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Exploring Wharton's jelly Mesenchymal stem cells: A frontier in neuronal trans-differentiation research

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Abstract
Nerve injuries, irrespective of tissue involvement, disrupt the continuity of nerve fibers, leading to compromised functionality. Mesenchymal stem cells (MSCs) have emerged as promising candidates for cell therapy across a spectrum of diseases, owing to their unique functional attributes including potent differentiation capacity, immunomodulation, and growth support. In particular, the secretome produced by Wharton's Jelly Mesenchymal Stem Cells (WJ-MSCs) plays a pivotal role in their trans-differentiation into neural stem cells, thus creating a conducive microenvironment for nerve regeneration. Through the secretion of trophic factors, WJ-MSCs provide a nurturing, protective, and activating milieu that fosters the regeneration of damaged nerves, engaging both neuronal and non-neuronal cells. This synergistic interplay holds immense potential for accelerating nerve regeneration processes and augmenting functional recovery. This review underscores the therapeutic promise of WJ-MSCs in the realm of nerve regeneration, offering insights into their mechanisms of action and future directions for clinical applications.

Keywords: Neuronal, Trans-differentiation, WJ MSCs, umbilical cord tissue, secretome

Introduction

Stem cells, distinguished by their ability for self-renewal, proliferation, differentiation, and tissue regeneration, play a pivotal role in biomedical research and therapeutic applications. They are broadly classified into embryonic and non-embryonic stem cells. Embryonic stem cells (ESCs), particularly pluripotent stem cells, possess the remarkable capacity to differentiate into various cell types. Despite their considerable therapeutic potential, concerns regarding their tumorigenicity and ethical considerations have hindered their clinical utilization (Blum and Benvenisty, 2008) [4].

Non-embryonic stem cells, also known as adult stem cells, exhibit multipotent or limited pluripotent capabilities compared to ESCs. These cells are derived from adult tissues such as bone marrow, fat, muscle, liver, skin, brain, dental pulp, retina, and orbicularis, as well as less mature sources like cord blood, cord tissue, placenta, and fetal body tissues such as pancreas and liver. The therapeutic potential of these cells is immense (Pittenger *et al*., 1999; Uchida *et al*., 2000; Gronthos *et al*., 2000; Minguell *et al*., 2001; Tuch, 2006) [46, 58, 20, 37, 56].

Initially, haematologists primarily concentrated on bone marrow mesenchymal stem cells (MSCs) because of their regenerative and immunomodulatory properties. However, MSCs derived from alternative sources, particularly Wharton's jelly of the umbilical cord tissue (WJ-MSCs), have garnered attention for several reasons. These include their primitive nature, ease of expansion *in vitro*, capacity for multi-lineage differentiation, immunomodulatory and antioxidant actions, as well as their release of trophic factors (Fong *et al*., 2007; Garzon *et al*., 2012; Lian *et al*., 2016) [14, 19, 32].

Wharton's jelly, the gelatinous connective tissue found in the umbilical cord, comprises myofibroblasts, collagen fibers, and stromal cells. Wharton's jelly-derived MSCs (WJ-MSCs), easily obtainable and cultured *in vitro*, demonstrate pluripotency and the remarkable ability to differentiate into various mesenchymal and non-mesenchymal cell types.

These include neurons, osteocytes, adipocytes, chondrocytes, cardiomyocytes, and hepatocytes (Wang *et al*., 2004; Saben *et al*., 2014; Ranjbaran *et al*., 2018; Mitchell *et al*., 2003; Fu *et al*., 2006; Fong *et al*., 2007; Moshrefi *et al*., 2010; Cardoso *et al*., 2012; Venugopal *et al*., 2011; Puranik *et al*., 2012; Singh *et al*., 2013; Sreekumar *et al*., 2014; Uranio *et al*., 2014; Eswari *et al*., 2016) [62, 49, 48, 38, 17, 14, 39, 7, 61, 47, 53, 53, 59, 13].

In various diseases, including neurodegenerative disorders, MSC-based therapies have shown promising potential for immune modulation and tissue repair (Uccelli *et al*., 2006; van Velthoven *et al*., 2010; Satheesan *et al*., 2020) [57, 60]. This versatility and therapeutic potential make WJ-MSCs an exciting prospect for regenerative medicine and the treatment of a wide range of medical conditions.

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

Various protocols are available for isolating cells from Wharton's jelly (WJ), which differ based on the removal of the umbilical artery and vein and the method of enzymatic or mechanical dissection. Enzymatic disruption involves the use of collagenase, trypsin, or hyaluronidase, followed by purification and culture of dissociated cells (Wang *et al*., 2004) [62]. On the other hand, mechanical dissection entails cutting tissue into small pieces or segments, which are then transferred to culture plates until cells migrate to the plastic bottom of the plate (Mitchell *et al*., 2003; La Rocca *et al*., 2009) [38, 29].

Cells derived from WJ require specific culture conditions, with media containing either low or high glucose levels and supplemented with platelet-rich plasma or other additives such as bovine fetal bovine serum (Mitchell *et al*., 2003; Eswari *et al*., 2016; Ranjbaran *et al*., 2018; Satheesan *et al*., 2020) [38, 13, 48, 50]. Studies have highlighted the faster doubling rate of umbilical cord Wharton's jelly-derived MSCs compared to fetal fibroblasts (Moshrefi *et al*., 2010) [39]. Additionally, it has been observed that WJ-MSCs have shorter doubling times than adult bone marrow mesenchymal cells, suggesting a relatively immature nature of WJ-MSCs compared to adult stromal cells (Campagnoli *et al*., 2001; Baksh *et al*., 2007; Karahuseyinoglu *et al*., 2007; Troyer and Weiss, 2008) [6, 2, 23, 55].

In efforts to optimize isolation procedures, Venugopal *et al.* (2011) [61] replaced porcine trypsin with TrypLE Express, a trypsin alternative devoid of animal and human components, eliminating the need for neutralization with serum-containing media. Garzon *et al.* (2012) [19] reported fluctuating cell viability across passages, with the highest viability observed at the 5th and 6th passages. Liang *et al.* (2016) [32] emphasized the stability of early and intermediate-stage WJ-MSCs, cautioning against the effects of serial passaging on lineage-specific differentiation.

Regarding morphology, WJ-MSCs exhibit diverse shapes, including spindle-shaped, rectangular, cuboidal, and fibroblast-like cells, as well as parallel arrays of confluent cells (Eswari *et al*., 2016; Ranjbaran *et al*., 2018; Satheesan *et al*., 2020) [38, 48, 50], which align with observations in bone marrow-derived MSCs (Colter *et al*., 2001; Hanabdari *et al*., 2016) [11, 24].

Clonogenicity of WJ-MSCs

The formation of cell colonies from single cells serves as a tangible demonstration of the self-renewal capacity inherent in stem cell populations (La Rocca *et al*., 2009) [29]. Factors such as coating density and oxygen content play crucial roles in determining the frequency and rate of colony formation from Wharton's jelly mesenchymal stem cells (WJ-MSCs). Studies involving WJ-MSCs derived from goat, buffalo, and sheep consistently demonstrate significant alkaline phosphatase activity and colony formation, underscoring the robustness of these cells (Sreekumar *et al*., 2014; Eswari *et al*., 2016; Satheesan *et al*., 2020) [53, 13, 60].

Wharton's jelly MSCs induced NSCs *in vitro*

Mitchell *et al.* (2003) [38], Fu *et al.* (2004; 2006) [18, 17], and Satheesan *et al.* (2020) [50] have noted distinctive morphological transformations in Wharton's jelly mesenchymal stem cells (WJ MSCs), such as cell body retraction and process elaboration by the third day of cultivation. By the fifth day, numerous WJ MSCs exhibited granular formations resembling Nissl bodies, indicating the initiation of neural differentiation.

Various protocols have successfully induced human umbilical cord (UC) WJ MSCs into neural stem cells (NSCs). Murakami *et al.* (2017) [40] employed commercial Mesenchymal Stem Cell Neurogenic Differentiation Medium, while Kruminis-Kaszkiel *et al.* (2020) [26] utilized NSCs induction medium composed of DMEM/F12 with Glutamax supplemented with FBS, Penicillin/Streptomycin, N2 supplement, and EGF.

In a recent investigation by Satheesan *et al.* (2020) [50], WJ-MSCs at passages 3 to 5 were cultured to near confluence and subjected to treatment with Neuronal Conditioned Medium (NCM) collected from ovine fetal brain suspension culture. This intervention effectively facilitated the transition of WJ-MSCs into neurons, yielding swift and substantial outcomes.

Regarding the morphology of WJ MSCs induced NSCs, cell body contraction and process elaboration were observed, with numerous cells showing granular formations akin to Nissl bodies by the fifth day. Murakami *et al.* (2017) [40] cultured UC WJ MSCs using commercially available mesenchymal stem cell neural gene differentiation medium and NSCs induction medium, following the approach outlined by Kruminis-Kaszkiel *et al.* (2020) [26].

In another recent study by Satheesan *et al.* (2020) [50], WJ-MSCs at passages 3 to 5 were exposed to neuronal-conditioned medium (NCM) obtained from fetal sheep brain suspension cultures. This treatment led to the transformation of WJ-MSCs into neurons, characterized by rounded cell bodies with multiple neurite-like extensions, resembling the morphology of neural stem cells. GFAP-positive cells exhibited a stellate morphology without the elongated processes of neuronal marker-positive cells. Peng *et al.* (2011) [44] induced a spindle-like shape in WJ-MSCs akin to Schwann cells by treating them with basic fibroblast growth factor, platelet-derived growth factor, and forskolin. Documented notable morphological changes in MSCs within hours after neural induction, including the assembly and extension of long dendritic processes. Guan *et al.* (2014) [21] and Satheesan *et al.* (2020) [50] observed significant morphological alterations in WJ-MSCs during neural induction, including the emergence of multiple dendrites and a singular axon-like process extending from the cell body, along with granular structures reminiscent of Nissl substances. Please refer to Figure 1 for a visual representation.

Expression of neuronal markers by UCT-WJMSCs and induced NSCs

Mitchell *et al.* (2003) [38] observed that Human UC-MSCs spontaneously produced Nestin, a biomarker indicative of

neural progenitors, even without exposure to differentiation cues. Additionally, WJ-induced NSCs displayed GFAP-positive cells, whose expression was detected in untreated WJ cells but was notably elevated post-induction. Satheesan *et al*. (2020) [50] found that upon induction of neuronal differentiation in WJ-MSCs, there was an increase in the expression of neuronal marker genes such as β III tubulin, nestin, and GFAP.

Frausin *et al*. (2015) [15] reported the capability of WJ-MSCs to differentiate into neuron-like cells *in vitro*, demonstrating both neuronal morphological and biochemical properties and expressing typical neuronal proteins like nestin and β-tubulin. Chen *et al*. (2016) [10] noted high expression of Nestin and NeuroD1 in developed neural stem cells derived from human UC, contrasting with low expression in undifferentiated MSCs, suggesting the activation of molecular mechanisms related to neuronal function in differentiated MSCs.

Lian *et al*. (2016) [32] showed that at early (P7) and intermediate (P14) stages, WJ MSCs exhibited positivity for nestin or βIII tubulin, with neural induction significantly enhancing the expression of these markers in both groups, confirmed via immunofluorescence and quantitative PCR. Expression of nestin was highlighted as crucial for MSC differentiation into neurons, with serum in the medium found to reduce nestin expression, as determined by RT-PCR to assess the expression of NSE and GFAP genes (Zhu *et al*., 2002) [69].

Murakami *et al*. (2017) [40] demonstrated the expression of neurogenic markers such as III-tubulin and Notch in cells before and after induction of neurogenic differentiation, with higher levels observed in differentiated cells through RT-PCR analysis.

Satheesan *et al*. (2020) [50] further indicated that WJMSCs underwent a mesenchymal-to-nervous fate change in the presence of neuronal-conditioned medium, as confirmed by immunocytochemistry and RT-PCR, showing elevated expression of nestin, III-tubulin, and neural lineage GFAP markers in induced NSCs compared to uninduced WJ-MSCs.

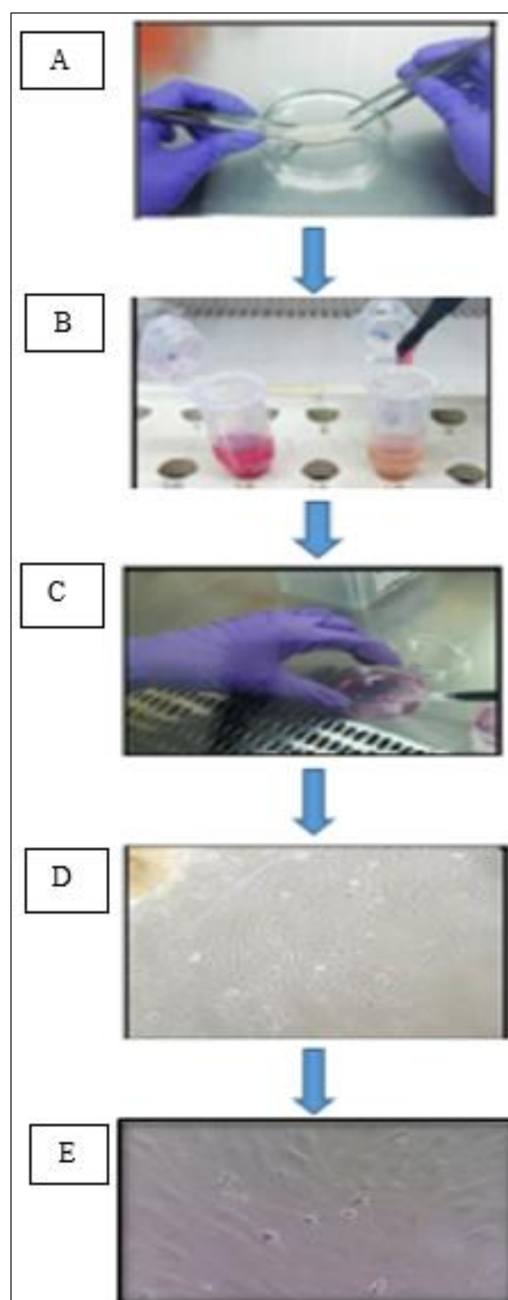

Fig 1: Diagram showing isolation and neuronal induction of umbilical cord tissue-wharton jelly mesenchymal stem cells. A. Processing of umbilical cord B. Collagenase Trypsinisation of extracted Wharton jelly C. Plating/culturing D. Migration of WJ- MSCs (200x) E. Induced Neuronal stem cell (200x) using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

Potential of WJ induced NSCs in neurodegenerative disorders: Fu *et al*. (2006) [17] reported that the transplantation of human umbilical cord MSCs partially alleviated amphetamine-induced rotational behavior caused by lesions, suggesting a potential application of UC-MSCs in Parkinson's disease treatment. When administered intravenously, UC-MSCs were shown to migrate spontaneously to damaged or inflamed areas (Chamberlain *et al*., 2007) [8], enabling targeted action with limited side effects, while most cells were retained in the lungs. Lim *et al*. (2007) [34] demonstrated that transplanting canine UC blood-derived MSCs restored neurological function in spinal cord-injured dogs, suggesting a therapeutic approach for spinal cord injury.

MSCs are known for their low immunogenicity and ability to modulate immune activation and provide trophic signals for tissue healing (Kurtz, 2008; van Velthoven *et al*., 2010) [28, 60]. Although the identification and characterization of neural stem cells (NSCs) in companion animals remain largely unexplored, studies have successfully isolated and cultured NSCs from various animal sources (Agarwal *et al*., 2014; Kumar *et al*., 2014; Lija *et al*., 2019) [1, 27, 33]. However, traditional methods of obtaining NSCs from brain tissue have limitations due to purity issues, technical difficulties, and ethical concerns.

MSC-induced NSCs have emerged as a promising alternative source for NSC transplant studies due to their low immunogenicity, multiple sources, and minimal ethical controversies. Umbilical cord MSC transplantation has shown promise in repairing the injured central nervous system. Enhancing the differentiation of MSCs into neuron-like cells is crucial in regenerative medicine and tissue engineering (Fong *et al*., 2007; Lian *et al*., 2016) [14, 32]. Satheesan *et al*. (2020) [50] demonstrated that umbilical cord tissue-derived WJ-MSCs possess stem cell properties, exhibit neuronal phenotypes *in vitro*, and are readily available and expandable. While each methodology for MSC differentiation into neurons has its advantages and disadvantages, improving trans-differentiation efficiency is essential for the success of future clinical applications of WJ MSCs. Further research is needed to ensure that neurons derived from MSCs using specific protocols retain normal nerve function.

Conclusion

Umbilical cord Wharton's jelly-derived MSCs possess several advantageous characteristics, including their ready availability, stem cell properties, abundance of progenitor cells, and ability to be expanded and maintained in culture. Additionally, these MSCs have demonstrated the capacity to develop neuronal phenotypes *in vitro*. Moreover, research suggests that WJ MSCs exhibit trans-differentiation potential, and their secretomes indicate their suitability as candidates for advanced cell-based therapies targeting neurodegenerative diseases.

Given these attributes, WJ MSCs and their derived NSCs hold significant promise for various therapeutic and biotechnological applications in neuroregenerative medicine. Their potential to differentiate into neural cell types opens avenues for innovative treatments targeting neurological disorders. Additionally, their ability to secrete beneficial factors further underscores their potential for facilitating tissue repair and regeneration in the central nervous system. Therefore, harnessing the capabilities of WJ MSCs and their derivatives represents a promising approach in the ongoing quest to develop effective therapies in neuroregenerative medicine.

Conflicts of Interest

Authors declares no conflicts of interest.

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