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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(2): 18-24

www.thepharmajournal.com
Received: 02-12-2022
Accepted: 09-01-2023

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Wharton jelly mesenchymal stem cells: A potential candidate for neuronal trans-differentiation

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Abstract
The nerve injury, whether it results in tissue loss or not, involves cutting off the nerve, making it impossible for the two portions to interact and, as a result, impairing functionality. Mesenchymal stem cells are one of the best options for cell therapy in many diseases due to their distinct functional properties, such as their potent differentiation capacity, immunomodulatory capacity and growth support. The secretome generated by WJ-MSCs is a key factor in their transdifferentiation into neural stem cells. These cells provide a favorable environment for damaged nerves to regenerate by secreting chemicals that nourish, protect, and activate both neuronal and non-neuronal cells involved in the process. The combination of these properties can accelerate nerve regeneration processes and improve functional recovery.

Keywords: NSCs, secretome, trans-differentiation, WJ MSCs, umbilical cord tissue

Introduction
Stem cells are undeveloped cells that have the ability to multiply, renew themselves, change into differentiated cells, and regenerate tissues. Embryonic and nonembryonic stem cells are the two primary categories of stem cells. Pluripotent stem cells (ESCs) are able to differentiate into all known types of cells. The extensive deployment of embryonic stem cells for clinical purposes has been constrained by their tumorigenicity and ethical issues (Blum and Benvenisty, 2008) [4]. Non-embryonic stem cells (non-ESCs) are multipotent because their potential to differentiate into cell types is more limited. Non-embryonic stem cells (non-ESCs) are pluripotent as they have more limited potential to differentiate into cell types. Non-embryonic stem cells are also called adult stem cells. This is because the cells are derived from adults, usually from adult tissue sources such as bone marrow, fat, muscle, liver, skin, brain, dental pulp, retina and orbicularis (Pittenger *et al*., 1999; Uchida *et al*., 2000; Gronthos *et al*., 2000; Minguell *et al*., 2001 and Zuk *et al*., 2001) [46, 58, 20, 37, 68]. The term "non-embryonic stem cells" also applies to less mature tissue sources, including cord blood, cord tissue, placenta, and foetal body tissue such as pancreas and liver (Tuch, 2006) [56].
Hematologists first paid much attention to bone marrow mesenchymal stem cells (MSCs) (Friedenstein *et al*., 1976) [16]. However, it soon became apparent that MSCs were superior to other stem cell types due to their suitability for uses such as in regenerative medicine and immunomodulation, ease in isolation, expansion, long-term cryopreservation, extraordinary plasticity, simple genetic manipulation and few ethical and legal concerns. Additionally, MSCs generated from other sources might have benefits over bone marrow and adipose derived cells in theory (Nekanti *et al*., 2010) [42]. MSCs derived from the Wharton's jelly of the umbilical cord tissue (WJ MSCs) in particular display distinctive characteristics like a primitive nature, ease of expansion *in vitro*, capacity for multi-lineage differentiation, immunomodulatory-antioxidative action, the release of tropic factors and homing to damage sites (Fong *et al*., 2007; Garzon *et al*., 2012 and Lian *et al*., 2016) [14, 19, 32].
Moreover, in theory, MSCs from other sources may be superior to bone marrow-and adipose-derived cells (Nekanti *et al*., 2010) [42]. In particular, MSCs obtained from Wharton's jelly of umbilical cord tissue (WJ-MSCs) are characterized by primitive properties, facile expansion *in vitro*, multi-lineage differentiation capacity, immunomodulatory-antioxidant activity, release of tropics, etc. homing to factors and defects (Fong *et al*., 2007; Garzon *et al*., 2012 and Lian *et al*., 2016) [14, 19, 32].
The gelatinous connective tissue of the umbilical cord is known as Wharton's jelly and is composed of myofibroblasts, collagen fibers, and proteoglycan-like stromal cells (Kobayashi *et al*., 1998) [25]. They are readily available, can be grown and maintained *in vitro* culture, are

pluripotent, and can produce a variety of mesenchymal cell types. WJ-MSCs can differentiate into osteocytes, adipocytes, chondrocytes (Wang *et al*., 2004; Saben *et al*., 2014 and Ranjbaran *et al*., 2018) [62, 49, 48] and non-mesenchymal lineages such as neurons. can even transdifferentiate into Cardiomyocytes, hepatocytes, endothelial cells, especially neuron-like cells have received attention (Mitchell *et al*., 2003; Wang *et al*., 2004; Fu *et al*., 2006; Fong *et al*., 2007 and Lian *et al*., 2016) [38, 62, 17, 14, 32]. Pluripotent WJ-MSCs are derived from goat (Moshrefi *et al*., 2010) [39], bovine (Cardoso *et al*., 2012) [7] and humans Venugopal *et al*., 2011 [61] and Puranik *et al*., 2012 [47], buffaloes (Singh *et al*., 2013 and Sreekumar *et al*., 2014) [52, 53], dogs (Uranio *et al*., 2014) [59] and sheep (Eswari *et al*., 2016) [13]. In several diseases, including neurodegenerative diseases, MSC-based therapies can be used to control immune activation and provide trophic signals for tissue repair. (Uccelli *et al*., 2006 and van Velthoven *et al*., 2010, Satheesan *et al*., 2020) [57, 60, 50].

Isolation, culture and expansion of WJ MSCs *in vitro*

To isolate cells from WJs, there are many protocols, depending on the possibility of removing the umbilical artery and vein, and the method of enzymatic or mechanical dissection. I have. The cell matrix is enzymatically disrupted using collagenase, trypsin, or hyaluronidase, and the dissociated cells are then purified and cultured (Wang *et al*., 2004) [62]. Using a mechanical device, the tissue is cut into very small pieces or a few centimeters in length, and the pieces are simply transferred to culture plates until these cells migrate to the plastic bottom of the plate (Mitchell *et al*., 2003 and La Rocca *et al*., 2009) [38, 29]. Cleaved or fragmented cells require a medium with low or high glucose levels. Dulbecco's Modified Eagle Medium uses platelet-rich plasma or other additives such as bovine foetal bovine serum. (Mitchell *et al*., 2003; Eswari *et al*., 2016, Ranjbaran *et al*., 2018 and Satheesan *et al*., 2020) [38, 13, 48, 50]. According to a study by Moshrefi *et al*. 2010 [39] Umbilical cord-WJ-MSCs have faster doubling rate than foetal fibroblasts. Mesenchymal stromal cells derived from foetal blood often have short doubling times (Campagnoli *et al*., 2001) [6]. It also highlighted the fact that adult bone marrow mesenchymal cells have a longer doubling time than WJ cells and cord blood mesenchymal cells (Baksh *et al*., 2007 and Karahuseyinoglu *et al*., 2007) [2, 23]. This property has been hypothesized to indicate a relatively immature nature of WJ-MSCs compared to adult stromal cells (Troyer and Weiss, 2008) [55]. Venugopal *et al*. (2011) [61] replaced porcine trypsin for cell dissociation with TrypLE Express, which is free of animal and human components. Additionally, TrypLE does not require a neutralization step using serum-containing media. According to Garzon *et al*. (2012) [19], WJ MSC cell viability first decreased from the 1st to 3rd cell passage, increased to the 6th passage, and decreased again from the 6th to the 10th cell passage. The highest level of cell viability was observed at the 5th and 6th cell passages. According to Liang *et al*. (2016) [32], early and intermediate stages of WJ-MSCs are relatively stable, and the effects of serial passaging on lineage-specific differentiation warrant careful consideration. Although the cardiac differentiation capacity of WJ-MSCs decreased, their propensity for neural differentiation increased significantly at metaphase. By taking advantage of its strong adhesive properties, adult MSCs can be easily isolated and propagated extensively. The cells are heterogeneous, with at least two subpopulations present in culture: small, spindle-shaped, rapidly self-renewing MSCs and larger, slower-regenerating MSCs (Colter *et al*., 2001) [11]. Hanabdari *et al*. (2016) showed that BM-MSCs with spindle-shaped morphology and clonal features adhered rapidly up to passage 3-4, but this adhesion slowed with increasing passage number. A uniform fibroblast-like shape of the cells could be seen in homogeneous populations. The same morphological features have been observed in WJ-MSCs in several studies (Eswari *et al*., 2016, Ranjbaran *et al*., 2018 and Satheesan *et al*., 2020) [13, 48, 50]. These studies revealed a variety of morphologies, including spindle-shaped, rectangular, cuboidal, and fibroblast-like cells, as well as confluent cells arranged in parallel arrays.

Clonogenicity of WJ-MSCs

Cell colonies formed from single cells are a formal example of the ability of stem cell populations to self-renew (La Rocca *et al*., 2009) [29]. According to Lopez *et al*. (2011), coating density and oxygen content are two important factors affecting the frequency and rate of CFU-F from WJ MSCs. UC-WJ-MSCs of goat, buffalo and sheep were each found to exhibit significant alkaline phosphatase activity and form colonies (Moshrefi *et al*., 2010; Sreekumar *et al*., 2014, Eswari *et al*. 2016 [13] and Satheesan *et al*., 2020 [50]) [39, 53, 13, 50].

Wharton's jelly MSCs induced NSCs *in vitro*

According to Mitchell *et al*. (2003) [38], Fu *et al*. (2004; 2006) [18] and Satheesan *et al*., (2020) [50] the WJ MSCs showed retraction of the cell body and elaboration of processes on day 3 and by day 5, granular structures resembling Nissl material were also seen in many of the WJ MSCs. Human UC WJ MSCs were induced to become neural stem cells using commercial Mesenchymal Stem Cell Neurogenic Differentiation Medium (C-28015, Promocell, Germany) as per the manufacturer's protocol (Murakami *et al*., 2017) [40] as well as NSCs induction medium (DMEM/F12 with Glutamax supplemented with 2% of FBS, 0.2% of Penicillin/Streptomycin, 1% of N2 supplement (100×) and EGF 10 ng/mL was used by Kruminis-Kaszkiel *et al*., 2020 [26] In a recent study of Satheesan *et al*., 2020 [50] reported that WJ-MSCs at P3-P5 were grown to near confluence and treated with Neuronal Conditioned Medium (NCM) collected ovine foetal brain suspension culture encouraged and facilitated their transformation into neurons quickly and with good yield.

Morphology of WJ MSCs induced NSCs

WJ-MSCs showed cell body contraction and process elaboration by day 3, and a granular structure resembling Nissl bodies was also observed in many WJ-MSCs by day 5. UC WJ MSCs were cultured using commercially available mesenchymal stem cell neural gene differentiation medium (C-28015, Promocell, Germany) following the manufacturer's protocol (Murakami *et al*., 2017) [40] and NSCs induction medium (Glutamax supplemented), cultured in DMEM/F12 with 2% FBS, 0.2% penicillin/streptomycin, 1% N2 supplement (100x), and EGF 10 ng/mL were used by Kruminis-Kaszkiel *et al*., 2020 [26]. In a recent study by Satheesan *et al*., 2020 [50], P3-P5 WJ-MSCs were grown to near confluence, treated with neuronal-conditioned medium (NCM) collected from foetal sheep brain suspension cultures,

and transferred to neurons and facilitated the transformation of rounded cell bodies with multiple neurite-like extensions, resembling the morphology of neural stem cells. The morphology of GFAP-positive cells was stellate and lacked the long processes of neuronal marker-positive cells (Mitchell *et al*., 2003; Satheesan *et al*., 2020) [38, 50]. Basic fibroblast growth factor, platelet-derived growth factor, and forskolin were used to treat WJ-MSCs, which have a spindle-like shape similar to Schwann cells (Peng *et al*, 2011) [44]. Within hours after neural induction, MSCs exhibited marked morphological changes, as reported by Li *et al*. (2012). Most cells assembled and extended long dendritic cell processes within hours. The flattened morphology of MSCs in the control group was unchanged. After 6 days of continuous induction, cells reached confluence and morphology was essentially distinguishable from control cells. WJ-MSCs underwent profound changes in morphology with the development of multiple dendrites and a single axon-like process extending from the cell body, as well as with granular structures reminiscent of Nissl substances (Guan *et al*., 2014 and Satheesan *et al*., 2020) [21, 50] as in figure 1.

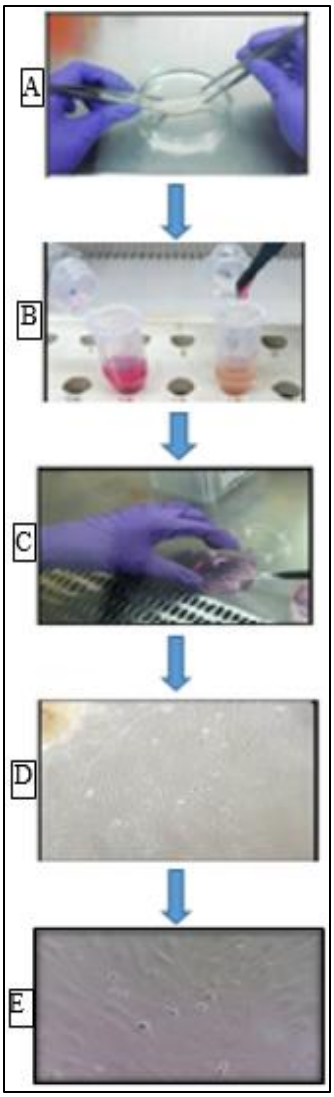

Fig 1: Diagram showing isolation and neuronal induction of umbilical cord tissue-wharton jelly mesenchymal stem cells A. Processing of umbilical cord B. Collagenase Trypsinisation of extracted Wharton jelly C. Plating/culturing D. Migration of WJ-MSCs (200x) E. Induced Neuronal stem cell (200x) using neuronal conditioned medium

Expression of neuronal markers by UCT-WJMSCs and induced NSCs

Human UC-MSCs produced Nestin, a biomarker of neural progenitors, without being exposed to differentiation cues, according to Mitchell *et al*. (2003) [38]. Moreover, GFAP-positive cells were identified in WJ-induced NSCs, their expression was observed in untreated WJ cells, but expressed at higher levels after induction. Neuronal marker genes (β III tubulin, nestin and GFAP) showed increased expression upon induction of neuronal differentiation in WJ-MSCs (Satheesan *et al*., 2020) [50]. Frausin *et al*. (2015) [15] reported that WJ-MSCs can differentiate into neuron-like cells *in vitro*. The cells exhibit both neuronal morphological and biochemical properties and express typical neuronal proteins such as nestin and β-tubulin. Expression of Nestin and NeuroD1 was high in developed neural stem cells and low in undifferentiated MSCs of human UC, suggesting that differentiated MSCs activated molecular mechanisms of neuronal function (Chen *et al*., 2016) [10]. At the early (P7) and intermediate (P14) stages, Lian *et al*. (2016) [32] showed that WJ MSCs were positive for either nestin or βIII tubulin. Neural induction significantly increased the expression of these two markers in both groups. This was corroborated by immunofluorescence and quantitative PCR. Expression of nestin is important for differentiation of MSCs into neurons, and serum in the medium reduces nestin expression, an RT-PCR to determine whether the NSE and GFAP genes were expressed (Zhu *et al*., 2017) [67]. RT-PCR analysis showed that neurogenic markers such as III-tubulin and Notch were expressed in cells both before and after induction of neurogenic differentiation, although the levels of these markers were appeared to be higher in differentiated cells (Murakami *et al*., 2017) [40]. According to Satheesan *et al*. (2020) [50] WJMSCs underwent a mesenchymal-to-nervous fate change in the presence of a neuronal-conditioned medium. This was confirmed by immunocytochemistry as well as by RT-PCR, which showed that the expression of nestin, III-tubulin, and neural lineage GFAP markers was higher in induced NSCs than in uninduced WJ-MSCs.

WJ MSCs 'Secretome'

Cytokines, chemokines and growth factors make up the stem cell secretome and have recently attracted attention for their multiple effects on healing, regeneration or repair of injured tissues (Drago *et al*., 2013) [12]. Chemicals secreted by mesenchymal stem cells (MSCs) in response to injury, including growth factors, cytokines, antioxidants and extracellular matrix proteins, are part of the MSC secretome and can directly or indirectly repair (Chan and Lam, 2013) [9]. According to Dasari *et al*. (2007) cord blood MSCs may also enhance the expression of local neurotransmitters such as BDNF and neurotrophin-3 (NTF3) and improve disease recovery. In addition to finding that WJ-MSCs express more genes, particularly secreted proteins involved in angiogenesis and neurogenesis, Hsieh *et al*. 2013 [22] showed that WJ-MSCs expressed more stemness and growth-related factors than BM-MSCs.

Human umbilical cord perivascular cell (HUCPVC) populations were studied by Pires *et al*. (2014) [45] to determine whether their secretomes or human BM-MSCs and WJs surrounding umbilical veins and arteries, exhibited neuronal phenotypes and neuron-like properties without the use of other exogenous substances to support growth. The

local distribution of UC CM may promote the proliferation of these cells in wounded tissue by encouraging the recruitment of cells from the surrounding tissues. Consequently, using UC CM for regenerative medicine may be appropriate (Shen *et al*., 2015) [51].

Both the adult and developing nervous system express a variety of growth factors from the neurotrophin family, including brain-derived neurotrophic factor. BDNF has been shown to increase survival of several neurons *in vitro*, including retinal ganglion cells and hippocampal neurons (Lindholm *et al*., 1996) [35]. BDNF regulates the number of neurons in the developing geniculate ganglion by preventing cell death instead of promoting cell proliferation (Patel and Krimm, 2010) [43]. Wilkins *et al*. (2009) [65] demonstrated that significant amounts of BDNF are secreted by MSCs. Serum-free chemically defined medium conditioned with MSCs (5 × 105 cells, medium conditioned for 24 hours in 1 ml of medium) contained 190.5 pg/ml of BDNF. NGF and BDNF present in BM-MSC conditioned media are known to reduce reactive astrogliosis. Several different types of chemicals are secreted into the medium by bone marrow MSCs, including trophic factors that support neuronal survival and neurite outgrowth (Nakano *et al*., 2010) [41].

Shen *et al*. (2015) [51] used liquid chip and ELISA techniques to analyse umbilical cord MSCs-derived conditioned media. These cells secreted various cytokines and chemokines, including BDNF at high concentrations (13,900-2,156 pg/mL), to promote neuronal survival, proliferation, and differentiation. Bierlein *et al*. (2017) [3] showed that transdifferentiation of BDNF-secreting mesenchymal stem cells significantly increases BDNF production and proteins that identify Schwann cells, which can stimulate neurite sprouting and regeneration. In rats with diabetic retinopathy, Zhang *et al*. (2017) [66] showed that intravitreal injection of neural stem cells obtained from human umbilical cord-derived MSCs has a BDNF-dependent mechanism (neuroprotective effect). Satheesan *et al*., (2020) [50] Quantification of BDNF levels was performed by ELISA in conditioned media of NCM, WJ-MSC-derived NSCs and WJ-MSCs of foetal ovine NPCs and found that BDNF was found at a concentration of 873.90 ± 12.16 pg/mL. 625.57 ± 6.00 pg/mL, and 481.12 ± 23.08 pg/mL, respectively. A highly significant (p<0.01) difference in BDNF levels in NCM compared to WJ-MSC-induced NSCs and conditioned media from WJ-MSCs was observed. Therefore, in the present study, neurite outgrowth, axonal outgrowth and neuroplasticity of WJ-MSCs may be affected by the soluble factor BDNF released both in cells and in the NCM.

Potential of WJ induced NSCs in neurodegenerative disorders: According to Fu *et al*. (2006) [17], transplanting human umbilical cord MSCs partially reversed amphetamine-evoked rotational behaviour caused by lesions, which raised the possibility of using UC-MSCs as a Parkinson's disease treatment. When administered intravenously, the UC-MSCs have been shown to spontaneously migrate to the damaged or inflamed area (Chamberlain *et al*., 2007) [8]. Selective homing allows us to focus the action of these cells where they are needed, limiting potential side effects, while retaining most of the infused cells in the lungs. According to Lim *et al*. (2007) [34], transplanting canine UC blood derived MSCs resulted in restoration of neurological function in spinal cord-injured dogs and can be considered a therapeutic approach for spinal cord injury. According to Kurtz (2008) [28], MSCs are poorly immunogenic and, depending on the preparation and method of cell distribution, can survive and function within the recipient for several weeks. Therefore, MSC-based therapies for various diseases can be built on the potential of MSCs to modulate immune activation and provide trophic signals for tissue healing (Van Velthoven *et al*., 2010) [60]. The identification and characterization of neural stem cells in companion animals remains largely unstudied. Agarwal *et al*., (2014) [1] successfully identified and described NSCs from foetal goat brain. According to Kumar *et al*., (2014) [27], adherent culture allows rapid isolation and expansion of buffalo neural stem cells. NSCs were effectively extracted and cultured from hippocampal and cortical tissues of foetal sheep by Li *et al*., (2017) [31] and Lija *et al*., (2019) [33]. NSCs are usually extracted from embryonic, foetal, or adult brain tissue, which are considered limited sources. Poor purity and technical difficulties in isolation have also slowed the development of NSC-based cell therapies. There were also moral and ethical issues. The methods for obtaining NSCs are currently being improved by emerging technologies on a constant basis; in particular, the identification of ESCs and the development with high purity typically take a lot of time and are accompanied by safety concerns, making this approach difficult to apply to clinical therapy. Somatic cell trans-differentiation into NSCs circumvents the aforementioned issues and offers a very alluring method for the creation of NSCs in large quantities for clinical use (Tang *et al*., 2017 and Satheesan *et al*., 2020) [54, 50].

MSC-induced NSCs have become a viable alternative source for NSC transplant studies in recent years. Due to its advantages of low immunogenicity, multiple sources and few ethical controversies, umbilical cord MSC transplantation has emerged as a promising and attractive cell-based therapeutic option for repairing the injured central nervous system. Therefore, one of the most important techniques in regenerative medicine and tissue engineering is to develop MSCs into neuron-like cells at a higher rate. There are many reports of chemo-induction therapy with a significantly higher rate of differentiation into neurons (Fong *et al*., 2007 and Lian *et al*., 2016) [14, 32]. Satheesan *et al*., 2020 [50] demonstrated that UCT-WJ-MSCs are readily available, have stem cell properties, are a rich source of progenitor cells, can be expanded and maintained in culture, and exhibit neuronal phenotypes *in vitro*. Each methodology, however, has advantages and disadvantages, and it is still debatable whether or not the neurons that are separated from MSCs using a particular protocol have normal nerve function. Therefore, more research must be done to increase the trans-differentiation efficiency in order to ensure the success of any future clinical uses of WJ MSCs.

Conclusion

Umbilical cord WJ-derived MSCs are readily available, have stem cell properties, are a rich source of progenitor cells, can be expanded and maintained in culture, and have the capacity to develop neuronal phenotypes *in vitro* as it is clear in this review. The WJ MSCs itself had the capacity for trans-differentiation, and its secretomes suggested that it would make a good candidate for cutting-edge cell-based treatments for neurodegenerative diseases. In light of this, the WJ MSCs produced NSCs may play a variety of therapeutic and biotechnological roles in neuroregenerative medicine.

Conflicts of Interest

Author declares no conflicts of interest.

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