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Discarded Wharton jelly of the human umbilical cord: a viable source for mesenchymal stromal cells

NATE WATSON*, RYAN DIVERS*, ROSHAN KEDAR, ANKUR MEHINDRU, ANUJ MEHINDRU, MIA C. BORLONGAN & CESAR V. BORLONGAN

Department of Neurosurgery and Brain Repair, University of South Florida Morsani College of Medicine, Tampa, Florida

Abstract

Mesenchymal stromal cells (MSCs) are multi-potent cells that have the capability of differentiating into adipogenic, osteogenic, chondrogenic and neural cells. With these multiple capabilities, MSCs have been highly regarded as an effective transplantable cell source for regenerative medicine. A large bank of these cells can be found in several regions of the human umbilical cord, including the umbilical cord lining, the subendothelial layer, the perivascular zone and, most important, in Wharton jelly (WJ). These cells, all umbilical cord-derived MSCs, are durable, have large loading capacities and are considered ethical to harvest because the umbilical cord is often considered waste. These logistical advantages make WJ as appealing source of stem cells for transplant therapy. In particular, WJ is a predominantly good source of cells because MSCs in WJ are maintained in an early embryologic phase and therefore have retained some of the primitive stemness properties. WJ-MSCs can easily differentiate into a plethora of cell types leading to a variety of applications. In addition, WJ-MSCs are slightly easier to harvest compared with other MSCs (such as bone marrow-derived MSCs). The fascinating stemness properties and therapeutic potential of WJ-MSCs provide great promise in many aspects of regenerative medicine and should be considered for further investigations as safe and effective donor cells for transplantation therapy in many debilitating disorders, which are discussed here. We previously reviewed the therapeutic potential of WJ-MSCs and now provide an update on their recent preclinical and clinical applications.

Key Words: *differentiation, MSCs, multi-potent cells, proliferation, transplantation*

Introduction

In recent years, medical research has focused on using stem cell therapy to alleviate a number of debilitating disorders. In particular, recent efforts have turned to the human umbilical cord (hUC) for new sources of mesenchymal stromal cells (MSCs). MSCs found in the hUC present several advantages over other stem cell tissue sources. First, hUC is seen as biological waste and is typically discarded after birth. Its use therefore presents no ethical concerns [1, 2]. Second, hUC cells exhibit reduced immunogenicity. Because these inactivated MSCs lack MHCII and other costimulatory molecules on their surface, they present no immune response in the host tissue. In laboratory studies, the allogenic transplantation of hUC cells into non-immune-suppressed animals did not produce rejection [3]. Third, hUC cells have an increased proliferative capacity, evidenced by a higher frequency of colony-forming-unit fibroblasts and a shorter population doubling time than other cells [4].

MSCs can be isolated from the umbilical cord (UC) lining, subendothelial layer, perivascular zone and Wharton jelly (WJ; the gelatinous matrix in the umbilical cord that provides insulation and protection of the vein and arteries of the umbilical cord) [5] (Figure 1). The MSCs found in these regions of the hUC are multipotent and can differentiate into adipogenic, osteogenic, chondrogenic and neuronal cells [6].

However, limitations remain for the isolation of UC-MSCs for clinical use. For cord lining MSCs, the isolation methods are incredibly time-consuming. In addition, the current procedure for isolation of WJ-MSCs involves fetal bone serum as its nutrient enhancement. The problem that results from this supplement is that viral and prion diseases become a

*These authors equally contributed to this work.

Correspondence: **Cesar V. Borlongan**, Ph.D., Center of Excellence for Aging and Brain Repair, Department of Neurosurgery and Brain Repair, University of South Florida Morsani College of Medicine, 12901 Bruce B. Downs Boulevard, Tampa, FL 33612, USA. E-mail: cborlong@health.usf.edu

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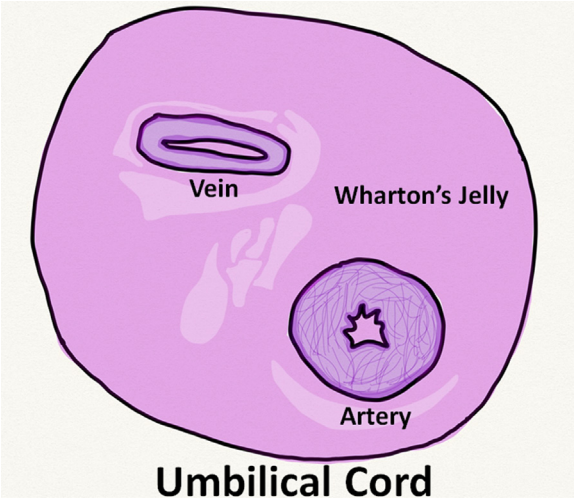

Figure 1. Anatomy of the human umbilical cord showing Wharton's jelly.

major concern. Thus, standard isolation still needs to be modified [7]. An additional problem with their application for clinical use is that UC-MSCs proliferate at high rates and, after tissue repair, differentiate into two daughter cells with asymmetric portions of the parent celles cytoplasm. Because of this, these MSCs present enigmatic problem because they cannot be tracked through magnetic resonance imaging after a short period of time. Therefore, determining whether the MSCs fully developed into the cell type they were primarily intended to and whether they are functioning correctly would be an arduous and inefficient process. Currently the stains of superparamagnetic iron oxide (SPIO) and Mn2+ are being used for the identification and tracking of MSCs. However, these both present problems. The Mn stain has a high cytotoxicity, and, although there is a solution to minimize the cytotoxicity, the impending risk is too high [1]. Although SPIO stain does not possess this property, it cannot track the MSC cells accurately and decreases in clarity as more time elapses [8]. An experiment conducted with Prussian blue staining demonstrates this lack of consistency [9]. Therefore, future research efforts must focus on methods to isolate MSCs more efficiently from the umbilical cord [10] and developing an appropriate method of staining to enable magnetic resonance imaging of the MSCs.

Despite the setbacks in isolation, WJ-MSCs still present perhaps the best opportunity for cell therapy in the future. With a greater proliferative capacity and tri-lineage differentiation potential, these cells can be induced to form a more diverse array of cell types than MSCs found in both the bone marrow and from other regions of the umbilical cord. This high differentiation potential may be able to target a variety of disorders (Table I). For example, UC stem cells exhibit an interesting potency to heal cutaneous burn wounds [11]. Both UC epithelial cells and UC-MSCs can be grown on scaffolds and grafted to treat partial- and full-thickness burns [11].

Table I. Milestone discoveries for WJ transplantation.

Disease indication	WJ cell therapy outcomes
Cancer	Cells do not pose a risk for metastasis of tumor cells [14] Cells promote proteins that halt the cell cycle of cancer cells and promote tumor suppressing genes [13] Cells invoke the body's immune system [13]
Liver disease	The high proliferative capacity of WJ-MSCs induces hepatocyte differentiation [18] The hepatocyte cells can reduce liver fibrosis [21,22]
Peripheral nerve damage	The cell has the ability to differentiate into Schwann cells [24] These can be transferred to the body and applied to the naturally degrading Schwann cells [24]
Cardiovascular repair	Biologically cardio-active leaflets can be manufactured using WJ-MSCs [26] The cells have been used in large animal studies with the sheep hearts functioning over 20 weeks into experimentation [27]
Connective tissue repair	Cartilage injuries are the result of a metabolic imbalance of chondrocytes and slow repair of the issue; WJ-MSCs can easily differentiate into the correct cell and be transplanted to repair the issue [31,32] Tendon injuries are also caused by an imbalance and disorganization of the collagen fibers; human umbilical cord perivascular cells can not only be transplanted to restore balance, they also help organize tendon collagen fibers [34,35]
Obesity and diabetes	In the prenatal environment, the growing child can have its cells be predisposed to grow into adipocyte cells based on the mother's condition [36] Using the differentiation ability of WJ-MSCs, researchers can grow insulin-producing cells in the lab that can be used to help treat obesity and type II diabetes [36,38]

Cell therapy for cancer

Compared with other sources of MSCs derived from bone marrow, WJ-MSCs exhibit reduced immunogenicity. WJ-MSCs lack costimulatory ligands, which activate an immune response from both B and T cells [12]. At the same time, WJ-MSCs express human leukocyte antigen G (HLA-G), a protein that induces the

expansion of regulatory T cells and suppresses cytotoxic T cells and natural killer cells at a high level [13].

Interestingly enough, WJ-MSCs [referred to as UCMSCs in Tamura *et al.* [13]] also possess properties that make them potential tools for cancer therapy. Cancer cells secrete cytokines and growth factors, and WJ-MSCs have receptors for these molecules in the cell membrane [13]. This interaction between cytokines and growth factors and their receptors results in WJ-MSCs exhibiting a tropism toward the inflammatory cancer and tumor tissues [13].

Moreover, WJ-MSCs also present tumorcidal abilities. Although bone marrow—derived MSCs have been shown to stimulate tumor growth, WJ-MSCs have been demonstrated to attenuate the growth of tumors [13]. Most of these properties are not fully understood, but there are two known mechanisms for cancer suppression. The first is that WJ-MSCs produce several secretory proteins that in turn promote the cell death of cancer cells and stop the cell cycle [13]. In addition, WJ-MSCs also caused more tumor-suppressing genes to be expressed thus aiding the cancer treatment. The second mechanism through which cancer can be suppressed is through the enhancement of the immune system reaction to the cancer cells [13]. Studies have shown that rats treated with rat WJ-MSC displayed great improvement in tumors [14]. The rats showed highly reduced tumors, and an abundant amount of lymphocytes in the area, which infiltrated the tissue of the tumor and assisted in therapy. In addition to their anti-cancer benefits, WJ-MSCs are also considered safe therapeutic cells because they do not pose a risk of spreading tumor tissue into other parts of the body or other adverse effects [15]. If research efforts can develop tumor-suppressor genes or anti-cancer drugs in the future, WJ-MSCs can potentially be used as vehicles for targeted cancer therapy because of their durability, large loading capacity, ability to be harvested in large numbers with no risk to the donor, and tumor tropism [13].

Cell therapy for liver disease

WJ-MSCs have also been explored as a method to cure liver diseases. Because of WJ-MSCs' excessive proliferation and ability to differentiate into various cell types, they are perfect candidates for this kind of treatment. These specific stem cells have been known to differentiate into adipocytes, osteoblasts and also neurons [16,17]. Because of the need for liver donors and the harmful side effects of the liver transplantation, stem cell therapies are becoming recognized as a better option for treating liver disease. Morphologic analysis demonstrates that WJ-MSCs express markers that correlate with the phenotype of hepatoblasts (the precursor to hepatocytes, the cell of the primary liver tissue), suggesting the ability for WJ-MSCs to differentiate into liver cells [18]. Hepatic commitment of these cells can potentially be induced in the presence of specific growth factors or the host liver cell environment. Moreover, in a model of fibrosis (the body's response to chronic liver damage), the introduction of WJ-MSCs into the injured livers of mice was able to relieve fibrosis and reduce hepatic inflammation [19], possibly by abrogating extracellular matrix accumulation (caused by fibrosis). However, these results are controversial because other studies found that MSCs produce no benefit to hepatic function in the long term [20].

Transplantation of WJ-MSCs has also been tested in liver fibrosis. Using carbon tetrachloride (CCl_4), rats were experimentally induced display liver fibrosis and 4 weeks later received WJ-MSCs injections [21]. After an additional 4 weeks, there was a remarkable decrease in the liver fibrosis in the rats treated with WJ-MSCs compared with the rats that were not treated with the WJ-MSCs. Some WJ-MSCs exhibited phenotypes of the liver, and those WJ-MSCs that did not differentiate had the capability to secrete cytokines that have the potential to restore liver function [22]. These observations indicate a multipronged reparative mechanism of WJ-MSCs involving specific lineage differentiation and therapeutic molecules that are key pathways in tissue repair.

Cell therapy for peripheral nerve damage

WJ-MSCs have also been proposed as a potential cure for peripheral nervous system injuries. When a peripheral neuron is damaged, the Schwann cells lose contact to the next axon and therefore self-degrade their own myelin sheaths. The body responds with a proliferation of Schwann cells that support axonal regrowth and regeneration of myelin [23]. Therefore, research in cell therapy has explored the efficacy of transplanting Schwann cells to heal the injury. This is difficult, however, because isolation of Schwann cells can cause damage to other peripheral nerves, and the amount of Schwann cells able to be isolated is typically low. MSCs offer a novel alternative stem cell source because they are easily accessible and highly proliferative. Because of the trans-differentiation potential of WJ-MSCs into ectoderm-derived cells, MSCs can potentially differentiate into functional Schwann cells and be applied to heal injured peripheral neurons [24]. To this end, the transplantation of WJ-MSCs into a rat with peripheral nerve damage revealed that the injected cells labeled with alentivirus green fluorescent protein to allow visualization of myelin of the regenerated axons, were able to differentiate and become

functioning Schwann cells with an efficiency of 97%. These findings indicate that WJ-MSCs appear as effective donor cells for cell therapy in the future [24].

Cell therapy for cardiovascular and connective tissue repair

Another potential use for WJ-MSCs is in cardiovascular tissue engineering. The cardiovascular system has low regenerative potential, which makes the use of WJ-MSCs an ideal alternative in cardiovascular tissue repair with their immunomodulatory properties and self-regenerating capacity [25]. Interestingly, biologically active heart valve leaflets could be engineered using only cells from the hUC [26]. These leaflets showed complex tissues that closely resembled native tissues. With such robust results, the use of WJ-MSCs became a large step in overcoming limiting factors in repairing congenital malformations. These efficacy readouts have also been confirmed in large animal studies in which engineered heart tissues were successfully transplanted into sheep, showing good functional performance after 20 weeks [27]. However, uncertainty remains over whether these engineered tissues can survive successfully in the long term [28]. Additional research studies have found that culturing UC-MSCs in the presence of certain growth factors and hormones more efficiently induces UC-MSCs to differentiate into cardiomyogenic lineages. In particular, the presence of the hormone oxytocin gave rise to the most efficient differentiation [29].

UC-MSCs may also be beneficial in the treatment of cartilage injuries. Cartilage injuries are the result of a metabolic imbalance of an organism's chondrocytes (the primary cell of cartilage tissue), and the natural growth and repair of cartilage tissue is slow [30]. Transplantation of UC-MSCs presents a potentially effective mechanism for cell therapy [31]. UC-MSCs, when cultured in a medium containing ascorbic acid, transferrin, dexamethasone and other molecules, can differentiate into chondrocyte-like cells [32]. Therefore, stem cell transplantation stands as a strategy to substantially increase the number of chondrocytes, enabling a quicker recovery of cartilage diseases [33].

The same is true for tendon injuries. hUC perivascular cells have been shown to produce collagen that repairs tendon injuries in rats [34]. Additionally, the presence of hUC perivascular cells facilitated a change in structure and organization of the collagen fibers [34]. Instead of being disorganized, these collagen fibers were arranged in linear parallel bundles, increasing the tendon's tensile strength in comparison to the control group [35].

Cell therapy for obesity and diabetes

The prenatal differentiation of WJ-MSCs may play a factor in determining an individual's susceptibility to obesity and related disorders later in life. Obesity can be affected by increased adipogenesis, the early determination of MSCs to adipocytes before birth. This phenomenon is influenced heavily by the prenatal environment [36]. The changes in the prenatal environment have been largely ascribed to the mother's health condition. When healthy mothers were compared with diabetic mothers, a major difference was observed in the prenatal environments were the protein levels [36]. The changes in the concentration of CD90 is related to the change in plasticity and the up-regulation of CD44, CD29, CD73, CD166 and SSEA4, whereas telomerase reverse transcriptase (TERT) is related to the increase of the proliferative ability of the cells. Studies show that WJ-MSCs from mothers with hyperglycemia or gestational diabetes mellitus have a higher affinity toward adipocyte differentiation and increased adipocyte differentiation efficiency than those from lean mothers [36]. The findings suggest that the changes in the prenatal environment in obese mothers predispose the child to becoming obese or having type II diabetes later in life [36]. Additional research also suggests that WJ-MSCs may have potential in the direct treatment of diabetes mellitus [37]. By using markers that indicate when certain genes are expressed, models have shown that WJ-MSCs have the capability to differentiate into all sorts of pancreatic cells including the insulin-producing β cells [38]. Using immunohistochemistry and enzyme-linked immunosorbent assays, a significantly greater amount of insulin and C-peptide protein was released from the differentiated cells than from the undifferentiated cells. These results suggest that the WJ-MSC did in fact turn insulin-producing cells. In just a week, WJ-MSCs can turn into the exact cells that this aggressive disease attacks. With more research and study, WJ-MSCs may provide a means to alleviate diabetes [39].

Moreover, new cell isolation methods have emerged to more efficiently culture and expand the population of WJ-MSCs in a laboratory setting. A lower oxygen concentration (5%) than room air (21%), as well as a low plate density (10 cells/cm^2), has powerful effects on WJ cell expansion, shortening population doubling time and substantially increasing the colony-forming efficiency of the cells [40]. These new methods are exciting and hold promise as researchers search for even more efficient methods to harvest stem cells for developing cell therapies [40].

As noted earlier, WJ-MSCs are potentially viable resources as donor cells for clinical transplantation use, primarily because of their high proliferative

Table II. Comparison of MSC sources.

MSC source	Potential benefits	Potential drawbacks
WJ	High proliferative capacity [4] Tri-lineage differentiation ability [4,6] Provokes little immune response when transplanted [3,4,8]	Currently no uniform isolation procedure [4,7] Difficult to extract from other anatomic zones of the hUC [4]
Bone marrow	Useful for hematopoietic stem cell transplantation [8] Ability to differentiate into multiple cell types in the mesodermal lineage [8]	Difficult and invasive procedures needed for isolation [8] Provokes a greater immune response than WJ-MSCs [8,12]
UC blood	Exhibit properties similar to WJ-MSCs, especially in broad differentiation abilities [4]	Difficult to extract and isolate cells in sufficient amounts for transplantation [4]
Amniotic fluid	High differentiation capacity Easy to obtain from the placenta [10]	Little research on functional effects conducted to date [10] More research needed on their potential uses and potential methods for isolation [10]
UC matrix	High differentiation capacity [4] Immunomodulatory properties similar to WJ-MSCs [3,4,8]	Difficult to extract and isolate cells in sufficient amounts for transplantation [4]

capacity and ability to differentiate between three tissue lineages (ectoderm, mesoderm and endoderm). WJ-MSCs are likely more beneficial than some other sources of MSCs (Table II). For example, for several years, bone marrow MSCs have been demonstrated as the future of hematopoietic stem cell transplantation, primarily because of their intrinsic micro-environmental support for hematopoietic stem cells and their ability to differentiate into various mesodermal lineages, much like WJ-MSCs, with limited difficulty in isolation. However, these stem cells are inferior because of the invasive procedures required for aspiration [8].

Another potent source of MSCs is UC blood. Much like WJ-MSCs, these are multi-faceted cells with the ability to differentiate into lineages while either *in vitro* or *in vivo* and possess higher proliferative capacity than those MSCs of bone marrow [4]. However, studies suggest that MSCs derived from the umbilical cord blood are limited in their utility because technical challenges in extracting sufficient amounts of these cells.

Amniotic fluid MSCs are similar to those of WJ in that they have a good differentiation capability and can be efficiently obtained from the placenta [10]. These multi-potent stem cells may have a future in cell therapy and clinical use. However, because they have only recently emerged in the field, most researchers believe that further studies must be conducted to develop a proficient method to culture and isolate these cells [10].

Lastly, similar to WJ-MSCs, UC matrix MSCs (UCM-MSCs) are useful in tissue engineering and possibly cell therapy. They also have differentiation abilities, immuno-modulatory properties and trophic activity [4]. However, the number of UCM-MSCs extracted is limited, and a unique *ex vivo* expansion method is necessary to obtain sufficient numbers of them [4].

Although all the sources of MSCs possess comparable stemness properties (differentiation, proliferation capacity) and present with potential for cell-based clinical therapeutic use, and all sources display nearly analogous post-transplantation effects, the ease in isolating and propagating ample supply of WJ-MSCs, combined with their high proliferative capacity and ability to differentiate into the three germ lineages, make WJ-MSCs appealing donor cells for transplantation therapy.

Conclusions

Recent evidence demonstrates that WJ-MSCs are potential transplantable cells for treatment of devastating diseases such as cancer and diabetes. Their use in cell therapy will be an integral addition to the field of regeneration. WJ-MSCs have a multitude of benefits, such as their high proliferation rate [41], lower doubling time and ability to function with non-immune-suppressed animals [42]. However, there remains a paucity of research in the translation of WJ-MSCs into clinical use, largely because of the cells' heterogeneity, which results from the current isolation methods and inefficient staining methods [43]. There are two primary explanations for heterogeneity. First, the hUC has multiple distinct anatomic zones, and previous attempts at isolating WJ-MSCs have inadvertently harvested cells from different anatomic structures of the hUC in addition to WJ. Second, there is currently wide variation in procedures to harvest WJ-MSCs, and this variation can produce inconsistent results between studies

[10]. Future research and refinement of isolation procedures can potentially overcome these obstacles [10]. Regardless of these drawbacks, WJ-MCSs are the ideal future for cell therapy; their properties of high proliferation capability and versatility to differentiate between three lineages allow them to lower immunogenicity and have the potential to treat an array of diseases and disorders [44].

In addition, WJ-MSCs stimulate immune responses from B and T cells [12] and suppress cytotoxic and natural killer cells [13]. WJ-MSCs possess cytokines and growth factor receptors, which allow them to be vital tools for cancer therapy. In such therapy, WJ-MSCs drastically weaken cancerous tumors by secreting therapeutic proteins that promote cell death of cancerous cells and stop the cell cycle [45]; moreover, WJ-MSCs enhance the immune response to cancer cells. With the minimal risk of spreading cancer cells throughout the body or to the MSC donor, WJ-MSCs have the potential to serve as vehicles for delivery of tumor suppressive genes and anti-cancer drugs.

Apart from cancer treatment, WJ-MCSs can also facilitate cell-based therapies for liver disease and diabetes mellitus because of their high proliferation and differentiation ability [46], (e.g., WJ-MSCs can express hepatoblastic phenotypes and can become liver cells or pancreatic cells) [47].

As we recognized the many versatile capabilities of WJ-MSCs, their documented efficacy in animal models and limited clinical trials as therapeutic cells advances the field of regenerative medicine. With more research, WJ-MSCs may someday become recognized as routine donor cells for cell-based therapies [48–50].

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