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Therapeutic Potential of Mesenchymal Stem Cells for Diabetes

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ABSTRACT

Mesenchymal stem cells (MSCs) are self-renewing multipotent cells that have the capacity to secrete multiple biologic factors that can restore and repair injured tissues. Preclinical and clinical evidence have substantiated the therapeutic benefit of MSCs in various medical conditions. Currently, MSCs are the most commonly used cell-based therapy in clinical trials because of their regenerative effects, ease of isolation, and low immunogenicity. Experimental and clinical studies have provided promising results using MSCs to treat diabetes. This review will summarize the role of MSCs on tissue repair, provide emerging strategies to improve MSC function, and describe how these processes translate to clinical treatments for diabetes.

INTRODUCTION

Advances in stem cell biology have seen the rise of an exciting new field of research known as regenerative medicine. Regenerative medicine is a multidisciplinary branch of translational research that aims at repairing injured tissues to restore normal cellular function. To date, the cell population most commonly studied in clinical trials includes mesenchymal stem/stromal cells (MSCs). The therapeutic potential of MSCs is based on their ease of isolation, ability to differentiate into multiple cell types, low immunogenicity, and most importantly their release of biologic factors shown to alleviate impaired tissues.

MSCs are multipotent cells, of mesodermal origin, that characteristically: a) adhere to plastic and self-renew, b) express specific surface antigen markers (CD73, CD90, CD105), and c) at a minimum, have the ability to differentiate into osteocytes, adipocytes, or chondrocytes (Dominici *et al.* 2006). MSCs are widely distributed in the body and can therefore be isolated from multiple sources, including the bone marrow, heart, bodily fluids, skin, and perinatal tissues. MSCs react to microenvironmental changes (pH, oxygen, stress) by releasing immune modulatory and trophic factors known to regenerate injured cells and tissues (Caplan & Correa 2011). Experimental findings in neurodegenerative and cardiovascular disease have supported the rapid growth of cell-based research (Murphy *et al.* 2013). To date, 695 US clinical trials are testing the utility of MSCs as therapeutic agents for an array of medical conditions.

The aim of this review is to provide a concise summary of the existing literature evaluating MSCs as novel therapeutic agents for diabetes mellitus. Additionally, this focused review will discuss recent methods used to bolster stem cell performance and how these discoveries are translating into endocrine research.

AVAILABLE AND RENEWABLE SOURCES OF MSCs

In 2012, Shinya Yamanaka was one of the awardees of the Nobel Prize in Physiology or Medicine for discovering that mature cells can be reprogrammed into pluripotent cells. This remarkable technique is an excellent and readily available source of autologous stem cells that overcomes issues with cell/tissue rejection. Bone marrow and adipose tissue are another source for MSCs but their drawback is that invasive instrumentation is necessary to collect the tissue.

An emerging approach to retrieve MSCs in a non-invasive, ethically sound manner, and is traditionally considered medical waste includes the placenta and/or the umbilical cord (Nagamura-Inoue & Mukai 2015). Furthermore, cells from these nascent tissues are postulated to have higher proliferative and differentiation abilities, as well as a heightened ability to express paracrine factors when compared to other MSC tissue sources. In the United States, the Centers for Disease Control and Prevention approximates 4 million births per year and 2.5 million deaths per year, which results in a surplus of MSCs available from perinatal tissue.

ISOLATION OF MSCs FROM THE HUMAN UMBILICAL CORD

Studies have established that MSCs can be isolated, expanded, and cryopreserved from both umbilical cord blood and Wharton's jelly (umbilical cord matrix). However, advantages to the isolation of MSCs from the Wharton's jelly (WJ) includes: a higher yield, more homogenous stem cell population, increased likelihood of successful MSC isolation, and better ability to differentiate into insulin-producing cells (Weiss & Troyer 2006; El-Demerdash *et al.* 2015; Vangsness *et al.* 2015; Arutyunyan *et al.* 2016). Several techniques have been described for the isolation of WJ-MSCs, but the two most common methods include an enzymatic digestion of cord tissue or an explant culture method (Figure 1).

Enzymatic method

In this method, the umbilical cord WJ tissue is exposed to enzymes that disrupt the collagen matrix and hence releases cells into the underlying solution. The solution is then collected into a conical tube that is centrifuged to separate the pellet (cells) from the suspension. The supernatant is removed and the cells are plated on a tissue culture dish with stem cell media. Collagenase, hyaluronidase, trypsin, and dispase are examples of enzymes used to dissociate WJ-MSCs from the matrix (Bruyn *et al.* 2011; Azandeh *et al.* 2012; Rostamzadeh *et al.* 2015).

Explant method

The derivation of MSCs under this method relies on the direct transfer of dissected umbilical cord tissue fragments onto a tissue culture dish (Fong *et al.* 2011; Mori *et al.* 2015; Talaei-Khozani *et al.* 2015). The culture dish is filled with media that stimulates the propagation of stem cells. Adherence of the WJ umbilical cord tissue to the bottom of the culture dish allows the migration of stem cells from the cord onto the surface of the dish. Within the first week, cells are visibly adherent to the surface of the plastic dish, at which point the tissue can be removed.

Although this technique is simple and involves less manipulation of the umbilical cord tissue, many researchers argue that this protocol results in a longer period for the cells to reach confluency when compared to the enzymatic method (Salehinejad *et al.* 2012; Hiew *et al.* 2016).

Flow cytometric characterization of MSCs

After growing the cells in a humidified incubator at 37°C with 5% CO_2 with stem cell media the International Society for Cellular Therapy states that cells must express specific cell surface antigen markers to meet the definition of an MSC (Dominici *et al.* 2006). Mesenchymal cells from the umbilical cord should express ≥ 95% of CD 73, CD 90, and CD 105. Furthermore,

MSCs should express ≤ 2% of CD 14 or CD 11b, CD34, CD 45, CD 19 or CD 79α, or HLA-DR, as they are markers of hematopoietic differentiation.

Differentiating MSCs into fat, bone, and cartilage

MSCs are idealized because of their multilineage potential, and have proven to consistently differentiate into at least three specialized cell types-chondrocytes, osteoblasts, and adipocytes. Cells should be stained with Alcian blue or collagen type II to demonstrate chondrocyte differentiation, Alizarin Red or von Kossa for osteoblast delineation, and Oil Red O to show an adipocyte lineage (McNamara; Mauck *et al.* 2006; Boeuf *et al.* 2010; Thibault *et al.* 2010; Scott *et al.* 2011; Baglio *et al.* 2015; Westhrin *et al.* 2015). Additional articles have reported the successful differentiation of MSCs into insulin-producing cells, Schwann cells, and neurons (KEILHOFF *et al.* 2006; Moshtagh *et al.* 2013; Feng *et al.* 2014). Figure 2 depicts a WJ-MSC that has adhered to plastic, expresses MSC surface antigens, that has also undergone differentiation into three cell types.

MSCs STIMULATE TISSUE REPAIR

It is well established that the beneficial outcomes of MSCs occur through a paracrine release of biologic factors, rather than engraftment of cells into the recipient tissue. For purposes of this review, studies examining the regenerative properties of MSCs will be generalized into the following major themes: vascular development, anti-inflammation, and anti-fibrosis (Figure 3).

Vascular development

Angiogenesis, the formation of new blood vessels, is a vital process in tissue wound healing that is a targeted by many pharmacologic agents to treat disorders such as myocardial ischemia, ischemic stroke, and diabetic retinopathy (Hammes *et al.* 2011; Johnson & Wilgus 2014).

Preclinical studies in cardiac and brain ischemia support the concept that MSCs improve structural and functional outcomes by repairing and stimulating the growth of blood vessels (Acosta *et al.* 2013; Hsuan *et al.* 2016). The angiogenic properties of MSCs is mediated through the release of hypoxia inducible factor, vascular endothelial growth factor, angiopoietin, and erythropoietin. (Wei *et al.* 2012). The ability to repair vascular injury after administration of MSCs has been supported in studies of diabetic peripheral vascular disease, cutaneous wound repair, and bone necrosis (Paneni *et al.*; Arno *et al.* 2014; Fan *et al.* 2015).

Immunomodulation

Although inflammation is the body's natural response to protect against harmful stimuli, excessive or prolonged inflammatory stress can be detrimental to cells and tissues. For instance, chronic inflammation has now emerged as an important contributor to the pathogenesis of metabolic syndrome (Monteiro & Azevedo 2010). As such, investigators have begun exploring the interactions between inflammation and MSC therapy. In particular, MSCs modulate key inflammatory cell types, including T-cells, natural killer cells, B-cells, and dendritic cells (Wang *et al.* 2012). The MSC interaction with these innate and adaptive immune cells results in downregulation of inflammatory markers (interleukin-1β, tumor necrosis factor α, interleukin-6) as well as an increase in protective cytokines (interleukin-10, prostaglandin E_2, indoleamine 2, 3-dioxygenase). Bone degenerative studies treated with MSCs also highlight their ability to decrease the secretion of macrophage inflammatory protein and monocyte chemoattractant protein (Pers *et al.* 2015). In rodent models of acute lung injury, Gupta et al demonstrated that MSCs increase expression of anti-inflammatory cytokine interleukin-10 (Gupta *et al.* 2015).

Anti-Fibrosis

Multiple groups have documented the anti-fibrotic effects of MSCs. In a study of radiation-induced pulmonary fibrosis in Sprague Dawley rats, Dong *et al* showed a decrease in pro-fibrotic transforming growth factor-β and tumor necrosis factor-α after systemic MSC instillation (Dong *et al.* 2015). The authors speculate that MSCs also inhibit lung fibrosis through the secretion of hepatocyte growth factor and prostaglandin. Similarly, a review article of preclinical and clinical studies recapitulates the anti-fibrotic effects of MSCs in liver fibrosis (Berardis *et al.* 2015).

Taken together, the growing body of literature demonstrates the potential benefits MSCs may offer in endocrine disorders.

STRATEGIES TO ENHANCE MSC SURVIVAL AND FUNCTION

To offer regenerative effects to injured cells, transplanted MSCs must first survive the harsh environment of the treated tissue. In this niche, MSCs must overcome various stressors including hypoxia, inflammation, high acidity, and decreased energy reserves. Strategies to prolong survival of MSCs long enough to deliver a rich source of restorative factors, include: i) preconditioning the cells (hypoxia, mechanical stimulation), ii) genetically modifying the MSCs (viral transfection with promoter-targeted small hairpin RNA to overexpress/silence specific proteins), and iii) delivering MSCs with biomaterials (scaffolds, hydrogels). This concise review will present two strategic examples.

Hypoxic preconditioning:

Preclinical studies of myocardial infarction revealed that intracardiac injection of hypoxic treated stem cells sustained viability of surrounding cardiac cells, preserved cardiac function, and engraftment of cells to the injured heart was higher (Baglio et al. 2015). Work by Zhang and Chacko suggests that MSCs grown in hypoxia induces a pro-survival state (Chacko et al. 2010;

Zhang et al. 2016). These findings have also been linked to decreases in nuclear damage, apoptosis, and production of lactate dehydrogenase (Bader et al. 2015). Hypoxic preconditioning also increases MSC homing/motility via the stromal-derived factor-1 receptor/ CXCR4 transduction pathway, as well as through the focal adhesion kinase and potassium channel Kv2.1 signaling mechanism (Hu *et al.* 2011).

Vascular endothelial growth factor (genetic) overexpression:

In a rat model of myocardial infarction, overexpressing vascular endothelial growth factor (VEGF) via transfection with a viral vector, protected MSCs against cell death, stimulated vascular growth, improved cardiac function, and lessened infarct size (Augustin *et al.* 2013). Using a mouse model of diabetes, islet transplants treated with MSCs virally transduced to express VEGF demonstrated a lower blood glucose, restored euglycemia quicker after surgery, and improved graft vascularization (Hajizadeh-Saffar *et al.* 2015).

MESENCHYMAL STEM CELLS TO TREAT DIABETES

The versatile properties of MSCs have generated their clinical interest as therapies for diabetes. To date, over 40 clinical trials are registered using MSCs as therapeutic agents for diabetes. These studies range in scope from diabetes related vascular complications, to wound healing, and even include MSC therapy to treat new-onset diagnosis. As of May 29th, 2017, forty-seven MSC studies for diabetes are registered on clinicaltrials.gov. Here, we will summarize findings from clinical investigations addressing the use of MSC-based therapy for new-onset, as well as chronic, diabetes.

Diabetes Mellitus:

In 2015, investigators from Sweden (NCT01068951) reported the first study aimed to evaluate safety and efficacy of autologous MSC treatment in newly-diagnosed type 1 diabetics. Stem cells were harvested from the patient's iliac crest bone marrow and the median systemic single dose was 2.75 x 10^6 cells/kg. They concluded that administration of MSCs did not result in adverse events in any of the 10 patients and provided promising C-peptide concentrations at the one-year follow-up. This phase I trial did not show any functional differences between the control and MSC group in hemoglobin A1c (HbA1c) or insulin dose.

Hu *et al* conducted a single-center double blind study examining the safety, feasibility, and preliminary outcomes of umbilical cord Wharton's jelly-derived MSCs for new-onset type I diabetics (Hu *et al.* 2013). The MSC-treated group underwent two intravenous infusions (mean cell count of 2.6×10^7) separated 4 weeks apart. Postprandial glucose and HbA1c measurements were lower in the experimental cohort between 9 months to 24 months after MSC infusion. Also, insulin usage and fasting C-peptide were significantly improved in the MSC group. The study authors concluded that in their small study, not powered to detect functional differences, the transplant of umbilical cord MSCs is feasible and safe.

A pilot study in China involving placenta-derived MSCs to patients with long-standing diabetes mellitus type 2 revealed the transplantation was safe, easy, and potentially efficacious (Jiang *et al.* 2011). This investigation included 10 patients with type 2 diabetes for a duration ≥3 years, insulin dependent (≥0.7 U/kg/day) for at least one year, and poorly controlled glucose. The subjects received on average 1.35×10^6/kg placental stem cells on three separate occasions with one-month intervals between intravenous infusions. Six months after treatment, the insulin dosage and HbA1c measurements for all the patients demonstrated a trend towards improvement. Moreover, C-peptide and insulin release were also higher after MSC treatment. In addition, this

study included a group of individuals that translate closer to actual clinical scenarios, as they also had other co-morbidities, including heart disease, kidney disease, and vascular complications.

Lately, researchers have developed insulin-secreting MSCs and delivered them, in combination with hematopoietic stem cells, to patients with type I diabetes. (Vanikar *et al.* 2010; Thakkar *et al.* 2015). Autologous transplantation via the intra-pancreatic route tended to have an improved C-peptide and postprandial glucose at 15-24 months when compared to allogenic transplantation. Both studies viewed the stem cell administration as a safe procedure with potential benefit; however, larger studies will need to be conducted to substantiate their findings.

Table 1 summarizes a list of clinical trials utilizing MSCs for the treatment of diabetes.

WHICH DIABETIC PATIENTS WOULD BENEFIT FROM MSC THERAPY

Given the findings in the meta-analysis by El-Badawy and El-Badri, patients with diabetes type I and II can benefit from MSC therapy (El-Badawy & El-Badri 2016). Furthermore, the authors discuss that patients in the early stages of diabetes may be among the best candidates for stem cell treatment. Although 22 studies were included in this review, only 6 studies (total of 112 patients) used MSCs, of which only 2 studies focused on early-onset diagnosis (total of 49 patients). Still, the four studies in patients with chronic diabetes type I/II (average 8-year duration) had improvements in diabetic measures, which strongly justifies further studies to clearly delineate potential diabetic populations that may benefit from MSC therapy.

REGULATION OF CELL-BASED PRODUCTS PRIOR TO CLINICAL APPLICATION

Thus far, no standardized method for the isolation, characterization, expansion, potency testing, nor pathogen screening for MSCs exists (Arutyunyan *et al.* 2016; Smith *et al.* 2016; Weiss *et al.* 2016). The regulation of cell based products by the US Food and Drug Administration (FDA)

238 focuses on three main themes: i) prevention of transmitting communicable disease via
239 contaminated tissue, ii) proper handling and processing of tissue, and iii) demonstration of
240 clinical safety and effectiveness of cells, especially after extensive manipulation. The FDA also
241 requires tissue processing facilities to register, list their products, and provide accurate labeling
242 of the products. Recent review articles have presented specifics focusing on standardization and
243 production of clinical-grade stem cells (Giancola *et al.* 2012; Sensebé *et al.* 2013; Arutyunyan *et*
244 *al.* 2016; Smith *et al.* 2016; Weiss *et al.* 2016).

245 **MAINTENANCE OF UMBILICAL CORD MSCs**

246 Public and private biobanks have been firmly established for the cryopreservation of
247 hematopoietic stem cells from the umbilical cord blood. There has now been a recent option
248 from private banks for the cryopreservation of MSCs from cord tissue, as well as cord blood.
249 However, the cost of banking MSCs can become a concern as the initial charge is between
250 $1,000 to $3,000 for collection, processing, and preservation (Roura *et al.* 2012) . In addition, the
251 banking centers charge storage costs that amount to a few hundred dollars per year. Researchers
252 from Loughborough University presented a provocative cost-effectiveness analysis of allogeneic
253 induced pluripotent stem cell-derived β-cell therapy. Assuming the cost of stem cell therapy was
254 approximately $200,000, the graft/transplant survival required to achieve cost-effectiveness
255 (when compared to insulin therapy) with/without immunosuppressive therapy was calculated to
256 range between 8-11 years. Yet, current evidence indicates that graft β-cell function for 8-11 years
257 is highly unlikely. A more cost-effective approach may entail a cord blood-derived mesenchymal
258 stem cell administration (Bart 2010).

259 **ALLOGENEIC TRANSPLANTATION OF MSCs**

Advantages to allogeneic administration of MSCs include: i) wide availability, ii) low cost, iii) and quality control (Sarkar *et al.* 2010). Although it is well established that MSCs reduce the clinical sequelae of graft versus host disease, some studies question the safety of allografts. For instance, donor MSC infusion in a rat model of skin allograft transplantation induced an immunogenic response (higher TNF-α levels) (Sbano *et al.* 2008). In Seifert's animal study, pretreating a solid organ transplantation with allogeneic MSCs resulted in a trend to higher inflammatory levels and signs of rejection (Seifert *et al.* 2012). Despite these findings in the preclinical setting, phase I clinical trials have yet to report rejection/severe immunologic reactions after allogeneic transplantation of MSCs (Haarer *et al.* 2015). Larger and long-term human studies will need to assess the risk of rejection and/or inflammation secondary to donor-derived MSCs.

FUTURE OBJECTIVES

Before widespread use of MSCs (or their derivatives) in clinical medicine, many unresolved questions remain:

- How do we ensure that the MSCs are consistently produced and controlled per standard measures?
- What is the best source, route, dose, and number of administrations for clinical effectiveness?
- What are the long-term consequences of cell-based therapies (stem cells, conditioned media, exosomes, *etc.*)?
- Which strategies and tissue sources yield the best results?
- How do we optimize a scalable line of MSCs that are cost-effective for clinical application?
- Should MSCs/cell-based products be conditioned/altered to induce insulin-secreting potential?

Unravelling the cross-talk between the endogenous stem cell, exogenous stem cell, and their response to the microenvironment is critical in unlocking the potential use of MSCs as therapeutic agents in endocrinologic disorders.

CONCLUSION

Given their ability to mitigate fibrosis, modulate inflammation, and promote vascular growth, MSCs provide a promising therapeutic strategy for patients with endocrine disorders. The boundless availability of MSCs from various tissues and organs, as well as their beneficial properties, reinforce the widespread use of these cell types in regenerative studies. Although our understanding of factors mediating the function of MSCs has improved, there is still much that is not clearly understood. For instance, newer evidence is demonstrating that preconditioning/genetically altering MSCs may influence their function and thereby translate to improved clinical effects. Although large studies examining human application of MSCs are still lacking, initial studies in endocrine-focused studies demonstrate the potential for a paradigm shift. In sum, regenerative medicine remains a new and exciting field of research that holds much promise into the treatment of patients with endocrinologic diseases of all ages.

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REFERENCES

Acosta SA, Franzese N, Staples M, Weinbren NL, Babilonia M, Patel J, Merchant N, Simancas AJ, Slakter A, Caputo M *et al.* 2013 Human Umbilical Cord Blood for Transplantation Therapy in Myocardial Infarction. *Journal of Stem Cell Research & Therapy*.

Arno AI, Amini-Nik S, Blit PH, Al-Shehab M, Belo C, Herer E, Tien CH & Jeschke MG 2014 Human Wharton's jelly mesenchymal stem cells promote skin wound healing through paracrine signaling. *Stem Cell Research & Therapy* **5** 28. (doi:10.1186/scrt417)

Arutyunyan I, Elchaninov A, Makarov A & Fatkhudinov T 2016 Umbilical Cord as Prospective Source for Mesenchymal Stem Cell-Based Therapy. *Stem Cells International* **2016** 6901286. (doi:10.1155/2016/6901286)

Augustin M, Mahar MAA, Lakkisto P, Tikkanen I, Vento A, Pätilä T & Harjula A 2013 VEGF overexpression improves mesenchymal stem cell sheet transplantation therapy for acute myocardial infarction. *Journal of Tissue Engineering and Regenerative Medicine* **7** 742–750. (doi:10.1002/term.1471)

Azandeh S, Orazizadeh M, Hashemitabar M, Khodadadi A, Shayesteh AA, Nejad DB, Gharravi AM & Allahbakhshi E 2012 Mixed enzymatic-explant protocol for isolation of mesenchymal stem cells from Wharton's jelly and encapsulation in 3D culture system. *J. Biomedical Science and Engineering* **5** 580–586. (doi:10.4236/jbise.2012.510071)

Baglio SR, Rooijers K, Koppers-Lalic D, Verweij FJ, Pérez Lanzón M, Zini N, Naaijkens B, Perut F, Niessen HWM, Baldini N *et al.* 2015 Human bone marrow- and adipose-mesenchymal stem cells secrete exosomes enriched in distinctive miRNA and tRNA species. *Stem Cell Research & Therapy* **6** 127. (doi:10.1186/s13287-015-0116-z)

348 Bart T 2010 Cost effectiveness of cord blood versus bone marrow and peripheral blood stem
349 cells. 141–147. (doi:10.2147/CEOR.S11210)

350 Berardis S, Dwisthi Sattwika P, Najimi M & Sokal EM 2015 Use of mesenchymal stem cells to
351 treat liver fibrosis: current situation and future prospects. *World Journal of*
352 *Gastroenterology* **21** 742–758. (doi:10.3748/wjg.v21.i3.742)

353 Boeuf S, Richter W, Richter W, Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O,
354 Peterson L, Friedenstein A *et al.* 2010 Chondrogenesis of mesenchymal stem cells: role of
355 tissue source and inducing factors. *Stem Cell Research & Therapy* **1** 31.
356 (doi:10.1186/scrt31)

357 Bruyn D, Najar M, Raicevic G, Meuleman N, Pieters K, Stamatopoulos B, Delforge A, Bron D
358 & Lagneaux L 2011 A Rapid , Simple , and Reproducible Method for the Isolation of
359 Mesenchymal Stromal Cells from Wharton ' s Jelly Without Enzymatic Treatment. **20**.

360 Cai J, Wu Z, Xu X, Liao L, Chen J, Huang L, Wu W, Luo F, Wu C, Pugliese A *et al.* 2016
361 Umbilical Cord Mesenchymal Stromal Cell with Autologous Bone Marrow Cell
362 Transplantation in Established Type 1 Diabetes: A Pilot Randomized Controlled Open-
363 Label Clinical Study to Assess Safety and Impact on Insulin Secretion. *Diabetes Care* **39**
364 149–157. (doi:10.2337/dc15-0171)

365 Caplan AI & Correa D 2011 The MSC: an injury drugstore. *Cell Stem Cell* **9** 11–15.
366 (doi:10.1016/j.stem.2011.06.008)

367 Carlsson P-O, Schwarcz E, Korsgren O & Le Blanc K 2015 Preserved β-Cell Function in Type 1
368 Diabetes by Mesenchymal Stromal Cells. *Diabetes* **64**.

Dave SD, Vanikar A V., Trivedi HL, Thakkar UG, Gopal SC & Chandra T 2015 Novel therapy for insulin-dependent diabetes mellitus: infusion of in vitro-generated insulin-secreting cells. *Clinical and Experimental Medicine* **15** 41–45. (doi:10.1007/s10238-013-0266-1)

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D & Horwitz E 2006 Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* **8** 315–317. (doi:10.1080/14653240600855905)

Dong L-H, Jiang Y-Y, Liu Y-J, Cui S, Xia C-C, Qu C, Jiang X, Qu Y-Q, Chang P-Y & Liu F 2015 The anti-fibrotic effects of mesenchymal stem cells on irradiated lungs via stimulating endogenous secretion of HGF and PGE2. *Scientific Reports* **5** 8713. (doi:10.1038/srep08713)

El-Badawy A & El-Badri N 2016 Clinical Efficacy of Stem Cell Therapy for Diabetes Mellitus: A Meta-Analysis. *PLOS ONE* **11** e0151938. (doi:10.1371/journal.pone.0151938)

El-Demerdash RF, Hammad LN, Kamal MM & El Mesallamy HO 2015 A comparison of Wharton's jelly and cord blood as a source of mesenchymal stem cells for diabetes cell therapy. *Regenerative Medicine* **10** 841–855. (doi:10.2217/rme.15.49)

Fan L, Zhang C, Yu Z, Shi Z, Dang X & Wang K 2015 Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and osteogenesis in rabbit femoral head osteonecrosis. *Bone* **81** 544–553. (doi:10.1016/j.bone.2015.09.005)

Feng Y, Wang J, Ling S, Li Z, Li M, Li Q, Ma Z & Yu S 2014 Differentiation of mesenchymal stem cells into neuronal cells on fetal bovine acellular dermal matrix as a tissue engineered

nerve scaffold. *Neural Regeneration Research* **9** 0. (doi:10.4103/1673-5374.145378)

Fong C-Y, Chak L-L, Biswas A, Tan J-H, Gauthaman K, Chan W-K & Bongso A 2011 Human Wharton's Jelly Stem Cells Have Unique Transcriptome Profiles Compared to Human Embryonic Stem Cells and Other Mesenchymal Stem Cells. *Stem Cell Reviews and Reports* **7** 1–16. (doi:10.1007/s12015-010-9166-x)

Giancola R, Bonfini T & Iacone A 2012 Cell therapy: cGMP facilities and manufacturing. *Muscles, Ligaments and Tendons Journal* **2** 243–247.

Gupta N, Su X, Popov B, Lee JW, Serikov V, Matthay MA, Gupta N, Su X, Popov B, Lee JW *et al.* 2015 Acute Lung Injury in Mice 1. (doi:10.4049/jimmunol.179.3.1855)

Haarer J, Johnson CL, Soeder Y & Dahlke MH 2015 Caveats of mesenchymal stem cell therapy in solid organ transplantation. *Transplant International* **28** 1–9. (doi:10.1111/tri.12415)

Hajizadeh-Saffar E, Tahamtani Y, Aghdami N, Azadmanesh K, Habibi-Anbouhi M, Heremans Y, De Leu N, Heimberg H, Ravassard P, Shokrgozar MA *et al.* 2015 Inducible VEGF expression by human embryonic stem cell-derived mesenchymal stromal cells reduces the minimal islet mass required to reverse diabetes. *Scientific Reports* **5** 9322. (doi:10.1038/srep09322)

Hammes H-P, Feng Y, Pfister F & Brownlee M 2011 Diabetic retinopathy: targeting vasoregression. *Diabetes* **60** 9–16. (doi:10.2337/db10-0454)

Hiew V, Fatimah S & Teoh L 2016 Comparison of explant-derived and enzymatic digestion-derived mesenchymal stem cells from Whartonðs jelly. *Frontiers in Bioengineering and Biotechnology* **4**. (doi:10.3389/conf.FBIOE.2016.02.00030)

Hsuan YC-Y, Lin C-H, Chang C-P & Lin M-T 2016 Mesenchymal stem cell-based treatments for stroke, neural trauma, and heat stroke. *Brain and Behavior* **6** e00526. (doi:10.1002/brb3.526)

Hu X, Wei L, Taylor TM, Wei J, Zhou X, Wang J-A & Yu SP 2011 Hypoxic preconditioning enhances bone marrow mesenchymal stem cell migration via Kv2.1 channel and FAK activation. *American Journal of Physiology - Cell Physiology* **301** C362–C372. (doi:10.1152/ajpcell.00013.2010)

Hu J, Yu X, Wang Z, Wang F, Wang L, Gao H, Chen Y, Zhao W, Jia Z, Yan S *et al.* 2013 Long term effects of the implantation of Wharton's jelly-derived mesenchymal stem cells from the umbilical cord for newly-onset type 1 diabetes mellitus. *Endocrine Journal* **60** 347–357.

Hu J, Wang Y, Gong H, Yu C, Guo C, Wang F, Yan S & Xu H 2016 Long term effect and safety of Wharton's jelly-derived mesenchymal stem cells on type 2 diabetes. *Experimental and Therapeutic Medicine* **12** 1857–1866. (doi:10.3892/etm.2016.3544)

Jiang R, Han Z, Zhuo G, Qu X, Li X, Wang X, Shao Y, Yang S & Han ZC 2011 Transplantation of placenta-derived mesenchymal stem cells in type 2 diabetes: a pilot study. *Frontiers of Medicine* **5** 94–100. (doi:10.1007/s11684-011-0116-z)

Johnson KE & Wilgus TA 2014 Vascular Endothelial Growth Factor and Angiogenesis in the Regulation of Cutaneous Wound Repair. *Advances in Wound Care* **3** 647–661. (doi:10.1089/wound.2013.0517)

KEILHOFF G, GOIHL A, LANGNASE K, FANSA H & WOLF G 2006 Transdifferentiation of mesenchymal stem cells into Schwann cell-like myelinating cells. *European Journal of Cell Biology* **85** 11–24. (doi:10.1016/j.ejcb.2005.09.021)

Liu X, Zheng P, Wang X, Dai G, Cheng H, Zhang Z, Hua R, Niu X, Shi J & An Y 2014 A preliminary evaluation of efficacy and safety of Wharton's jelly mesenchymal stem cell transplantation in patients with type 2 diabetes mellitus. *Stem Cell Research & Therapy* **5** 57. (doi:10.1186/scrt446)

Mauck RL, Yuan X & Tuan RS 2006 Chondrogenic differentiation and functional maturation of bovine mesenchymal stem cells in long-term agarose culture. *Osteoarthritis and Cartilage* **14** 179–189. (doi:10.1016/j.joca.2005.09.002)

McNamara LM Osteogenic differentiation of mesenchymal stem cells is regulated by osteocyte and osteoblast cells in a simplified bone niche. *European Cells and Materials* **23** 2012–2013. (doi:10.22203/eCM.v023a02)

Monteiro R & Azevedo I 2010 Chronic inflammation in obesity and the metabolic syndrome. *Mediators of Inflammation* **2010**. (doi:10.1155/2010/289645)

Mori Y, Ohshimo J, Shimazu T, He H, Takahashi A, Yamamoto Y, Tsunoda H, Tojo A & Nagamura-Inoue T 2015 Improved explant method to isolate umbilical cord-derived mesenchymal stem cells and their immunosuppressive properties. *Tissue Engineering. Part C, Methods* **21** 367–372. (doi:10.1089/ten.TEC.2014.0385)

Moshtagh PR, Emami SH & Sharifi AM 2013 Differentiation of human adipose-derived mesenchymal stem cell into insulin-producing cells: an in vitro study. *Journal of Physiology and Biochemistry* **69** 451–458. (doi:10.1007/s13105-012-0228-1)

Murphy MB, Moncivais K & Caplan AI 2013 Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. *Experimental & Molecular Medicine* **45** e54. (doi:10.1038/emm.2013.94)

Nagamura-Inoue T & Mukai T 2015 Umbilical Cord is a Rich Source of Mesenchymal Stromal Cells for Cell Therapy. *Current Stem Cell Research & Therapy*.

Paneni F, Beckman JA, Creager MA & Cosentino F Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. (doi:10.1093/eurheartj/eht149)

Pers Y-M, Ruiz M, Noël D & Jorgensen C 2015 Mesenchymal stem cells for the management of inflammation in osteoarthritis: state of the art and perspectives. *Osteoarthritis and Cartilage* **23** 2027–2035. (doi:10.1016/j.joca.2015.07.004)

Rostamzadeh A, Anjomshoa M, Kurd S, Chai J-K, Jahangiri F, Nilforoushzadeh MA & Zare S 2015 The Role of Wharton's Jelly Mesenchymal Stem Cells in Skin Reconstruction. *Journal of Skin and Stem Cell* **2**. (doi:10.17795/jssc30347)

Roura S, Pujal J-M, Gálvez-Montón C & Bayes-Genis A 2012 Generation of disease-specific induced pluripotent stem cells from patients with different karyotypes of Down syndrome. (doi:10.1186/s13287-015-0113-2)

Salehinejad P, Alitheen NB, Ali AM, Omar AR, Mohit M, Janzamin E, Samani FS, Torshizi Z & Nematollahi-Mahani SN 2012 Comparison of different methods for the isolation of mesenchymal stem cells from human umbilical cord Wharton's jelly. *In Vitro Cellular & Developmental Biology - Animal* **48** 75–83. (doi:10.1007/s11626-011-9480-x)

Sarkar D, Vemula PK, Zhao W, Gupta A, Karnik R, Karp JM & Hu D 2010 Engineered mesenchymal stem cells with self-assembled vesicles for systemic cell targeting. *Biomaterials* **31** 5266–5274. (doi:10.1016/j.biomaterials.2010.03.006)

Sbano P, Cuccia A, Mazzanti B, Urbani S, Giusti B, Lapini I, Rossi L, Abbate R, Marseglia G, Nannetti G *et al.* 2008 Use of donor bone marrow mesenchymal stem cells for treatment of skin allograft rejection in a preclinical rat model. *Archives of Dermatological Research* **300** 115–124. (doi:10.1007/s00403-007-0827-9)

Scott MA, Nguyen VT, Levi B & James AW 2011 Current methods of adipogenic differentiation of mesenchymal stem cells. *Stem Cells and Development* **20** 1793–1804. (doi:10.1089/scd.2011.0040)

Seifert M, Stolk M, Polenz D & Volk H-D 2012 Detrimental effects of rat mesenchymal stromal cell pre-treatment in a model of acute kidney rejection. *Frontiers in Immunology* **3** 202. (doi:10.3389/fimmu.2012.00202)

Sensebé L, Gadelorge M & Fleury-Cappellesso S 2013 Production of mesenchymal stromal/stem cells according to good manufacturing practices: a review. *Stem Cell Research & Therapy* **4** 66. (doi:10.1186/scrt217)

Skyler JS, Fonseca VA, Segal KR & Rosenstock J 2015 Allogeneic mesenchymal precursor cells in type 2 diabetes: A randomized, placebo-controlled, dose-escalation safety and tolerability pilot study. *Diabetes Care* **38** 1742–1749. (doi:10.2337/dc14-2830)

Smith JR, Pfeifer K, Petry F, Powell N, Delzeit J & Weiss ML 2016 Standardizing Umbilical Cord Mesenchymal Stromal Cells for Translation to Clinical Use: Selection of GMP-Compliant Medium and a Simplified Isolation Method. *Stem Cells International* **2016** 1–14. (doi:10.1155/2016/6810980)

Talaei-Khozani T, Borhani-Haghighi M, Ayatollahi M & Vojdani Z 2015 An in vitro model for hepatocyte-like cell differentiation from Wharton's jelly derived-mesenchymal stem cells

by cell-base aggregates. *Gastroenterology and Hepatology from Bed to Bench* **8** 188–199.

Thakkar UG, Trivedi HL, Vanikar A V. & Dave SD 2015 Insulin-secreting adipose-derived mesenchymal stromal cells with bone marrow–derived hematopoietic stem cells from autologous and allogenic sources for type 1 diabetes mellitus. *Cytotherapy* **17** 940–947. (doi:10.1016/j.jcyt.2015.03.608)

Thibault RA, Scott Baggett L, Mikos AG & Kasper FK 2010 Osteogenic differentiation of mesenchymal stem cells on pregenerated extracellular matrix scaffolds in the absence of osteogenic cell culture supplements. *Tissue Engineering. Part A* **16** 431–440. (doi:10.1089/ten.TEA.2009.0583)

Vangsness CT, Sternberg H & Harris L 2015 Umbilical Cord Tissue Offers the Greatest Number of Harvestable Mesenchymal Stem Cells for Research and Clinical Application: A Literature Review of Different Harvest Sites. *Arthroscopy: The Journal of Arthroscopic & Related Surgery* **31** 1836–1843. (doi:10.1016/j.arthro.2015.03.014)

Vanikar A V., Dave SD, Thakkar UG & Trivedi HL 2010 Cotransplantation of Adipose Tissue-Derived Insulin-Secreting Mesenchymal Stem Cells and Hematopoietic Stem Cells: A Novel Therapy for Insulin-Dependent Diabetes Mellitus. *Stem Cells International* **2010** 1–5. (doi:10.4061/2010/582382)

Wang L, Zhao Y & Shi S 2012 Interplay between mesenchymal stem cells and lymphocytes: implications for immunotherapy and tissue regeneration. *Journal of Dental Research* **91** 1003–1010. (doi:10.1177/0022034512460404)

Wei L, Fraser JL, Lu Z-Y, Hu X & Yu SP 2012 Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and neurogenesis after cerebral

ischemia in rats. *Neurobiology of Disease* **46** 635–645. (doi:10.1016/j.nbd.2012.03.002)

Weiss ML & Troyer DL 2006 Stem cells in the umbilical cord. *Stem Cell Reviews* **2** 155–162. (doi:10.1007/s12015-006-0022-y)

Weiss ML, Rao MS, Deans R & Czermak P 2016 Manufacturing Cells for Clinical Use. *Stem Cells International* **2016** 1–5. (doi:10.1155/2016/1750697)

Westhrin M, Xie M, Olderøy MØ, Sikorski P, Strand BL, Standal T, Szpalski C, Wetterau M, Barr J, Warren S *et al.* 2015 Osteogenic Differentiation of Human Mesenchymal Stem Cells in Mineralized Alginate Matrices. *PLOS ONE* **10** e0120374. (doi:10.1371/journal.pone.0120374)

541 **Table 1:** Summary of clinical studies using mesenchymal stem cells as a treatment for diabetes

Author, year, MSC sample size, country	Objective	Inclusion criteria	MSC source	MSC dose & delivery	Outcomes
(Cai *et al.* 2016) n=21 China 1-year study	Investigate the potential benefits on metabolic control and safety of combined UC-MSC and autologous bone marrow mononuclear cell transplantation without immunotherapy in patients with established T1D	-18–40 years -both genders -history of T1D ≥2 and ≤16 yrs -HbA1c ≥7.5% & ≤10.5% -fasting serum C-peptide <0.1 pmol/mL -daily insulin requirements <100 IU	Umbilical cord Wharton's jelly-derived MSC from single term neonate + Autologous bone marrow mononuclear cells from iliac crests	UC-MSCs (1 x 10^6/kg) BM-MNCs (107 x 10^6/kg) intrapancreatic	No severe adverse events in MSC cohort 1 pt with transient abdominal pain; 1 pt with puncture site bleeding Less self-reported hypoglycemic events in MSC group C-peptide AUC improved by 106% in MSC group, while control group had decrease by 8% Serum insulin AUC increased 49% in MSC group, control group decreased by 6% HbA1c, FBG, insulin dose levels decreased at 3, 6, 9, and 12 months, whereas they remained stable in the control group
(Hu *et al.* 2016) n=31 China 3-year study	Explore the long-term safety and efficacy of WJ-MSCs infusion in T2DM patients with a follow-up period of 36 months	-18-60 years of age with T2DM -both genders -diabetes diagnosis according to ADA	Umbilical cord Wharton's jelly-derived MSC from single term neonate	Two intravenous infusions separated by 1 month Dose per infusion: 1 x 10^6/kg	No serious adverse reactions noted, including: fever, chills, liver toxicity, hypersensitivity, infection, hemorrhage, proteinuria, myocardial infarction, or thromboembolic events None of the patients experienced severe hypoglycemia Improvements in C-peptide and insulin dosage were observed in MSC group Mild benefit in HbA1c and fasting plasma glucose

(Skyler *et al.* 2015) n=45 United States 12-week study	Assess the safety, tolerability, and feasibility of adult allogeneic bone marrow-derived mesenchymal precursor cells in T2D inadequately controlled with metformin either alone or with one additional oral antidiabetic agent	-<80 years of age with T2D -HbA1 ≥7.0% to <10.5% -metformin either alone or in combination with one other oral antidiabetic medication (except a thiazolidinedione) for at least 3 months -Women of childbearing potential who were surgically sterile or agreed to use contraception during the entire study were eligible	Bone marrow-derived mesenchymal precursor cells	0.3 x 10^6/kg (n=15) 1 x 10^6/kg (n=15) 2 x 10^6/kg (n=15) intravenous	Treatment emergent adverse events were comparable between MSC and placebo groups 1 subject with severe abdominal pain in MSC group No serious adverse events during 12-week study No discontinuations or serious hypoglycemic events in MSC group Experimental group did not have immunologic response to MSCs
(Carlsson *et al.* 2015) n=9 Sweden 1-year study	Evaluate the safety and efficacy of autologous MSCs in treatment of patients recently diagnosed with type 1 diabetes	-18–40 years of age with T1D -diagnosed <3 weeks before enrollment and with a stimulated C-peptide level >0.1 nmol/L	Autologous bone marrow mononuclear cells from iliac crests	median 2.75 × 10^6 cells/kg intravenous	MSC group tolerated transplant with no side effects No tumors or chronic infections have been diagnosed in any of the study None of the study patients have had any episodes of either hyperglycemic ketoacidosis AUC for C-peptide values (after meal tolerance test) in MSC group were preserved/increased
(Dave *et al.* 2015) n=10 India 3-year study	Describe experience of treating IDDM with co-infusion of in vitro MSC-differentiated insulin-secreting cells with hematopoietic stem cells	-8-45 years of age with IDDM -any gender -diagnosis at least for 6 months, with low levels of serum C-peptide levels (<0.5 ng/mL)	Autologous adipose tissue MSC-differentiated into insulin-secreting cells + Autologous bone marrow-derived HSC	Autologous: 2.7 x 10^4/kg insulin secreting MSC Allogeneic: adipose MSCs-2.1 x 10^4/kg insulin secreting MSC infused into portal circulation, thymus and	There were no untoward effects of stem cell infusion All pts had improved C-peptide, Hb1Ac, blood sugar status and exogenous insulin requirement Pts returned to normal lifestyle and unrestricted diet

				into subcutaneous tissue	
(Thakkar *et al.* 2015) n=20 (10 autologous; 10 allogeneic) India 2-year study	Assess safety and efficacy of autologous vs. allogeneic stem cell transplantation	-8-45 years of age with T1DM -diagnosed >12 months ago -presence of glutamic acid decarboxylase (GAD) antibodies -low serum C-peptide	Autologous group: abdominal fat MSCs and bone marrow HSCs Allogeneic group: non-diabetic abdominal fat MSCs and bone marrow HSCs	Autologous: 2.7 x 10^4/kg insulin secreting MSC Allogeneic: adipose MSCs-2.1 x 10^4/kg insulin secreting MSC infused into portal circulation, thymus and abdominal subcutaneous tissue	No untoward effect, morbidity, or mortality Sustained improvement in mean insulin requirement, serum C-peptide, mean HbA1c
(Hu *et al.* 2013) n=15 China 2-year study	Assess the long-term effects of WJ-MSCs for newly-onset T1DM	-patients of both sexes ≤25 years with T1DM according to ADA -≤6 months with fasting C-peptide ≥ 0.3 ng/mL	Umbilical cord Wharton's jelly-derived MSC from neonates	2.6 x 10^7 cells intravenous	No obvious adverse reactions occurred No difference in the fasting blood glucose between control and experimental group After 9 months, the HbA1c, insulin dosage, and C-peptide improved in the MSC group
(Vanikar *et al.* 2010) n=11 India 1-year study	Present findings of insulin replacement therapy by co-transplantation of insulin-secreting adipose derived MSCs and bone marrow HSCs	-5-45 years of age with IDDM for at least 6 months -any gender - low levels of serum C-peptide levels (<0.5 ng/mL)	adipose tissue and bone marrow derived MSCs and HSCs, respectively	Mean total cell quantum transplanted was 96 mls with nucleated cell counts of cultured bone marrow: average of 28×10^3/μL and MSC-1.2×10^3/μL	No adverse/untoward side effect related to stem cell infusion or administration of induction therapy No DKA in any of the patients

(Liu *et al.* 2014) n=22 China 1-year study	Explored the efficacy and safety of WJ-MSC transplantation in T2DM patients and followed up with them for 12 months after treatment	-18-70 years of age with T2DM according to ADA criteria -any gender, not pregnant or nursing -poor glycemic control with recent anti-diabetic therapies, including drugs and/or insulin injection for at least three months -negative for glutamic acid decarboxylase antibody -fasting blood glucose level ≥7.0mmol/L and HbA1c ≥ 7% -organic sufficiency: heart, liver, kidney and lung	Umbilical cord Wharton's jelly-derived MSC from term neonate	1st transplant: Intravenous 2nd transplant: Intrapancreatic Dose for each infusion:1 × 10^6 cells/kg	3 patients with fever after operative day 1 patient with subcutaneous hematoma 1 patient with nausea, vomiting, and headache Mild improvement in HbA1c, insulin dosage, and fasting C-peptide Markers of systemic inflammation were decreased at 6 months
(Jiang *et al.* 2011) n=10 China	Evaluate the safety and clinical feasibility of placenta-derived MSCs in T2DM	-30-85 years of age with T2DM -duration of diabetes ≥3 years -requiring insulin for optimal glycemic control in a dose of ≥0.7 U/kg/day at least for 1 year	Placenta-derived MSCs	Average total of 1.35 x 10^6/kg Three intravenous infusions separated by 1 month	No systemic manifestations were observed after cell transplantation At 6 months, average insulin dosage, C-peptide, and HbA1c improved after treatment

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543 UC-MSC-umbilical cord-derived mesenchymal stem cell; T1D-type I diabetes; AUC-area under
544 the curve; FBG-fasting blood glucose; WJ-Wharton's jelly; T2DM-type II diabetes; ADA-
545 American Diabetes Association; IDDM-Insulin dependent diabetes mellitus; HSC-
546 Hematopoietic stem cells; DKA-Diabetes ketoacidosis

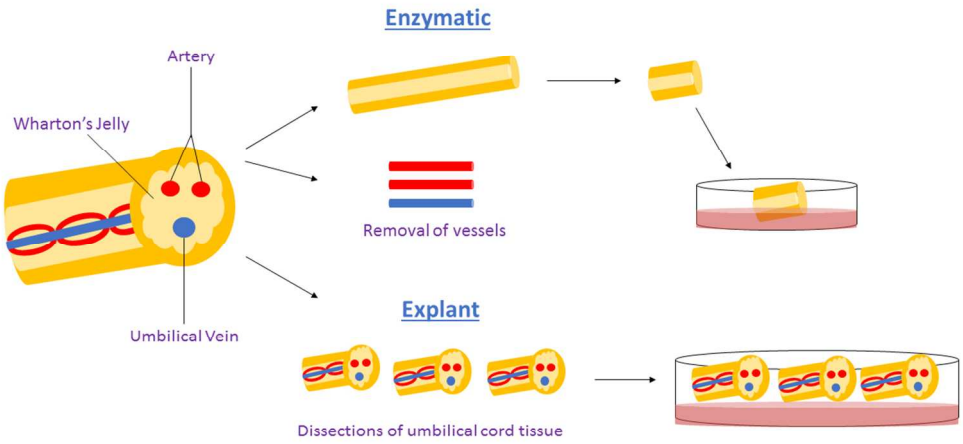
FIGURE 1. Enzymatic versus Explant method for obtaining WJ-MSCs

WJ-MSCs-Wharton's Jelly-derived mesenchymal stem cells

FIGURE 2. Characterization of WJ-MSCs. A) Cross-section of human umbilical cord. B) Plastic adherence of fibroblast-like appearance of WJ-MSCs. Magnification at 10x. C) Flow cytometry of WJ-MSC surface antigen markers. D) Multi-lineage differentiation of WJ-MSCs into a) Osteogenic (Alizarin Red stain) cells, b) Adipogenic (Oil Red O stain), and c) Chondrogenic (Alcian blue) cells. Magnification at 10x.

FIGURE 3. Therapeutic effects of mesenchymal stem cells

VEGF-vascular endothelial growth factor; ANG-angiopoietin; EPO-erythropoietin; HIF-hypoxia inducible factor, TNF-tumor necrosis factor, FAK-focal adhesion kinase



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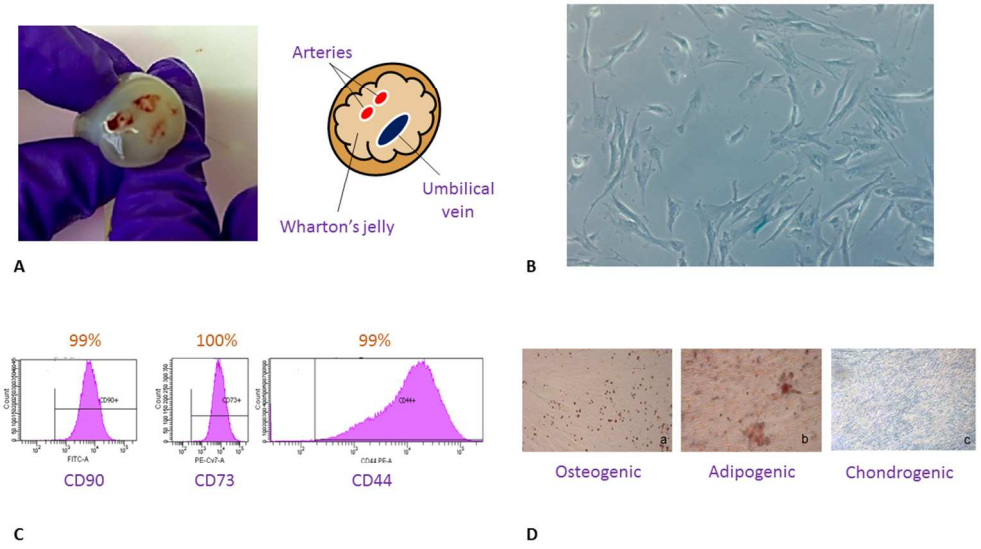

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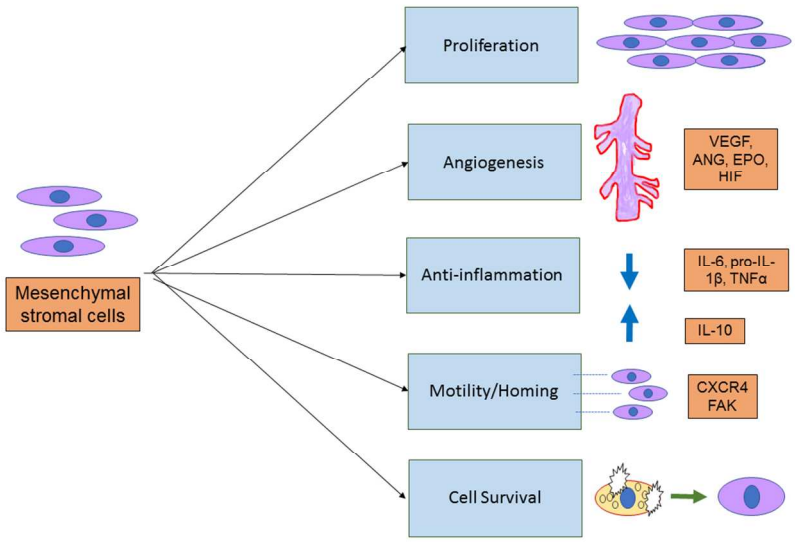

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