

THE ROLE OF WHARTON'S JELLY MESENCHYMAL STEM CELLS IN DIABETES MELLITUS

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ABSTRACT

Diabetes mellitus is one of the most common chronic disorders in the world, which characterized by progressive destruction of insulin-producing beta cells in the pancreatic islets of Langerhans. Currently, great interest has been generated in human umbilical cord mesenchymal stem cells (huMSCs), which are potential applications for the treatment of diabetes mellitus. Therefore, the aim of this study is that WJMSCs will be induced to differentiate into pancreatic beta-like cells *in vitro*. At this stage, we isolated and induced cultured huMSCs from the UC's WJ under specific culture conditions, and then the morphology of huMSCs were monitored under an inverted phase contrast microscope. We envision that these results will provide a basis for the potential use of Wharton's Jelly MSCs as a source in stem cell-based therapy for diabetes mellitus.

INTRODUCTION

Diabetes mellitus is one of the most common chronic disorders in the world. The prevalence of this disease was globally estimated to be 4.4% in 2030 and the total number of patients will be increased to 366 million [1]. Diabetes mellitus is characterized by progressive destruction of insulin-producing beta cells in the pancreatic islets of Langerhans. There are three major types of diabetes, i.e. type 1 diabetes, type 2 diabetes and gestational diabetes.

Type 2 diabetes mellitus (T2DM) comprises 90% of all causes of diabetes, which is a metabolic stress resulting from over-nutrition- and insufficient activity-induced insulin resistance and beta cell impairment [2, 3]. The routine therapies for T2DM involve insulin sensitizers with exogenous insulin supply, but these drugs temporarily ameliorate hyperglycemia, and ultimately progressive beta cell dysfunction happens [4].

Type 1 diabetes (T1DM) comprises 10% of all causes of diabetes, which is an insulin-dependent autoimmune disorder characterized by the destruction of beta cells with serious short-term and long-term implications. In addition, the impaired quality of life associated with life-long daily injections of insulin and potentially fatal complications due to micro- and macro-angiopathy require novel therapeutic opportunities [5]. On the other hand, islet transplantation is one of the promising medical treatments, which may efficiently prevent diabetic nephropathy, retinopathy and other complications [6, 7]. However, the islet transplantation leads

to immune rejection, therefore the lifelong immunosuppressant drugs must be used [5, 8].

Stem cells, having the ability to differentiate into functional insulin-producing beta cells, could become a promising source of islet cells [9]. Currently, great interest has been generated in human umbilical cord mesenchymal stem cells (huMSCs), which are potential applications in regenerative medicine. Human Wharton's Jelly MSCs (WJMSCs) could be induced to form other cell lines, such as neurons and glial cells, germ like cells, endothelial and insulin-producing beta cells [10].

Furthermore, the MSCs –under specific culture conditions–, hold the ability to differentiate into a variety of lines, such as bone, skin, adipose, cartilage, endothelium, muscle and neuronal cells [10]. MSCs can also be isolated from different sources, such as adipose tissue, muscle, skin, bone marrow, amniotic fluid, umbilical cord (UC) blood, the umbilical vein and UC's WJ [11]. In 2003, Mitchell et al. differentiated WJMSCs in low serum medium containing fibroblast growth factor (FGF) into neurons and glial cells. Compared to others sources, WJMSCs have some advantages, such as 1) WJMSCs have no ethical problems, 2) WJMSCs can be easily isolated and without invasive procedures, 3) WJMSCs are more primary cells and 4) WJMSCs are more cost effective than other source of MSCs [12].

The WJMSCs express stem cell mesenchymal markers (CD44 and CD105), whereas they do not express the hematopoietic markers such as CD34 and CD45. Moreover, WJMSCs express major histocompatibility complex (MHC) class I molecules (e.g. human leukocyte antigen (HLA)-A, -B, -C), but they do not express MHC class II (e.g. HLA-DR) molecules on their surface [10]. Most importantly, the graft-vs-host disease (GVHD) markers, such as CD80, CD86 and CD40, are not detectable or weakly expressed in WJMSCs [13].

WJMSCs-derived from umbilical cord have attracted much interest for today. Wharton's jelly is a mature mucous tissue within the UC, connecting the umbilical vessels to the amniotic epithelium as shown in Figure 1. At birth, UC weighs about 40 g and measures approx. 30-65 cm in length and 1.5 cm in width [14].

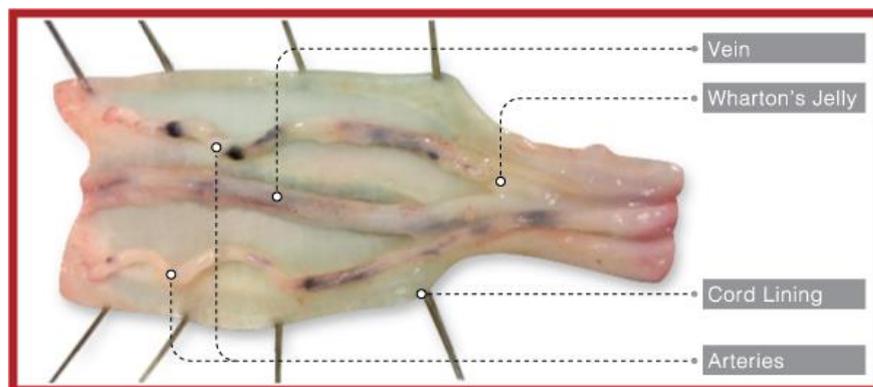


Figure 1. The anatomy of the human umbilical cord [15]

Thus, the aim of this study is that WJMSCs will be induced to differentiate into pancreatic beta-like cells *in vitro*. Differentiation will be confirmed by the expression of pancreatic beta cell markers and insulin secretion by induced cells.

We envision that these results will provide a basis for the potential use of Wharton's Jelly MSCs as a source in stem cell-based therapy for diabetes mellitus.

At this stage, we isolated and induced cultured huMSCs from the UC's WJ under specific culture conditions, and then the morphology of huMSCs were monitored under an inverted phase contrast microscope.

MATERIALS AND METHODS

Collection of human umbilical cords

The required approvals for this study were obtained from the Regional Committee of Science and Research Ethics (Borsos-Abaúj-Zemplén, Heves and Nógrád County, Szentpéteri kapu 72-76, Miskolc H-3526). Umbilical cords (UCs) were collected from healthy full-term infants delivered by caesarean section in Borsod-Abaúj-Zemplén County Hospital affiliated to University of Miskolc, Hungary. All UCs were negative for hepatitis B virus (HBV), hepatitis C (HCV), human immunodeficiency virus (HIV) Epstein-Barr virus (EBV), cytomegalovirus (CMV) and syphilis in umbilical cord serum.

Preparation of human umbilical cords

The umbilical cords were stored at 4°C in sterile 0.9% saline solution for transportation to the laboratory and were processed within 24 hours of collection. After disinfection in 75% (v/v) ethanol (Sigma-Aldrich, Hungary) for 30 second, the cords were rinsed twice in sterile phosphate buffered saline (1xPBS, AppliChem, Germany), supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin (Sigma-Aldrich) and placed in a sterile petri dish. After that the cords were cut into pieces of approximately 2-3 cm. Following removal of umbilical cord arteries and veins by blunt dissection, the remaining umbilical cord tissues, including Wharton's Jelly components, were diced into cubes of about 0.5 cm³.

The cubed explants were transferred to six, twelve-well plates and T25 flask (Becton Dickinson, Hungary) containing medium composed of low-glucose Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) supplemented with 10% (v/v) fetal bovine serum (FBS, Sigma-Aldrich), and 100 units/mL penicillin/streptomycin, and then the culture dishes were incubated at 37°C in a humidified tissue culture incubator containing 5% (v/v) CO₂ and 95% air. The medium was changed every four to seven days and the cells were passed when they reaching 70-80% confluence. After 28 days in culture, the cord cubes were removed from the culture flask and the adherent cells from individual explanted cord tissue were recovered by treatment with 0.25% trypsin (Sigma-Aldrich) at 37°C in culture incubator for five to nine minutes and then passaged into a new flask for further expansion. The UC tissues were processed according to the schematic representation presented in Figure 2.

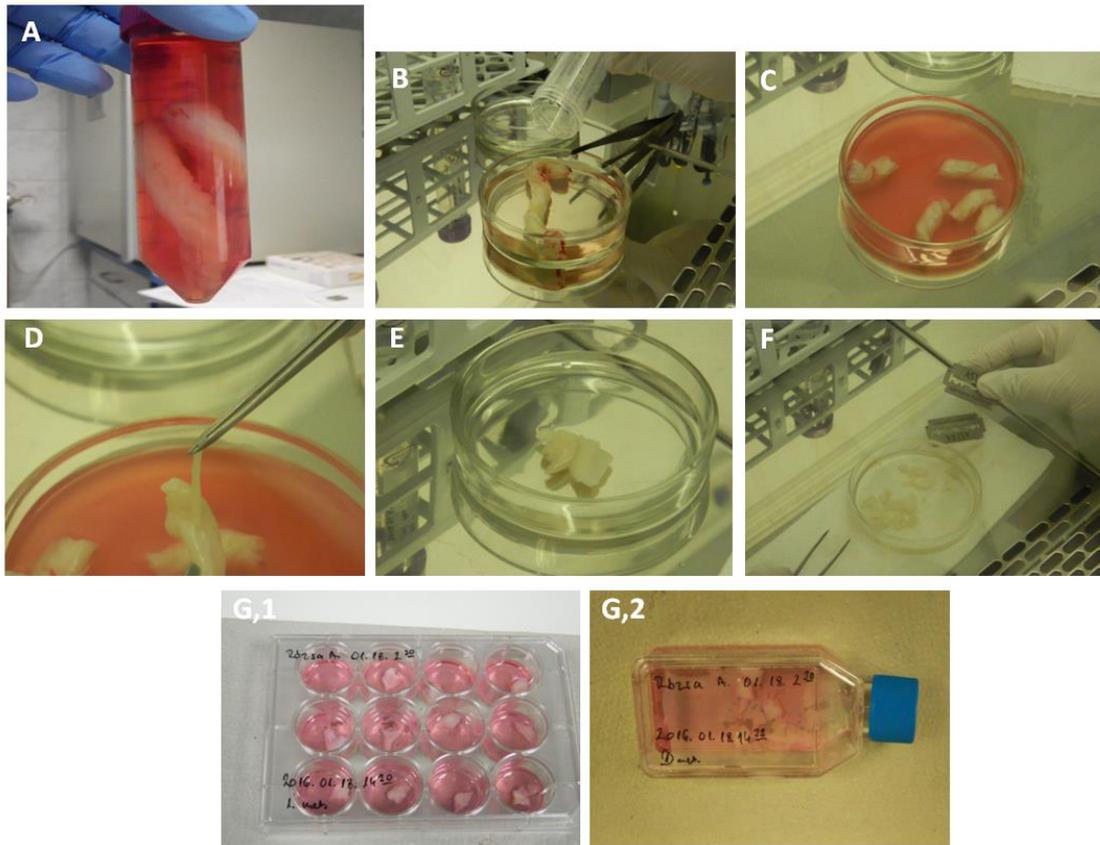


Figure 2. Isolation and culture of Wharton's Jelly mesenchymal stem cells from umbilical cord

RESULTS AND DISCUSSION

Morphology of human WJMSCs cultured in vitro

Over the first 7-10 days of culture, freshly isolated cells from umbilical cord displayed fibroblast or spindle-like appearance as shown in Figure 3, Panel B. During week 2, they typically appeared as slender cells with few lamellipodia and narrow cytoplasm as illustrated in Figure 3, Panel C. When cells reached 70-80% confluency after 26-28 days of primary culture, the cells principally formed bipolar spindle-like cells with parallel or whirlpool-like arrangements as illustrated in Figure 3, Panel D.

Please note that the primary cells did not efficiently grow in six-well or twelve-well plates than in T25 flask (data not shown).

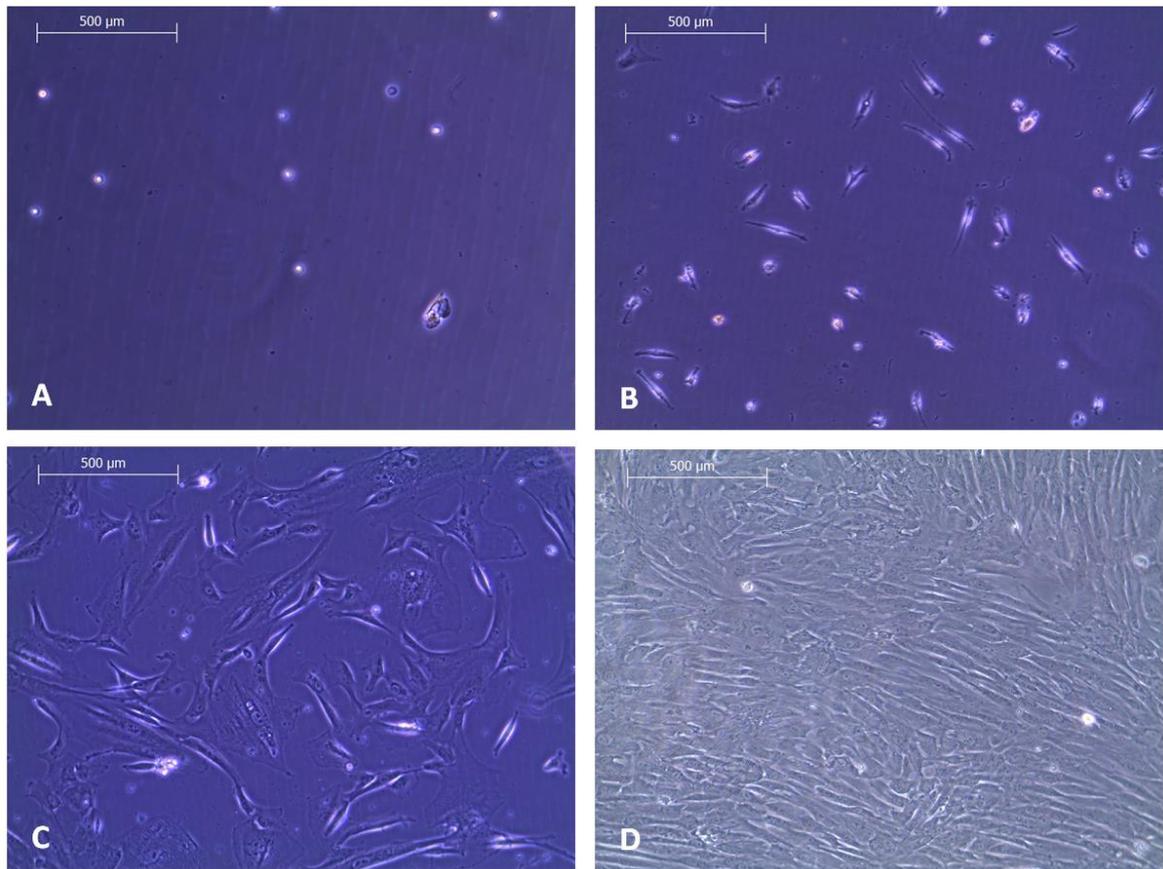


Figure 3. Morphology of human umbilical cord-derived mesenchymal stem cells at 2 days (A), 10 days (B), 17 days (C) and 28 days (D) under inverted microscope (Magnification= 10x)

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REFERENCES

- [1] LIAO, Y.H., VERCHERE, C.B., WARNOCK, G.L.: **Adult stem or progenitor cells in treatment for type 1 diabetes: current progress.** *Can. J. Surg.*, 2007, 50 (2), 137-142.
- [2] KOLB, H., MANDRUP-POULSEN, T.: **An immune origin of type 2 diabetes?.** *Diabetologia*, 2005, 48 (6), 1038-1050.
- [3] TYSSELING-MATTIACE, V.M., SAHNI, V., NIECE, K.L., BIRCH, D., CZEISLER, C., FEHLINGS, M.G., STUPP, S.I., KESSLER, J.A.: **Self-**

- assembling nanofibers inhibit glial scar formation and promote axon elongation after spinal cord injury.** *J. Neurosci.*, 2008, 28 (14), 3814-3823.
- [4] **HATZIAVRAMIDIS, D.T., KARATZAS, T.M., CHROUSOS, G.P. Pancreatic islet cell transplantation: an update.** *Ann. Biomed. Eng.*, 2013, 41 (3), 469-476.
- [5] **HUSSAIN, M.A., THEISE, N.D. Stem-cell therapy for diabetes mellitus.** *Lancet*, 2004, 364 (9429), 203-205.
- [6] **NOGUCHI, H. Pancreatic islet transplantation.** *World J. Gastrointest. Surg.*, 2009, 1 (1), 16-20.
- [7] **MATSUMOTO, S. Islet cell transplantation for type 1 diabetes.** *J. Diabetes*, 2010, 2 (1), 16-22.
- [8] **SHAPIRO, A.M., LAKEY, J.R., RYAN, E.A., KORBUTT, G.S., TOTH, E., WARNOCK, G.L., et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunostippressive regimen.** *N. Engl. J. Med.*, 2000, 343 (4), 230-238.
- [9] **WANG, H.W., LIN, L.M., HE, H.Y., YOU, F., LI, W.Z., HUANG, T.H., MA, G.X., MA, L. Human umbilical cord mesenchymal stem cells derived from Wharton's jelly differentiate into insulin-producing cells in vitro.** *Chin. Med. J.*, 2011, 124 (10), 1534-1539.
- [10] **ROSTAMZADEH, A., ANJOMSHOA, M., KURD, S., CHAI, J.K., JAHANGIRI, F., NILFOROUSHZADEH, M.A., ZARE, S. The role of Wharton's jelly mesenchymal stem cells in skin reconstruction.** *J. Skin Stem Cell*, 2015, 2 (2), e30347.
- [11] **CHATZISTAMATIOU, T.K., PAPASSAVAS, A.C., MICHALOPOULOS, E., GAMALOUTSOS, C., MALLIS, P., GONTIKA, I., PANAGOULI, E., KOUSSOULAKOS, S.L., STAVROPOULOS-GIOKAS, C. Optimizing isolation culture and freezing methods to preserve Wharton's jelly's mesenchymal stem cell (MSC) properties: an MSC banking protocol validation for the Hellenic Cord Blood Bank.** *Transfusion*, 2014, 54 (12), 3108-3120.
- [12] **ANGELUCCI, S., MARCHISIO, M., DI GIUSEPPE, F., PIERDOMENICO, L., SULPIZIO, M., ELEUTERIO, E., ET AL. Research Proteome analysis of human Wharton's jelly cells during in vitro expansion.** *Promote Sci.*, 2010, 8 (1), 18.
- [13] **MA, L., FENG, X.Y., CUI, B.L., LAW, F., JIANG, X.W., YANG, L.Y., et al. Human umbilical cord Wharton's Jelly-derived mesenchymal stem cells differentiation into nerve-like cells.** *Chin. Med. J.*, 2005, 118 (23), 1987-1993.
- [14] **CONCONI, M.T., DI LIDDO, R., TOMMASINI, M., CALORE, C., PARNIGOTTO, PP. Phenotype and differentiation potential of stromal populations obtained from various zones of human umbilical cord: An overview.** *Open Tissue Eng. Regen. Med. J.*, 2011, 4 (1), 6-20.
- [15] <http://stemlife.com/stemlife/cord-tissue/what-is-cord-lining>