Comparison between the 2D and 3D groups was performed using Bonferroni's post hoc test. GraphPad Prism 9 software (GraphPad Software, lnc, La Jolla, California) was utilized to visualize the results. A significance level of 0.05 was used for statistical analysis, indicating that any p-value less than this threshold were considered statistically significant.

## 3. Results

### 3.1. Characterization of isolated WJ-MSCs

WJ-MSCs were characterized by analyzing the cells at passage 3 through flowcytometry analysis. We subjected the isolated cells to a flowcytometric analysis to examine the expression of specific markers. The results verified that WJ-MSCs expressed specific cell surface markers, including CD73 (100%), CD90 (91.4%), CD105 (99.5%), and CD166 (96%). On the other hand, the expression of CD34, CD31 and CD45 were insignificantly expressed in these cells, with only 1.71% and 2.51% expression, respectively as shown in Fig. 1.

### 3.2. Changes in the morphology of differentiated WJ-MSCs

We employed an inverted microscope to observe and track the morphological changes of differentiated cells over multiple phases. After isolating MSCs from WJ, the cells revealed a morphology characterized by fibroblast-like and spindle-shaped appearance during the third passage, as represented in Fig. 2A. In Fig. 2B, the experimental group (2D) underwent an 18-day differentiation process, resulting in WJ-MSCs exhibiting cellular structures and spherical morphologies that were comparable to pancreatic islet structures. The fibrous scaffolds were effectively manufactured, revealing numerous interconnected pores of suitable dimensions, as illustrated in Fig. 2C. SEM was employed to obtain the images.

Moreover, the SEM analysis results revealed a statistically significant distinction between the control and experimental groups (3D) during the last phase of cell differentiation. The cell structure in the experimental group exhibited similarities to cells resembling pancreatic islets (Fig. 2D).

### 3.3. Metabolic and cellular activity evaluation

The MTT experiment assessed the metabolic and cellular activity of WJ-MSCs on a scaffold consisting of PES/Fish gelatin. The investigation lasted seven consecutive days, during which the objective was to assess the feasibility of cultivating cells on a 3D scaffold compared to a 2D, which was utilized as the control group. The study consequences demonstrate that the metabolic activity and expansion of WJ-MSCs on a scaffold were notably more significant than cells cultivated in the 2D group across many time points, precisely at 1, 3, 5, and 7 days. Nevertheless, the study's results did not reveal substantial differences between the 2D and 3D groups, as illustrated in Fig. 3.

### 3.4. Gene expression pattern

The experiments were carried out in triplicate. During cellular differentiation into IPCs, specific markers for the pancreas, such as Pdx-1, insulin, glucagon, and Glut-2, were constantly up-regulated. Subsequently, during a differentiation period of 18 days, the mRNA gene expression levels of these indicators were assessed by quantitative PCR. According to our outcomes in Fig. 4, our study consisted of two experimental groups (2D and 3D) and two control groups (2D and 3D). Both control groups showed no noticeable difference, incorporating them into a solitary control group. In comparison to both the 2D group and the control group, the 3D group meaningfully increased the expression levels of Pdx-1 and Insulin. There were no notable disparities observed among the other genes; however, the expression levels of all genes in the 3D groups were demonstrated to be more elevated compared to both the 2D and control groups.

### 3.5. Immunofluorescence staining for glucagon and C peptide detection

The expression of glucagon and C-peptide in differentiated WJ-MSCs was assessed by utilizing Immunocytochemistry staining. In contrast to the cells that were grown in 2D environment (Fig. 5A), the outcomes of the study revealed that the cells that were cultured on the PES/Fish gelatin scaffold demonstrated more robust protein expression (Fig. 5B). The control groups were analyzed for protein expression in both experimental groups, and no detectable protein expression levels were observed.

### 3.6. Insulin and C-peptide release in response to glucose stimulation

This study intended to investigate the levels of C-peptide and insulin secretion in response to various glucose concentrations (5.5 mM, 15 mM, and 25 mM) using ELISA kit. Across all glucose concentrations, the control groups showed insignificant release of C-peptide and insulin, according to our findings. Minimal secretion of C-peptide and insulin was observed when the glucose volume was at 5.5 mM in both the experimental groups (2D and 3D). Nevertheless, when exposed to heightened glucose concentrations of 15 mM and 25 mM, the 3D and 2D groups exhibited a considerable boost in the release of C-peptide and insulin. Elevated levels

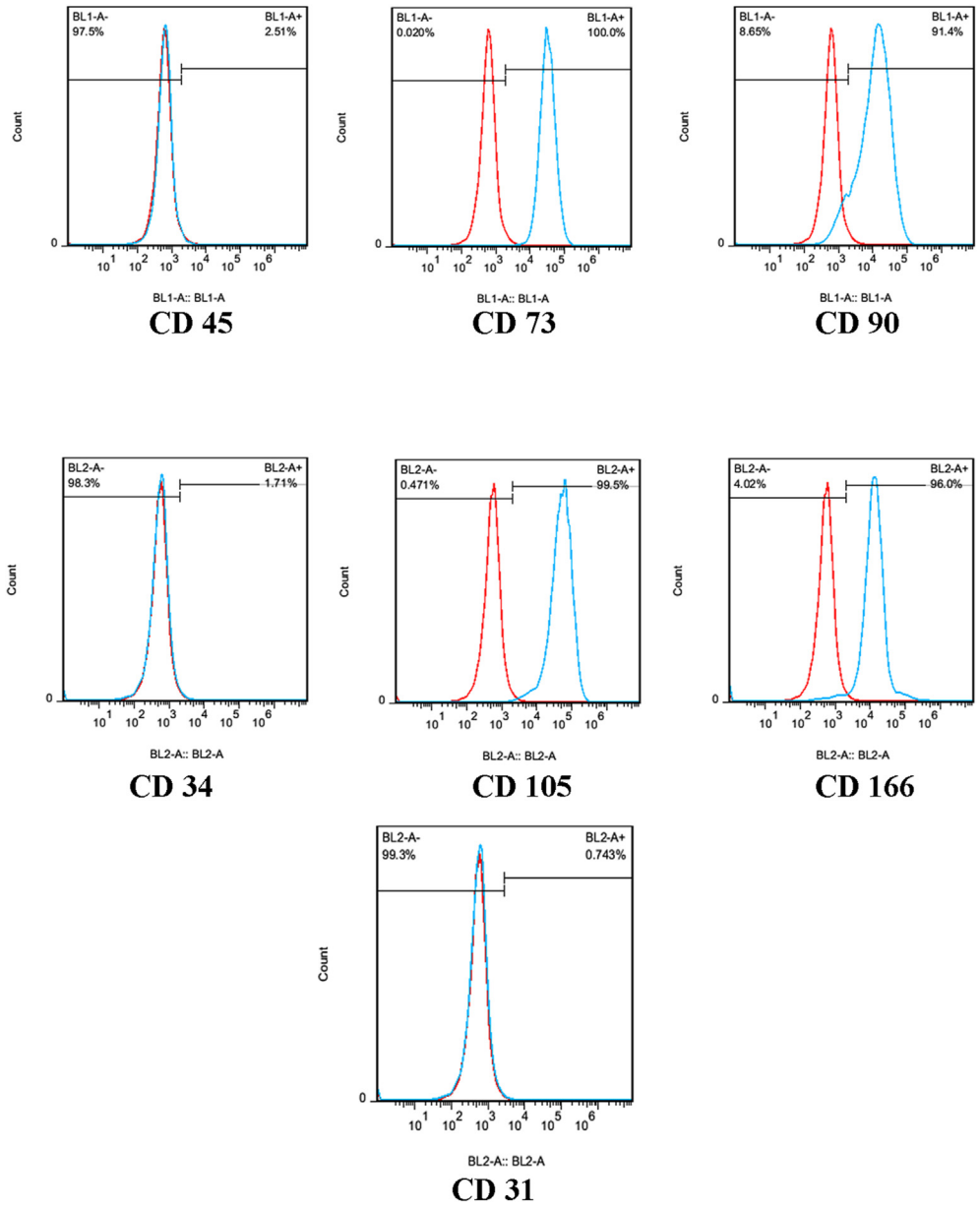


**Fig. 1.** Isolated WJ-MSC had a positive expression for mesenchymal markers CD73, CD90, CD105, and CD166 and a negative expression for hematopoietic markers CD45, CD31 and CD34.

of C-peptide and insulin release were observed in the IPCs that were differentiated on the fabricated scaffold, compared to those that were expanded in a conventional 2D culture medium (Fig. 6A and B).

## 4. Discussion

T1DM is caused by the devastation of β cells in the endocrine pancreas due to autoimmune response. The autoimmune destruction process is initiated by one or more environmental factors in genetically susceptible individuals and takes several months to years to progress. Patients are asymptomatic and maintain normal blood glucose levels during this time, but pertinent auto-antibodies are detected in their tests, indicating an approving result. The onset of symptomatic hyperglycemia and frank diabetes arises just after a notable amount of β cells have been impaired, which accounts for the extended period of latency [24]. Despite various treatment approaches available, clinical research is ongoing to find the most effective cure for T1DM. For many years, insulin injection has been a suggested treatment for diabetic patients despite the possible complications it may result in Refs. [25,26]. It is common for young patients and children with T1DM to experience local intricacies as a result of insulin injection. The use of insulin can lead to consequential adverse effects on the skin, including lipoatrophy and lipohypertrophy (LH). These effects can result in blood glucose levels that fluctuate above or below the target range [27–29]. The growing numeral of T1DM cases and the compliances, as mentioned above, suggest the importance of formulating novel approaches to attain insulin independence and restore glycaemic control.

Islet transplantation is a medical strategy that is supposed to be a definitive solution for treating T1DM. Although this treatment has shown encouraging results, it is currently restricted by the deficit of available islets and the necessity for immunosuppressive

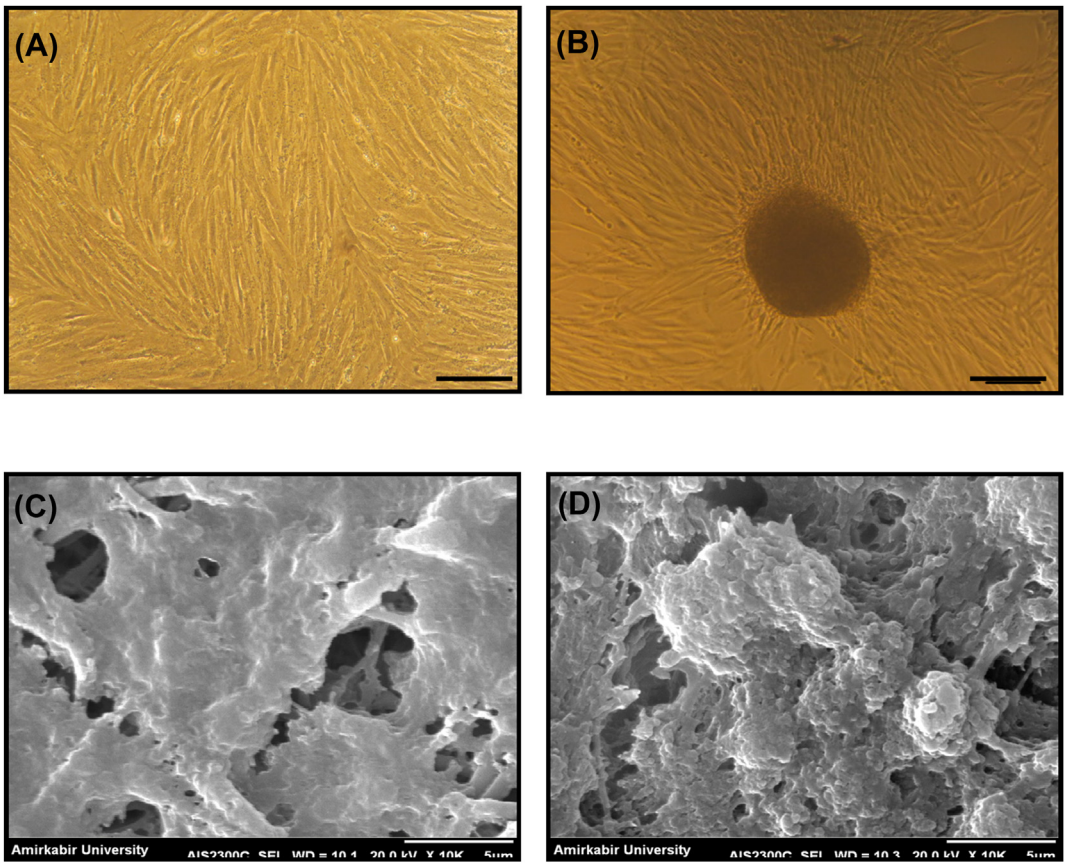


**Fig. 2.** Morphological investigation. (A) The undifferentiated WJ-MSCs exhibited a fibroblast-like spindle shape. (B) The differentiated cells into IPCs in the 2D experiment group. (C) Unseeded PES/Fish gelatin scaffold. (D) Round and spherical clusters derived from differentiated WJ-MSCs into IPCs.

medications to preclude the patient's immune system from striking the transplanted islets. Over the past few years, there has been an increasing request for alternative therapies to treat degenerative diseases such as T1DM. Tissue engineering and stem cell-based therapies have emerged as bright options to address this need. However, these therapies face certain limitations, particularly in creating an in vivo-like condition in cell culture that is necessary for successful treatments. To overcome this challenge, researchers have turned to various biological and artificial-based polymers to mimic the in vivo conditions [30,31]. In this study, we use a natural and synthetic polymer combination scaffold to prepare an in vivo condition for inducing WJ-MSCs into IPCs. Gelatin, a protein widely used in biomedical applications, is derived primarily from porcine and calfskin sources. However, there has been a growing attraction to fish gelatin due to its potential as a biomaterial. Recent studies have shown that nanofibrous fish gelatin demonstrates optimistic in vitro behavior, but its inferior mechanical properties compared to mammalian gelatin may limit its use in structural applications. Additionally, it may undergo rapid degradation and swelling in a biological environment. Therefore, incorporating fish gelatin with other polymers may be required to improve its properties and make it suitable for various biomedical applications [32]. PES stands out as a highly remarkable polymeric material, known for its

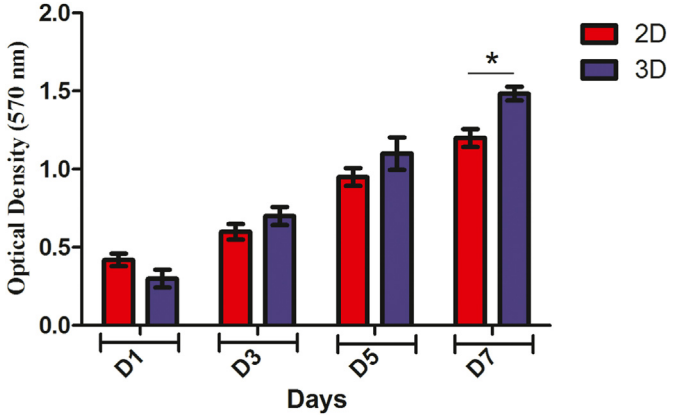


**Fig. 3.** To assess the viability of WJ-MSCs, an MTT test was conducted. The cells were seeded on PES/Fish gelatin and cultured in 2D medium for a period of 1, 3, 5, and 7 days. The experiment was repeated three times to ensure accurate results.

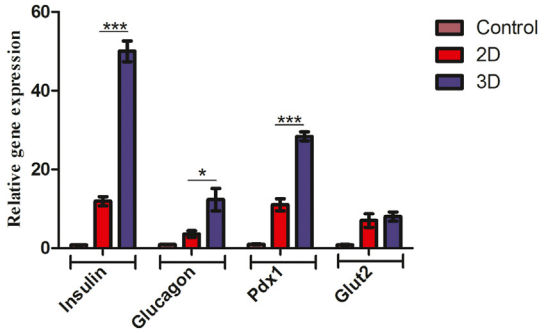


**Fig. 4.** The real-time PCR analysis revealed that the expression of Pdx-1, insulin, glucagon, and Glut-2 genes was significantly elevated in cells cultured under 3D conditions compared to the 2D group and control.*$p < 0.05$, ***$p < 0.001$.

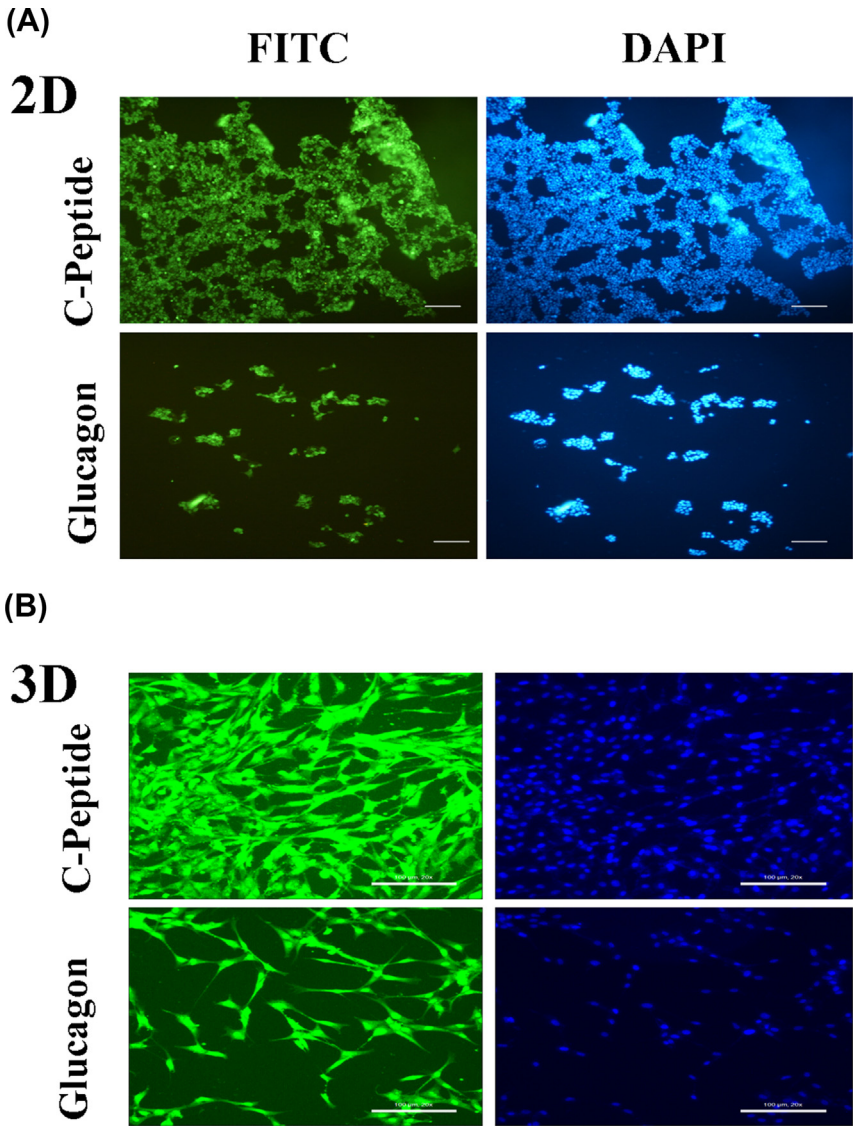


**Fig. 5.** Immunocytochemistry assessment of C-peptide and glucagon protein in WJ-MSCs cultivated on (A) 2D medium and (B) 3D scaffold.

excellent biocompatibility properties. In recent years, its benefits as blood purification membranes have been reported. Also, stem cells have been reported to differentiate effectively on PES due to its exceptional properties such as mechanical, hydrolytic, thermal, and oxidative stability. These unique characteristics of PES make it an ideal polymer for various applications in stem cell research [33].

In our study, the 3D scaffold was assembled from the combination of fish gelatin and PES polymers (to increase the mechanical properties) and produced using electrospinning to enhance the differentiation of WJ-MSCs into IPCs. The 3D matrix was utilized for cell culture instead of the conventional 2D medium. Stem cells expansion and differentiation have been facilitated using hybrid scaffolds in various studies [26,30,34]. Favorable stem cell growth and proliferation conditions have been observed in scaffolds characterized by a specific structure and pore size. These scaffolds provide a microenvironment that has been proven to promote the development of stem cells. Based on our research, it had discovered that the created scaffolds demonstrated remarkable biocompatibility, which effectively supported the growth of IPCs. Various investigations have used different biological scaffolds to produce IPCs using MSCs. These findings confirm the results of previous studies that used diverse scaffolds and sources for MSCs and differentiation to IPCs [35,36].

Subsequently, various analyses were carried out to verify the superior functionality of 3D scaffold-based cell culture compared to 2D medium-based cell culture. It was observed that culturing WJ-MSCs on a PES/fish gelatin scaffold in a 3D environment resulted in a significantly better outcome than culturing them in a 2D environment. Both groups had similar cell morphology in differentiated cells, forming islet-like clusters in the last stage. Meanwhile, the 3D group exhibited significantly higher gene and protein expression compared to the 2D group. The study analyzed the mRNA expression of various pancreatic markers and found that the levels of Pdx-1, Insulin, Glucagon, and Glut-2 were markedly boosted in the differentiated cells that were grown on the 3D scaffold. Insulin and Pdx-1 expression were notably more heightened in the 3D group compared to the 2D group. Still, the expression of Glucagon and Glut-2 did not match the findings of a previous study conducted by Enderami et al. [37].

The analysis of immunocytochemistry images and protein expression showed a considerable increment in the levels of Glucagon and C-peptide proteins in the 3D group than in 2D group.

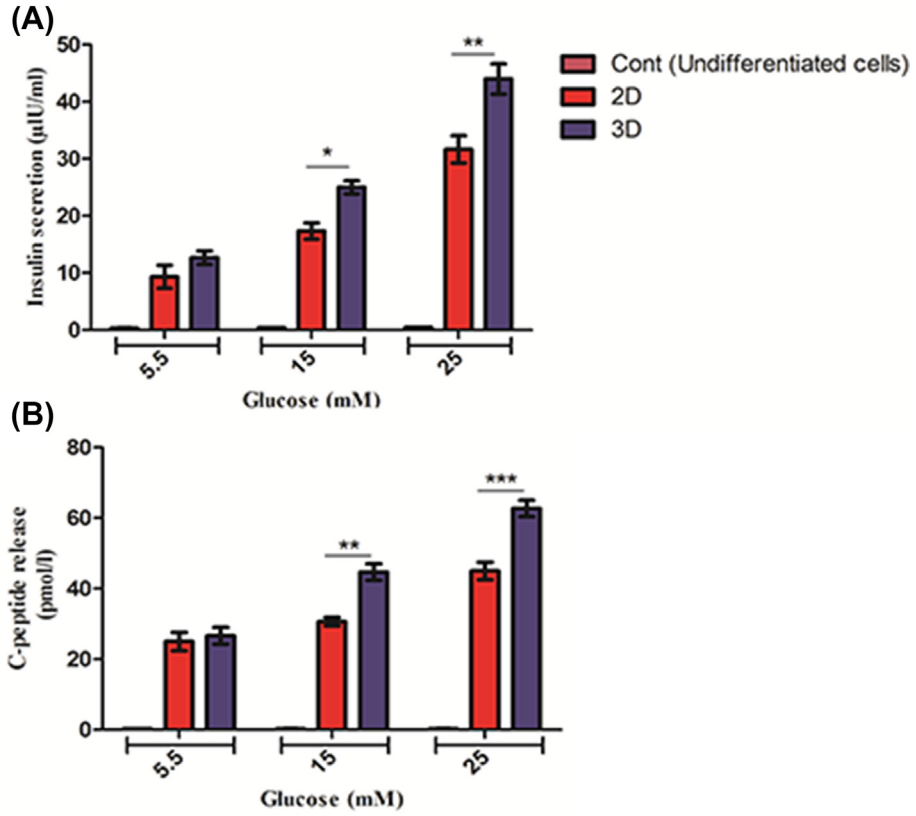


**Fig. 6.** In vitro responses of differentiated WJ-MSCs and secretion of insulin (A) and C-peptide (B) to the various glucose concentrations. Cells were examined in the experimental (cultured on PES/Fish gelatin scaffold and 2D medium) and control groups. *p < 0.05, **p < 0.01, ***p < 0.001.

Despite the experiment groups being exposed to low glucose concentration of 5.5 Mm, both groups exhibited insignificant insulin and C-peptide secretion. Glut-2 is a crucial facilitator of glucose transport within beta cells, and its expression has been shown to be closely correlated to its function in this process. Recent studies have highlighted the relevance of Glut-2 expression and its ability to effectively carry glucose within beta cells [38]. The study found that in high glucose levels (15 and 25 Mm), in the group that was grown in 3D, insulin and C-peptide secretion were observed to be notably greater than in the group grown in 2D. These results have shown that there is a new way to approach cell therapy for treating T1DM. This new approach could be a game-changer in treating T1DM in the near future. This approach holds notable promise for a cure, as it aims to restore the damaged pancreatic islet cells that produce insulin. By using stem cells or other cell types, scientists are exploring strategies to replace these cells and boost insulin production in the body [39]. The importance of these findings is profound, as they offer hope for millions of people living with T1DM worldwide.

## 5. Conclusion

Finally, we have shown and confirmed that using a nanofibrous scaffold can remarkably enhance the differentiation potential of WJ-MSCs to produce high levels of insulin when exposed to glucose. Specifically, we used a novel scaffold made of a PES/fish gelatin. This scaffold has proven to be a highly supportive 3D substrate for WJ-MSCs in vitro and has improved their ability to differentiate into IPCs. However, we acknowledge that further investigation is required to determine the role of these IPCs in vivo and explore their potential for clinical applications in treating DM.

## Ethical approval

The Medical Research Ethics Committee of Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

This work was supported by the Mazandaran University of Medical Sciences (Grant ID: 8806).

## References

[1] Dilworth L, Facey A, Omoruyi F. Diabetes mellitus and its metabolic complications: the role of adipose tissues. Int J Mol Sci 2021;22.
[2] Ong KL, Stafford LK, McLaughlin SA, Boyko EJ, Vollset SE, Smith AE, et al. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. Lancet 2023;402:203–34.
[3] Kumar A, Gangwar R, Ahmad Zargar A, Kumar R, Sharma A. Prevalence of diabetes in India: a review of IDF diabetes atlas. tenth ed. Current diabetes reviews; 2023.
[4] Kumar R, Saha P, Kumar Y, Sahana S, Dubey A, Prakash O. A review on diabetes mellitus: Type 1 & Type 2. World J Pharm Pharm Sci 2020;9:838–50.
[5] Syed FZ. Type 1 diabetes mellitus. Ann Intern Med 2022;175:Itc33–48.
[6] Pathak V, Pathak NM, O'Neill CL, Guduric-Fuchs J, Medina RJ. Therapies for type 1 diabetes: current scenario and future perspectives. Clin Med Insights Endocrinol Diabetes 2019;12:1179551419844521.
[7] Kahanovitz L, Sluss PM, Russell SJ. Type 1 diabetes - a clinical perspective. Point Care 2017;16:37–40.

[8] Chen S, Du K, Zou C. Current progress in stem cell therapy for type 1 diabetes mellitus. Stem Cell Res Ther 2020;11:275.
[9] Mummery C, Wilmut SI, van de Stolpe A, Roelen BAJ. Chapter 3 - what are stem cells? In: Mummery C, Wilmut SI, van de Stolpe A, Roelen BAJ, editors. Stem cells. San Diego: Academic Press; 2011. p. 45–57.
[10] Lu B, Atala A. Chapter 6 - small molecules: controlling cell fate and function. In: lee SJ, Yoo JJ, Atala A, editors. In situ tissue regeneration. Boston: Academic Press; 2016. p. 87–110.
[11] Costela-Ruiz VJ, Melguizo-Rodríguez L, Bellotti C, Illescas-Montes R, Stanco D, Arciola CR, et al. Different sources of mesenchymal stem cells for tissue regeneration: a guide to identifying the most favorable one in orthopedics and dentistry applications. Int J Mol Sci 2022;23.
[12] Hmadcha A, Martin-Montalvo A, Gauthier BR, Soria B, Capilla-Gonzalez V. Therapeutic potential of mesenchymal stem cells for cancer therapy. Front Bioeng Biotechnol 2020;8:43.
[13] Nadri S, Barati G, Mostafavi H, Esmaeilzadeh A, Enderami SE. Differentiation of conjunctiva mesenchymal stem cells into secreting islet beta cells on plasma treated electrospun nanofibrous scaffold. Artif Cell Nanomed Biotechnol 2018;46:178–87.
[14] Mansour RN, Soleimanifar F, Abazari MF, Torabinejad S, Ardeshirylajimi A, Ghoraeian P, et al. Collagen coated electrospun polyethersulfon nanofibers improved insulin producing cells differentiation potential of human induced pluripotent stem cells. Artif Cell Nanomed Biotechnol 2018;46:734–9.
[15] Dang Le Q, Rodprasert W, Kuncorojakti S, Pavasant P, Osathanon T, Sawangmake C. In vitro generation of transplantable insulin-producing cells from canine adipose-derived mesenchymal stem cells. Sci Rep 2022;12:9127.
[16] Stendahl JC, Kaufman DB, Stupp SI. Extracellular matrix in pancreatic islets: relevance to scaffold design and transplantation. Cell Transplant 2009;18:1–12.
[17] Chaicharoenaudomrung N, Kunhorm P, Noisa P. Three-dimensional cell culture systems as an in vitro platform for cancer and stem cell modeling. World J Stem Cell 2019;11:1065–83.
[18] Zhang S. Designer self-assembling peptide nanofiber scaffolds for study of 3-D cell biology and beyond. In: Advances in cancer research. Academic Press; 2008. p. 335–62.
[19] Abazari MF, Soleimanifar F, Nouri Aleagha M, Torabinejad S, Nasiri N, Khamisipour G, et al. PCL/PVA nanofibrous scaffold improve insulin-producing cells generation from human induced pluripotent stem cells. Gene 2018;671:50–7.
[20] Elango J, Zamora-Ledezma C, Maté-Sánchez de Val JE. Natural vs synthetic polymers: how do they communicate with cells for skin regeneration—a review. J Compos Sci 2023;7:385.
[21] Mansour RN, Barati G, Soleimani M, Ghoraeian P, Nouri Aleagha M, Kehtari M, et al. Generation of high-yield insulin producing cells from human-induced pluripotent stem cells on polyethersulfone nanofibrous scaffold. Artif Cell Nanomed Biotechnol 2018;46:733–9.
[22] Shetty P, Cooper K, Viswanathan C. Comparison of proliferative and multilineage differentiation potentials of cord matrix, cord blood, and bone marrow mesenchymal stem cells. Asian J Transfus Sci 2010;4:14–24.
[23] Shabani I, Haddadi-Asl V, Seyedjafari E, Babaeijandaghi F, Soleimani M. Improved infiltration of stem cells on electrospun nanofibers. Biochem Biophys Res Commun 2009;382:129–33.
[24] Paschou SA, Papadopoulou-Marketou N, Chrousos GP. Kanaka-Gantenbein C: on type 1 diabetes mellitus pathogenesis. Endocrine connections 2018;7:R38–46.
[25] Mozaffari MS. New strategies to advance pre/diabetes care: integrative approach by PPPM. EPMA J 2014;5:A14.
[26] Mirtaghi SM, Hassannia H, Mahdavi M, Hosseini-khah Z, Mellati A, Enderami SE. A novel hybrid polymer of PCL/fish gelatin nanofibrous scaffold improves proliferation and differentiation of Wharton's jelly-derived mesenchymal cells into islet-like cells. Artif Organs 2022;46:1491–503.
[27] Demir G, Er E, Atik Altınok Y, Özen S, Darcan Ş, Gökşen D. Local complications of insulin administration sites and effect on diabetes management. J Clin Nurs 2022;31:2530–8.
[28] Sahasrabudhe RA, Limaye TY, Gokhale VS. Insulin injection site adverse effect in a type 1 diabetes patient: an unusual presentation. J Clin Diagn Res 2017;11:Od10–1.
[29] van Munster HE, PM van de Sande C, Voorhoeve PG, van Alfen-van der Velden JA. Dermatological complications of insulin therapy in children with type 1 diabetes. Eur Diabetes Nurs 2014;11:79–84.
[30] Hosseini M, Dadashi-Noshahr K, Islami M, Saburi E, Nikpoor AR, Mellati A, et al. A novel silk/PES hybrid nanofibrous scaffold promotes the in vitro proliferation and differentiation of adipose-derived mesenchymal stem cells into insulin producing cells. Polym Adv Technol 2020;31:1857–64.
[31] Hong I-S. Enhancing stem cell-based therapeutic potential by combining various bioengineering Technologies. Front Cell Dev Biol 2022;10.
[32] Lacy HA, Nealy SL, Stanishevsky A. Degradation and mechanical behavior of fish gelatin/polycaprolactone AC electrospun nanofibrous meshes. Macromol Mater Eng 2023;308:2300106.
[33] Ardeshirylajimi A, Hosseinkhani S, Parivar K, Yaghmaie P, Soleimani M. Nanofiber-based polyethersulfone scaffold and efficient differentiation of human induced pluripotent stem cells into osteoblastic lineage. Mol Biol Rep 2013;40:4287–94.
[34] Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. 2020.
[35] Taherpour A, Hosseini-Khah Z, Zargari M, Ehsan Enderami S. The microenvironment of silk/gelatin nanofibrous scaffold improves proliferation and differentiation of Wharton's jelly-derived mesenchymal cells into islet-like cells. Gene 2022;833:146586.
[36] Azarbarz N, Khorsandi L, Nejaddehbashi F, Neisi N, Nejad DB. Decellularized Wharton's jelly scaffold enhances differentiation of mesenchymal stem cells to insulin-secreting cells. Tissue Cell 2022;79:101938.
[37] Enderami SE, Soleimani M, Mortazavi Y, Nadri S, Salimi A. Generation of insulin-producing cells from human adipose-derived mesenchymal stem cells on PVA scaffold by optimized differentiation protocol. J Cell Physiol 2018;233:4327–37.
[38] Sun B, Chen H, Xue J, Li P, Fu X. The role of GLUT2 in glucose metabolism in multiple organs and tissues. Mol Biol Rep 2023;50:6963–74.
[39] Silva IBB, Kimura CH, Colantoni VP, Sogayar MC. Stem cells differentiation into insulin-producing cells (IPCs): recent advances and current challenges. Stem Cell Res Ther 2022;13:309.