



(43) International Publication Date
4 October 2012 (04.10.2012)

(10) International Publication Number
WO 2012/131618 A1

- (51) **International Patent Classification:**
C12N 5/0775 (2010.01) *A61P 37/00* (2006.01)
A61K 35/44 (2006.01)
- (21) **International Application Number:**
PCT/IB2012/051519
- (22) **International Filing Date:**
29 March 2012 (29.03.2012)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
1004/CHE/2011 30 March 2011 (30.03.2011) IN
- (71) **Applicant (for all designated States except US):** **STEM-PEUTICS RESEARCH PRIVATE LIMITED** [IN/IN];
9th Floor, Manipal Hospital, 98, Rustom Bagh, Airport Road, Bangalore, Karnataka 560 017 (IN).
- (72) **Inventors; and**
- (75) **Inventors/Applicants (for US only):** **TA, Malancha,** [IN/IN]; C/o Stempeutics Research Pvt. Ltd, 9th Floor, Manipal Hospital, 98, Rustom, Bagh, Airport Road, Bangalore, Karnataka, 560 017 (IN). **MAJUMDAR, Anish, Sen,** [IN/IN]; C/o Stempeutics Research Pvt. Ltd, 9th Floor, Manipal Hospital, 98, Rustom, Bagh, Airport Road, Bangalore, Karnataka, 560 017 (IN).
- (74) **Agents:** **BHOLA, Ravi** et al.; # 4121/B, 6th Cross, 19th A Main, HAL II Stage [Extension], Bangalore, Karnataka 560 038 (IN).
- (81) **Designated States (unless otherwise indicated, for every kind of national protection available):** AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) **Designated States (unless otherwise indicated, for every kind of regional protection available):** ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))
- of inventorship (Rule 4.17(iv))

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) **Title:** A COMPOSITION COMPRISING POOLED WHARTON'S JELLY DERIVED MESENCHYMAL STEM CELLS AND METHODS THEREOF

(57) **Abstract:** The present disclosure discloses methods of isolating, culturing and pooling of Wharton's jelly derived cells to obtain a composition of pooled allogeneic mesenchymal stem cells (MSCs). The method comprises of collecting umbilical cords from multiple donors followed by isolation, culturing and pooling of WJ-MSCs from which the final therapeutic composition is formulated. The composition thus obtained has reduced biological variability. Since the present composition is also immune-modulatory in nature, it can be used for managing immune-mediated disorders and additional immuno-suppression may not be required when using this composition for managing immune-mediated disorders.



WO 2012/131618 A1

**“A COMPOSITION COMPRISING POOLED WHARTON’S JELLY
DERIVED MESENCHYMAL STEM CELLS AND METHODS
THEREOF”**

5 TECHNICAL FIELD

The present disclosure relates to stem cell science in general while, in particular it relates to methods of isolating, culturing and pooling of Wharton’s jelly derived cells to obtain a composition of pooled allogeneic mesenchymal stem cells (MSCs) having reduced immune variability (immunogenicity/increased immunosuppression) for clinical
10 application. The disclosure also relates to the said composition and the method of managing immune-mediated disorders by application of the said composition.

BACKGROUND AND PRIOR ART OF THE DISCLOSURE

Stem cells are characterized by their self-renewal ability and differentiation potential and
15 can be divided into embryonic and adult stem cells. Most adult stem cells are minor populations found in adult organs and can differentiate to specific cell lineages; Mesenchymal stem cells (MSCs) belong to this group. Several lines of evidence have shown that under appropriate environments, MSCs are able to differentiate into mesodermal, endodermal and even ectodermal cells. M.F. Pittenger et al (Science 1999;
20 284:143-147) disclose Multilineage potential of adult human mesenchymal stem cells.

In addition, MSCs have the ability to migrate and engraft into host tissues. They tend to home to sites of tissue growth and repair and enhance tissue regeneration. Another intriguing feature of MSCs is they are hypoimmunogenic cells, i.e., they possess immunomodulatory effects and can escape immune recognition. Le Blanc K (
25 Cytotherapy 2003; 5:485-489) discloses in her paper immunomodulatory effects of fetal and adult mesenchymal stem cells and Keyser KA et al., (Cell Transplant 2007; 16:555-562) discloses comparison of mesenchymal stem cells from different tissues to suppress T-cell activation. They lack surface expression of major histocompatibility complex class II molecules in the resting state and also lack expression of costimulatory molecules for
30 T cell induction (CD40, CD40 ligand, and the B7 molecules CD80 and CD86) Tse WT et al., (Transplantation.2003; 75(3): 389-397), in his paper he discussed on

immunosuppression of allogeneic T cell proliferation by bone marrow stromal cells in cell transplantation. MSCs downregulate lymphocyte proliferation and suppress the maturation and function of various other immune cells, which make them a very promising tool for regenerative and cell transplantation therapy.

- 5 The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cell Therapy (ISCT) suggested three defining criteria for mesenchymal stromal cells (MSCs). MSCs must be a plastic-adherent cell population, must express specific surface markers (e.g., positive for CD73, CD29, CD105 and negative for CD14, CD 19, CD34, CD45, and HLA class II), and must be able to differentiate into various lineages including bone,
10 cartilage and adipose under standard differentiating culture conditions *in-vitro* as per 'Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement.'

MSCs were first identified by Friedenstein and his colleagues, who described bone-forming progenitor cells from rat bone marrow (BM). (See, A.J. Friedenstein et al., (J. Embryol. Exp. Morphol. 1966; 16: 381–390)). Though BM is considered to be the best-
15 characterized source of MSCs, its harvest is a highly invasive and painful procedure for the donor and the number, proliferation and differentiation potential decreases with age. There are now various other sources of MSCs and one such alternative source is the umbilical cord which, though a fetus-derived tissue, it can be easily obtained, processed
20 and is noncontroversial. Originating from epiblast of the human embryo, umbilical cord is composed of two arteries and one vein, all of which are surrounded by a unique connective tissue stroma, rich in proteoglycans and mucopolysaccharides, called Wharton's Jelly (WJ). WJ was first described by Thomas Wharton in 1656, (see Wharton, TW: 1656, Adenographia (translated by Freer S.), Oxford, UK, Oxford Univ.
25 Press, pp. 242-248;1996)) while isolation of fibroblast-like cells from the WJ of the umbilical cord was originally described in 1991. McElreavey KD et al., (Biochem Soc Trans.1991; 19:29S) discloses method of isolating, culturing and characterizing fibroblast like cells derived from the Wharton's jelly portion of human umbilical cord.

WJ-derived MSCs possess multipotent properties between embryonic stem cells and
30 adult stem cells and have faster proliferation and greater ex vivo expansion capabilities than BMSCs, (see A. Bongso et al., (J.Cell. Biochem. 2008; 105:1352-1360); and A. Can

et al., (Stem Cells. 2007; 11: 2886-2896)). WJ-MSCs have unique properties of high proliferation rates, wide multipotency, hypoimmunogenicity and they do not induce teratomas (Human Wharton's Jelly Stem Cells Have Unique Transcriptome Profiles Compared to Human Embryonic Stem Cells and Other Mesenchymal Stem Cells). On
5 studying in detail the immune properties of WJ-MSCs, Troyer and Weiss reported that there is no evidence for immune rejection of undifferentiated WJ-MSCs in vivo, thus concluding that they are well suited for allogeneic transplantations, (Troyer, D. L et al., (Stem Cells. 2008;26:591–599)).

MSC transplantation requires harvesting a large number of cells. They are present in very
10 low percentage in their niches and a vast number of cells are required to provide sufficient quantity for subsequent therapeutic applications. MSC seeding density as well as choice of medium and type of tissue culture plastic can influence cell proliferation. It has been reported previously that human cells proliferate most rapidly and maximally retain the multipotentiality when seeded at low densities. Sekiya, et al., (Stem Cells.
15 2002; 20 :530–541) have evaluated conditions that maximized the yields of early progenitors and their quality by studying the expansion of human adult stem cells from bone marrow stroma. Basic fibroblast growth factor (bFGF) is a well-established growth factor which can enhance cell proliferation. There are reports of bFGF being used as a potent mitogen for MSCs in culture while maintaining their differentiation potential and
20 telomere length S. Tsutsumi et al., (Biochem. Biophys. Res. Commun. 2001;288: 413–419)).

MSC-mediated immunoregulatory effects can be applied towards the management of various autoimmune disorders (see Uccelli A et al., (*Curr Opin Immunol.* 2010; 22(6):768-74)). In fact, many clinical trials using MSCs for the management of
25 autoimmunity are currently in progress in settings of Crohn's disease, type 1 diabetes, MS, systemic lupus erithematosus, Sjo"gren syndrome and systemic sclerosis (see www.clinicaltrials.gov). Weiss et al showed that WJ-MSCs have low immunogenicity and suppress the proliferation of stimulated immune cells (see Weiss M.L et al., *Stem Cells.* 2008; 26(11):2865-74). Human WJ-MSCs do not stimulate proliferation of
30 allogeneic or xenogeneic immune cells and also, produce an immunosuppressive isoform of human leukocyte antigen (HLA), HLA-G6. Several studies have demonstrated that

autologous MSCs from patients with autoimmune diseases differ in their surface receptor expression and in some cases in their differentiation potential, when compared to MSCs isolated from healthy donors. WJ-MSCs, which are targeted to be an off-the-shelf allogeneic product along with their immunosuppressive properties, could be evaluated in
5 autoimmune experimental models.

The current management for many autoimmune diseases include the systemic use of anti-inflammatory drugs and potent immunosuppressive and immunomodulatory agents such as steroids and inhibitor proteins that block the action of inflammatory cytokines. However, despite their profound effect on immune responses, these therapies are unable
10 to induce clinically significant remissions in certain patients. In recent years, researchers have contemplated the use of stem cells to manage autoimmune disorders.

U.S. Patent No. 5,919,702 by Purchio et al., is about isolated chondrogenic progenitor cells from Wharton's Jelly. It discloses the isolation of cells from human umbilical cord Wharton's Jelly by removing blood and blood vessels and preconditioning using
15 exogenous growth factor to form prechondrocytes cells.

In WO 2007/099534 Hyman discloses a composition comprising of stem cells derived from umbilical cord, and methods of obtaining such composition. One or more umbilical cord is processed to obtain the stem cell population. Cell population includes mesenchymal stem cells derived from Wharton's Jelly, endothelial progenitor cells
20 derived from umbilical cord blood vessels and optionally, and hematopoietic stem cells from umbilical cord blood. The presence of endothelial progenitors and hematopoietic stem cells would call for HLA-matching or else, there would be increased chances of immune rejection since these cells are known to elicit strong immunological reaction in allogeneic hosts. Thus being unsuitable for allogeneic cell therapy.

Weiss et al., in U.S. Patent application number 2004/0136967 disclose the usefulness of stem cell from human sources in disease management. The disclosure relates to isolating, culturing stem cells and transforming the cells using genetic or other transformation
25 technologies.

Davies et al., in their U.S. Patent application 2005/0148074 discloses about Wharton's
30 Jelly extract, method of isolation and rapid expansion of progenitor cells from Wharton's

Jelly having rapid proliferation, and immune-incompetent to obtain enriched oestoprogenitor cells.

However, none of the approaches so far has been able to obtain off-the shelf allogeneic MSC product for therapeutics application in large scale, having minimized donor variability. Furthermore, rapid and efficient methods which are ethically acceptable is very much needed for isolating and preparing cell composition which are non-immunogenic in nature having reduced donor and biological variability among them for various applications in cell therapy. Present disclosure discloses a method to overcome this issue.

STATEMENT OF THE DISCLOSURE

Accordingly, the present disclosure relates to a composition comprising pooled Wharton's Jelly derived Mesenchymal stem cells, optionally along with pharmaceutically acceptable carrier, cryopreservant, serum, excipient or any combination thereof; a method of obtaining composition comprising pooled Wharton's Jelly derived Mesenchymal stem cells, optionally along with pharmaceutically acceptable carrier, cryopreservant, serum, excipient or any combination thereof, said method comprising acts of a) exposing Wharton's Jelly in plurality of mammalian umbilical cords and treating each individual cord matrix enzymatically or non-enzymatically in presence of a culture medium to obtain a cell suspension, b) straining and centrifuging the cell suspension to obtain a pellet and resuspending the pellet in the culture medium optionally along with serum, amino acids and growth factors, followed by passaging the cells, c) harvesting the passaged cells and establishing Master Cell Bank (MCB) of each individual umbilical cord, optionally followed by cryopreserving the established MCB in first cryopreservation medium to obtain Master Cell Bank (MCB) composition, d) pooling mesenchymal stem cells from plurality of established or optionally cryopreserved Master Cell Bank and establishing a Working Cell Bank (WCB) comprising pooled Mesenchymal stem cells optionally followed by cryopreserving the established WCB in the first cryopreservation medium to obtain Working Cell Bank (WCB) composition, and e) resuspending the WCB cells as carried above and passaging the cells for one or more generations followed by harvesting the passaged cells and adding the second

cryopreservation medium for obtaining the said composition; a Master Cell Bank composition or a Working Cell Bank composition comprising Mesenchymal Stem Cells at an amount ranging from about 1 million to about 5 million cells, along with: (a) either animal protein-free, defined cryopreservation medium having 5 or 10% DMSO, or (b) serum selected from a group comprising fetal bovine serum (FBS), Human serum albumin (HSA), cord serum and allogeneic human AB serum or any combination thereof at concentration ranging from about 85% to about 95% and DMSO at concentration ranging from about 5% to about 10%, and (c) optionally along with pharmaceutically acceptable carriers selected from a group comprising Plasmalyte A and Hank's balanced Salt Solution (HBSS) or a combination thereof, at concentration ranging from about 80% to about 95% or excipients or a combination thereof. and method of managing immune-mediated disorders, said method comprising step of administering a composition comprising pooled Wharton's Jelly derived Mesenchymal stem cells, optionally along with pharmaceutically acceptable carrier, cyropreservant, serum, excipient or any combination thereof, to a subject in need thereof.

BRIEF DESCRIPTION OF ACCOMPANYING FIGURES

In order that the disclosure may be readily understood and put into practical effect, reference will now be made to exemplary embodiments as illustrated with reference to the accompanying figures. The figure together with a detailed description below, are incorporated in and form part of the specification, and serve to further illustrate the embodiments and explain various principles and advantages, in accordance with the present disclosure where:

Figure 1 shows comparison of proliferation kinetics of human Wharton's Jelly-derived MSCs from multiple donors and against that of the pooled sample.

Figure 2 shows immunosuppressive capacity of individual WJ-MSC samples and comparison of immunosuppressive capacity between individual and pooled samples of WJ-MSCs at different MSC:PBMC ratios. In figure 2 (A), the graph represents % inhibition of PBMCs by individual WJ-MSC samples [D3, E3 and I3] when compared to the pooled WJ-MSCs sample. The white bars in the graph depicts percentage inhibition

of allogeneic PBMSc when co cultured with individual WJ-MSC and the solid bars shows the same obtained from the pooled samples.

In Figure 2 (B), the graph represents % proliferation of PBMCs in presence of individual WJ-MSC samples when compared to the pooled WJ-MSCs sample. The white bars in the graph depicts mean percentage proliferation of allogeneic PBMSc when co cultured with individual WJ-MSC, while the solid bars shows the same obtained from the pooled samples.

Figure 3 shows immunophenotype of individual and pooled samples of WJ-MSCs by flow cytometric analysis.

Figure 4 shows flow cytometric analysis for expression of co-stimulatory molecules on pooled sample of WJ-MSCs.

Figure 5 shows phase contrast images of cell morphology of individual and pooled samples of WJ-MSC.

Figure 6 shows RT-PCR analysis of tumor suppressor and oncogenes in individual samples highlighting sample to sample variability. Also a comparison of gene expression analysis of chemokine related genes between individual and pooled samples is shown where pooled sample shows stronger expression for some genes.

Figure 7 shows phase contrast images of morphology of passage 3 WJ-MSCs cultured in (A) 5% HS + 2 ng/mL bFGF and (B) standard medium containing 10% FBS; (C) Comparison of total cell yield and (D) cumulative population doublings over five passages in 5% HS + 2 ng/mL bFGF versus 10% FBS; (E) Analysis of mean population doubling time (hour $\pm$ SEM) of WJ-MSCs cultured in 5% HS + 2 ng/mL bFGF and standard medium containing 10% FBS. Results represent the average of three WJ-MSC samples (n = 3) with SEM. Scale bar = 100 μ M.

Note: bFGF- basic fibroblast growth factor; FBS- fetal bovine serum; HS- human AB serum; SEM- standard error of the mean; WJ-MSC- Wharton's jelly-derived mesenchymal stem cell.

Figure 8 shows reduction of hyperglycemia in STZ injected diabetic mice following WJ-MSCs administration. It shows the blood glucose detection before streptozotocin (STZ) injection, 4 weeks after STZ injection and 2 months after WJ-MSC transplantation in STZ-induced diabetic swiss albino mice.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present disclosure relates to a the present disclosure relates to a composition comprising pooled Wharton's Jelly derived Mesenchymal stem cells, optionally along with pharmaceutically acceptable carrier, cryopreservant, serum, excipient or any combination thereof.

In an embodiment of the present disclosure, the Mesenchymal Stem Cells are at an amount ranging from about 25 to about 200 million cells; and wherein the pharmaceutically acceptable carrier is selected from a group comprising Plasmalyte A and Hank's balanced Salt Solution (HBSS) or a combination thereof, at concentration ranging from about 80% to about 95%; the cryopreservant is Dimethyl Sulfoxide (DMSO) at concentration ranging from about 2% to about 5% or animal protein-free, defined cryopreservation medium having 2% or 5% DMSO; serum selected from a group comprising Human serum albumin (HSA), pooled cord serum and allogeneic pooled human AB serum or any combination thereof at concentration ranging from about 1% to about 10%.

The present disclosure further relates to a method of obtaining composition comprising pooled Wharton's Jelly derived Mesenchymal stem cells, optionally along with pharmaceutically acceptable carrier, cryopreservant, serum, excipient or any combination thereof, said method comprising acts of a) exposing Wharton's Jelly in plurality of mammalian umbilical cords and treating each individual cord matrix enzymatically or non-enzymatically in presence of a culture medium to obtain a cell suspension, b) straining and centrifuging the cell suspension to obtain a pellet and resuspending the pellet in the culture medium optionally along with serum, amino acids and growth factors, followed by passaging the cells, c) harvesting the passaged cells and establishing Master Cell Bank (MCB) of each individual umbilical cord, optionally followed by cryopreserving the established MCB in first cryopreservation medium to obtain Master Cell Bank (MCB) composition, d) pooling mesenchymal stem cells from plurality of established or optionally cryopreserved Master Cell Bank and establishing a Working Cell Bank (WCB) comprising pooled Mesenchymal stem cells optionally followed by cryopreserving the established WCB in the first cryopreservation medium to obtain Working Cell Bank (WCB) composition, and e) resuspending the WCB cells as

carried above and passaging the cells for one or more generations followed by harvesting the passaged cells and adding the second cryopreservation medium for obtaining the said composition.

In an embodiment of the present disclosure, the exposing comprises acts of rinsing the umbilical cord with normal saline and clearing blood clots followed by cutting the rinsed cord into small pieces of about 1 centimeter to about 5 centimeters each and storing the pieces in normal saline containing antibiotic for time period ranging from about 2 hours to about 4 hours, washing the stored pieces of cord with buffered saline and treating with iso-propyl alcohol for time period ranging from about 20 seconds to about 45 seconds, followed by rewashing with the buffered saline to remove the alcohol, and cutting the small pieces of cord longitudinally to expose the Wharton's Jelly.

In another embodiment of the present disclosure, the buffered saline is selected from a group comprising Dulbeccos Phosphate-Buffered Saline (DPBS), phosphate-buffered saline (PBS) and Hank's balanced Salt Solution (HBSS) or any combination thereof.

In yet another embodiment of the present disclosure, the culture medium is selected from a group comprising Dulbecco's modified Eagle's medium- Knock out (DMEM-KO), Dulbecco's modified Eagle's medium- F12 (DMEM-F12), Dulbecco's modified Eagle's medium- High glucose (DMEM-HG) and Dulbecco's modified Eagle's medium- Low glucose (DMEM-LG) or any combination thereof.

In still another embodiment of the present disclosure, the serum is selected from a group comprising Fetal Bovine serum (FBS), allogeneic pooled human AB serum, Human serum albumin (HSA) and cord serum or any combination thereof at concentration ranging from about 5% to about 15%; the amino-acid is L-Glutamine at concentration ranging from about 1mM to about 3mM and the growth factor is basic fibroblast growth factor (bFGF) at amount ranging from about 1 ng/ml to about 4 ng/ml.

In still another embodiment of the present disclosure, the enzymatic treatment is carried out in presence of collagenase and trypsin-EDTA; and the non-enzymatic treatment is carried out by explant culturing.

In still another embodiment of the present disclosure, the enzymatic treatment comprises contacting the cord matrix with collagenase dissolved in the culture medium, preferably DMEM-KO medium, having concentration ranging from about 400 ug/ml to about 600

ug/ml, for time period ranging from about 14 hours to about 16 hours, at temperature ranging from about 36°C to about 38°C.

In still another embodiment of the present disclosure, the treated cord matrix is digested with trypsin-EDTA at concentration ranging from about 0.01% to about 0.1%, for time
5 period ranging from about 7 minutes to about 13 minutes, at temperature ranging from about 36°C to about 38°C.

In still another embodiment of the present disclosure, the digested cord matrix is neutralized with the culture medium, preferably DMEM-KO medium containing Fetal Bovine Serum having concentration ranging from about 5% to about 15%.

10 In still another embodiment of the present disclosure, the straining is carried out by a strainer having pore size ranging from about 0.15µm to about 0.30µm; and wherein the centrifugation is carried out at about 1000rpm to about 1500rpm for time period ranging from about 4 minutes to about 6 minutes.

In still another embodiment of the present disclosure, the passaged cells have a
15 confluency ranging from about 70% to about 80%.

In still another embodiment of the present disclosure, the first cryopreservation medium:
(a) comprises animal protein-free, defined cryopreservation medium having 5% or 10% DMSO, or (b) comprises serum selected from a group comprising fetal bovine serum (FBS), Human serum albumin (HSA), cord serum and allogeneic human AB serum or any
20 combination thereof at concentration ranging from about 85% to about 95% and DMSO at concentration ranging from about 5% to about 10%, and (c) optionally along with pharmaceutically acceptable carriers selected from a group comprising Plasmalyte A and Hank's balanced Salt Solution (HBSS) or a combination thereof, at concentration ranging from about 80% to about 95%.

25 In still another embodiment of the present disclosure, the Master Cell Bank composition and the Working Cell Bank composition comprises Mesenchymal Stem Cells at an amount ranging from about 1 million to about 5 million cells, along with: (a) either animal protein-free, defined cryopreservation medium having 5% or 10% DMSO, or (b) serum selected from a group comprising fetal bovine serum (FBS), Human serum albumin (HSA), cord serum and allogeneic pooled human AB serum or any combination
30 thereof at concentration ranging from about 85% to about 95% and DMSO at

concentration ranging from about 5% to about 10%, and (c) optionally along with pharmaceutically acceptable carriers selected from a group comprising Plasmalyte A and Hank's balanced Salt Solution (HBSS) or a combination thereof, at concentration ranging from about 80% to about 95%.

- 5 In still another embodiment of the present disclosure, the second cryopreservation medium: (a) comprises animal protein-free, defined cryopreservation medium having 5% or 10% DMSO, or (b) comprises pharmaceutically acceptable carriers selected from a group comprising Plasmalyte A and Hank's balanced Salt Solution (HBSS) or a combination thereof, at concentration ranging from about 80% to about 95%, serum
10 selected from a group comprising Human serum albumin (HSA), cord serum and allogeneic human AB serum or any combination thereof at concentration ranging from about 1% to about 10%, and DMSO at concentration ranging from about 2% to about 5%.

- The disclosure further relates to a Master Cell Bank composition or a Working Cell Bank
15 composition comprising Mesenchymal Stem Cells at an amount ranging from about 1 million to about 5 million cells, along with: (a) either animal protein-free, defined cryopreservation medium having 5% or 10% DMSO, or (b) serum selected from a group comprising fetal bovine serum (FBS), Human serum albumin (HSA), cord serum and allogeneic human AB serum or any combination thereof at concentration ranging from
20 about 85% to about 95% and DMSO at concentration ranging from about 5% to about 10%, and (c) optionally along with pharmaceutically acceptable carriers selected from a group comprising Plasmalyte A and Hank's balanced Salt Solution (HBSS) or a combination thereof, at concentration ranging from about 80% to about 95% or excipients or a combination thereof.

- 25 The present disclosure further relates to a method of managing immune-mediated disorders, said method comprising step of administering a composition comprising pooled Wharton's Jelly derived Mesenchymal stem cells, optionally along with pharmaceutically acceptable carrier, cryopreservant, serum, excipient or any combination thereof, to a subject in need thereof.

In still another embodiment of the present disclosure, the immune mediated disorder is selected from a group comprising diabetes type 1, multiple sclerosis, Rheumatoid arthritis and lupus or any combination thereof.

5 In still another embodiment of the present disclosure, the composition is administered through modes selected from a group comprising intravenous administration, intra articular administration, pancreatic duodenal artery administration, intraperitoneal administration, hepatportal administration and intramuscular administration or any combination thereof.

10 In still another embodiment of the present disclosure, the composition is formulated into dosage forms selected from a group comprising tablet, troches, lozenges, aqueous or oily suspensions, ointment, patch, gel, lotion, dentifrice, capsule, emulsion, creams, spray, drops, dispersible powders or granules, emulsion in hard or soft gel capsules, syrups, elixirs, phytoceuticals, nutraceuticals and food stuffs or any combination thereof.

15 In still another embodiment of the present disclosure, the excipient is selected from a group comprising granulating agents, binding agents, lubricating agents, disintegrating agents, sweetening agents, glidants, anti-adherents, anti-static agents, surfactants, anti-oxidants, gums, coating agents, coloring agents, flavouring agents, coating agents, plasticizers, preservatives, suspending agents, emulsifying agents, plant cellulosic material and spheronization agents or any combination thereof.

20 As used herein, the term "pooled" means a plurality of MCB's from multiple donors that have been combined to create a WCB having more constant or consistent characteristics as compared to the individual MCB's.

As used herein, "management" or "managing" refers to preventing a disease or disorder from occurring in a subject, decreasing the risk of death due to a disease or disorder, 25 delaying the onset of a disease or disorder, inhibiting the progression of a disease or disorder, partial or complete cure of a disease or disorder and/or adverse affect attributable to the said disease or disorder, obtaining a desired pharmacologic and/or physiologic effect (the effect may be prophylactic in terms of completely or partially preventing a disorder or disease or condition, or a symptom thereof and/or may be 30 therapeutic in terms of a partial or complete cure for a disease or disorder and/or adverse affect attributable to the disease or disorder), relieving a disease or disorder (i.e. causing

regression of the disease or disorder). Further, the present disclosure also envisages treating the said disorder by administering the therapeutic composition of the instant disclosure.

5 The term Wharton's Jelly, cord, umbilical cord or cord matrix are all used as interchange terms in this disclosure.

As used herein, the term Wharton Jelly derived Mesenchymal stem cells also refer to as multipotent or mesenchymal Stromal cells, cord derived Mesenchymal stem cells and mesenchymal stem cells like cells.

'HSA' means Human Serum Albumin

10 'Human AB Serum' means pooled human AB serum also refer to as allogeneic human AB serum or allogeneic pooled AB serum from various/multiple donors

'Cord blood serum' means serum obtained from the cord blood. When the cord blood is obtained from the same cord it is referred to as autologous cord serum or when it's pooled from multiple cords it is referred to as allogeneic cord serum.

15 It is an objective of the present disclosure to provide a method for isolation, culturing, expansion and pooling of mesenchymal stem cell from Wharton's Jelly from multiple umbilical cords.

It is another objective to provide a method for generating a cell based composition having averaged biological variability between sample to sample generally seen in WJ-MS.

20 It is also an objective of the present disclosure to provide a method for culturing and expansion of WJ-MS in large-scale and in a medium comprising of pooled human AB serum, thus making the cell composition completely xeno-free.

It is yet another objective of the present disclosure to provide a method of culturing and maintaining WJ-MS in undifferentiated state using bFGF in culture medium.

25 It is still another objective of the present disclosure to provide a composition comprising of WJ-MS with or without pharmaceutical acceptable carriers having reduced variability by pooling of MSCs.

It is further an objective to provide allogeneic composition comprising of pooled WJ-MS having reduced biological variability and increased immunosuppression for
30 therapeutic use and more specifically for managing autoimmune disorders.

Briefly stated, present disclosure discloses a method of processing Wharton's Jelly (WJ) derived Mesenchymal Stem Cells (MSC) from Umbilical cord (UC) and to obtain a therapeutic composition for managing autoimmune disorders. The processing step comprises of collecting UC from multiple donors, isolation, culturing and pooling of WJ-MSC to obtain the final therapeutic composition comprising of pooled allogeneic WJ-MSC cells with reduced biological variability. Since the present composition is also immune-modulatory in nature, additional immuno-suppression treatment may be not required when using this composition for managing autoimmune disorder.

Wharton's Jelly-derived MSCs possess multipotent properties between embryonic stem cells and adult stem cells and have faster proliferation and greater ex vivo expansion capabilities than BMSCs. WJ-MSCs have unique properties of high proliferation rates, wide multipotency, hypoimmunogenicity and they do not induce teratomas.

There may be significant differences between different samples of human tissues and umbilical cord is no exception to this. In one of the embodiment of present disclosure, pooling of- WJ-MSCs from umbilical cords obtained from several/multiple donors is employed to reduce product variability and is practical since HLA matching is not required with the recipient. Such pooling of cells obtained from WJ-derived or cord – derived MSC does not pose a barrier for transplantation of these cells, since MSC mediate therapy does not require HLA matching between the donors and host. Since WJ-MSC would serve as a third party MSC to be used as an off-the shelf product, pooling of MSCs from different donors would not only assure constant cell quality, but also regular availability of cells for a larger patient population while maintaining consistent biological activity.

Present disclosure discloses a method of isolating, culturing and pooling of WJ-MSC to obtain allogeneic composition. The method comprises of obtaining human umbilical cords (HUC) from multiple donors. The cords are then rinsed in normal saline to clear off blood clots and are cut into small pieces - and stored in normal saline in presence of Antibiotic-Antimycotic (Invitrogen) at 4°C for 2-3 hours before processing. The cord pieces are washed with DPBS and disinfected by dipping them in 70% iso-propyl alcohol following thorough washes with DPBS to remove any traces of alcohol. Each cord piece is cut open longitudinally to expose the Wharton's jelly and the blood vessels. The cord

vessels are removed using sterile forceps and scalpel. The pieces are next placed in a dish such that the exposed jelly is now facing down and treated with Collagenase blend type H dissolved in plain Knock out Dulbecco's modified Eagle's medium (DMEM-KO) (Invitrogen) for 30 mins-18 hours at 37°C. Tissue is further digested with 0.05% Trypsin-
5 EDTA and neutralized with DMEM-KO medium containing 10-15% fetal bovine serum (FBS). The cell suspension thus obtained is strained using cell strainer and collected in a 50 ml Falcon tube. This solution is centrifuged and the pellet is washed in DPBS before resuspending in DMEM-KO supplemented with FBS, L-Glutamine and bFGF and plated on T-75 flask (BD-falcon) for culturing to establish master cell bank (MCB), which is
10 pooled to obtain the working cell bank (WCB). Both MCB and WCB is cryopreserved in cryopreservation composition for further use or used directly. The final composition is obtained from WCB.

Culturing WJ-MSC in pooled AB human Serum

15 Most of the existing protocols for *in vitro* culture of MSCs utilize fetal bovine serum (FBS) as nutrient supplement and porcine trypsin for dissociating cells to generate single-cell suspensions. However, for safe therapeutic use of culture expanded stem cells in humans or clinical application, use of animal derived products in the culture is not preferred. FBS raises potential safety concerns like transmission of viral/prion disease
20 and may initiate xenogeneic immune reactions against bovine antigens. Therefore, for therapeutic applications, there is a need to establish an alternative nutrient supplement which would favor cell proliferation, retain MSC characteristics and yet, prove safe in human subjects. In one of the embodiment WJ-MSCs is successfully isolated and expanded in 5-10 % pooled, allogeneic human AB serum supplemented with 2ng/ml of
25 basic fibroblast growth factor (bFGF). Porcine trypsin is replaced with TrypLE express, which is free of animal and human derived components, for the dissociation of cells.

In one of the embodiment the isolation and expansion of WJ-MSCs is carried under xeno-free conditions wherein the explants culture (or non-enzymatic) protocol is followed which allows reproducible isolation of WJ-MSCs from umbilical cord (n = 12) without
30 the use of any proteases to attain cellular dissociation. At the end of every passage, cells cultured in pooled, allogeneic HS are harvested using TrypLE Express, while trypsin is

used for those cultured in 10% FBS. In an attempt to reduce the content of allogenic HS in the culture medium, the medium is supplemented with 2 ng/mL bFGF. This concentration of bFGF is an optimal condition for the culture of WJ-MSCs. 10% FBS supplemented culture medium is used as reference. Therefore, WJ-MSCs are established in parallel cultures supplemented with either 5% HS + 2 ng/mL bFGF or 10% FBS. Cell morphology of WJ-MSCs cultured in FBS and pooled allogenic HS + bFGF is confirmed by phase-contrast microscopic examination as shown in Figures 7A and 7B, respectively. The estimation of the total number of WJ-MSCs generated at the end of five passages is carried out. The cumulative cell yield ranges between $1.8 \times 10^8 \pm 2.1 \times 10^7$ for 5%HS + 2 ng/mL bFGF and $1.4 \times 10^9 \pm 1.1 \times 10^9$ for 10% FBS (Figure 7C). The difference is not significant ($P = 0.39$). The Cumulative Population Doublings (CPDs) reach a peak of 10.4 ± 0.4 at passage 5 in 5% HS + 2 ng/mL bFGF when compared with 12.2 ± 1.3 ($P=0.05$) in 10% FBS (Figure 7D). Mean population doubling time of 35.3 ± 2.4 hours and 28.2 ± 2.5 hours ($P = 0.113$) is noted for 5% HS + 2 ng/mL bFGF and 10% FBS, respectively (Figure 7E).

WJ-Derived MSC based Composition and their applications

The pooled allogenic WJ-MSCs of the present disclosure is used in human or animal for managing certain disease conditions in them. Management involves using the cells of the disclosure to produce new tissue to replace injured/diseased tissues. Cells are implanted, administered or injected directly to the site of damage so that they will produce new tissue *in vivo*.

Accordingly, these cells are useful for preparing medicament or therapeutic or pharmaceutical composition (all interchangeable terms), whereby in one of the embodiments such a composition is formulated for managing autoimmune diseases.

The pooled allogenic WJ-MSC described herein, are administered either *per se* or, preferably as a part of composition that further comprises a pharmaceutically acceptable carrier or excipients. Pharmaceutically acceptable carrier as an example includes without limitation to Plasmalyte, or Hank's balanced Salt Solution (HBSS) having 5% glucose/dextrose and other such suitable carrier known in the art. The purpose of the

pharmaceutically acceptable carrier is to facilitate administration of WJ-MSCs to the subjects along with serum and cryopreservent.

Mode of administration

5 The pooled allogeneic WJ-MSCs of the present disclosure are administered to the individual using variety of transplantation approaches, depending on the site of implantation which includes, but not limited to intravenous, intraperitoneal, intraarticular administration, pancreatic duodenal administration, intramuscular or via hepatoportal vein or artery.

10 Due to their potent immunomodulatory properties, WJ-MSCs of present disclosure is explored has a new therapeutic composition for managing a variety of immune-mediated diseases.

The immune-mediated injury in autoimmune diseases can be organ-specific, such as type 1 diabetes which is the consequence of the destruction of the pancreatic beta islet cells or
15 multiple sclerosis which results from the breakdown of the myelin covering of nerves or rheumatoid arthritis. These autoimmune diseases are amenable for management involving the repair or replacement of damaged or destroyed cells or tissue. In contrast, non-organ-specific autoimmune diseases, such as lupus, are characterized by widespread injury due to immune reactions against many different organs and tissues.

20 Drawback of using non-self hematopoietic stem cells is the immune rejection of the transplanted cells. Immune rejection is caused by MHC protein differences between the donor and the patient (recipient). The transplanted hematopoietic stem cells and their progeny are rejected by the patient's own T cells, which are originating from the patient's surviving bone marrow hematopoietic stem cells. In this regard, embryonic and adult
25 stem cell offers distinct advantages over cord blood and bone marrow hematopoietic stem cell lines in avoiding rejection of the transplant.

Human mesenchymal cells isolated from the Wharton's jelly of the umbilical cord in the present disclosure, are easily obtained and processed compared to embryonic and bone marrow stem cells, possess stem cell properties and recent studies have shown that no
30 rejections occur even after xenotransplantation of post-differentiated umbilical cord

mesenchymal stem cells without immunosuppression therapy and they can be used without requiring any genetic alteration.

Additional embodiments and features of the present disclosure will be apparent to one of ordinary skill in art based upon description provided herein. However, the examples and the figures should not be construed to limit the scope of the present disclosure.

Example 1

Isolation and culturing of WJ-MSCs:

Human umbilical cords is collected from full term births from both normal vaginal delivery and Cesarian sections with informed consent as per the guidelines approved by the Institutional Committee for Stem Cell Research and Therapy (ICSCRT) and Institutional Ethics Committee (IEC) at the Manipal Hospital, Bangalore, India. In one of the embodiments umbilical cord is collected from multiple donors processed and pooled. In an exemplary embodiment, human umbilical cords are collected from multiple donors of child bearing age groups and provided in the table below:

Table-1

Donor I.D	Donor age
D3	25 years
F3	27 years
I3	22 years

After collecting the human umbilical cords from multiple donors, the following steps are performed:

1. The cords are rinsed in 50-200 ml of normal saline and blood clots are cleared off at room temperature.
3. The cords are then cut into small pieces of 1-5 cms each and stored in 20-40 ml of normal saline containing Antibiotic-Antimycotic (Invitrogen) at 4°C for 2-3 hours before processing.
4. The cord pieces are washed with 10-40 ml of Dulbeccos Phosphate-Buffered Saline (DPBS) 2-4 times and disinfected by dipping them in 10-30 ml of 70% iso-propyl alcohol for 20-45 seconds and preferably not more than 30 seconds following which, 2-5

thorough washes with 10-40 ml of DPBS are given to remove any traces of alcohol. All steps are carried out at room temperature.

5. Each cord piece is then cut open longitudinally to expose the Wharton's jelly and the blood vessels. The cord vessels are removed using sterile forceps and scalpel.

5 6. The pieces are next placed in a 10cm dish such that the exposed jelly is now facing down and treated with Collagenase blend type H (Sigma) dissolved in plain Knock out Dulbecco's modified Eagle's medium (DMEM-KO) (Invitrogen) for 14-16 hours at 36-38°C.

10 7. Tissue are further digested with 0.04-0.06% Trypsin-EDTA for 8-10 minutes at 36-38°C and neutralized with DMEM-KO medium containing 10-15% fetal bovine serum (FBS) (Hyclone).

8. The cell suspension thus obtained is strained using 0.22 µm cell strainer and collected in a 50 ml Falcon tube. This solution is then centrifuged at 1000-1500 rpm for 4-6 minutes at room temperature.

15 9. One-three DPBS washes are given before resuspending the pellet in DMEM-KO and is supplemented with 10-15% FBS, 2mM L-Glutamine (Invitrogen) and 1-4 ng/ml basic Fibroblast growth Factor (bFGF) (Sigma) and plated on T-75 flask (BD-falcon) at Passage 0 (P0).

20 10. At the end of P0, harvested cells from individual cords are seeded at 1000-5000 cells/cm² on respective 2 Cell STACK chambers (Corning).

11. At 70-80% confluency (day 5-6), passage 1 (P1) cells are harvested from the respective cords and the WJ-MSCs at this stage is referred as the Master Cell Bank (MCB), most of which are cryopreserved in cryopreservation medium composition comprising FBS and DMSO; alternatively it can be cryo preserved in the commercially available animal protein-free, defined cryopreservation medium [CryoStorTMCS10] or [CryoStorTMCS5] from Biolife Solutions.

25 In an embodiment of the present disclosure, the Master Cell Bank composition comprises Mesenchymal stem cells ranging from about 1 million cells to 5 million cells, Fetal Bovine Serum (FBS) ranging from about 85% to 95% and Dimethyl Sulphoxide (DMSO) ranging from about 5% to 15%. Alternatively, 1-5 million cells are cryopreserved in 30 CryoStorTMCS10 or in CryoStorTMCS5.

12. Remaining MCB cells are counted using hemocytometer and equal number of MSCs from each cord is pooled to form a single working cell bank (WCB) before replating on a single Cell STACK at 1000-5000 cells/cm² in DMEM-KO supplemented with 10-15% FBS, 2mM, L-Glutamine (Invitrogen) and 1-4ng/ml bFGF (Sigma). Alternatively method
5 to pool is to use varying cell numbers from each donors depending on the rate of proliferation so that after pooling and expansion they are represented in similar proportion in WCB.

13. Passage 2 (P2/P3) WJ-MSCs, harvested at the end of this passage and subsequent passages, will be used for as therapeutic composition and additional aliquots will be
10 cryopreserved in cryopreservation medium composition comprising FBS and DMSO. Alternatively, it can be cryo preserved in the commercially available [CryoStorTMCS10] or [CryoStorTMCS5] animal protein-free, defined cryopreservation medium from Bioline Solutions. CryoStorTMCS10 is uniquely formulated serum free, animal protein free and commercially available defined cryopreservation medium containing 10% DMSO.
15 Similarly CryoStorTMCS5 cell-specific, optimized freeze media pre-formulated with 5% DMSO.

In an embodiment of the present disclosure, the Working Cell Bank composition comprises Mesenchymal stem cells ranging from about 1 million cells to 5 million cells, Fetal Bovine Serum (FBS) ranging from about 85% to 95% and Dimethyl Sulphoxide
20 (DMSO) ranging from about 5% to 15%. Alternatively, 1-5 million cells are cryopreserved in CryoStorTMCS10 or in CryoStorTMCS5.

In another embodiment the cryopreservation medium composition comprises of pharmaceutical acceptable carrier, serum and DMSO, wherein, the pharmaceutical acceptable carrier is selected from plasmalyte A or HBSS; while serum is selected from
25 human serum albumin, pooled human AB serum or cord serum.

In another embodiment, the cryopreservation composition comprises of either Plasmalyte A or Hank's solution in about 80-90% along with Human AB serum or cord serum or human serum albumin in about 1-10% along with cryoprotectant the DMSO in about 5-10%. The MCB, WCB and the final composition can be cryopreserved in the
30 cryopreservation medium composition for further use.

The cells harvested at the WCB stage can be further cultured to generate therapeutic composition and cryopreserved. The final therapeutic composition does not comprise FBS.

The final therapeutic composition contains about 15 ml of cryopreservation medium composition having about 12.75 ml of Plasmalyte- A, about 1.5 ml of DMSO and about 0.75 ml of Human Serum Albumin, for about 200 million cell pellet. The entire contents are transferred to a cryobag.

In another embodiment, the final therapeutic composition comprises of 25 to 200 million pooled WJ-MSC in 80-95% Plasmalyte-A or Hank's (HBSS) solution along with 2-5% DMSO in a cryobag.

In another embodiment, the final therapeutic composition comprises of 25 to 200 million pooled WJ-MSC in 2-14ml CryoStorTMCS5.

In another embodiment, the composition comprises of 25 to 200 million pooled WJ-MSC in 80-95% Plasmalyte A, 1-10% pooled cord serum and 2-5% DMSO in a cryobag.

In yet another embodiment, the composition comprises of 25 to 200 million pooled WJ-MSC in 80-95% Plasmalyte A, 1-10% of human serum albumin and 2-5% DMSO in a cryobag.

In still another embodiment the composition comprises of 25 to 200 million pooled WJ-MSC in 80-95% Plasmalyte A, 1-10% of pooled human AB albumin and 2-5% DMSO in a cryobag.

Alternatively, the final composition comprises of 25 to 200 million pooled WJ-MSC - in 2-14ml CryoStorTMCS5.

In another embodiment of the disclosure, the following table (Table-2) provides for the various possibilities and alternatives of the MCB/WCB composition and the final therapeutic composition.

Table-2

Cell	Serum	Cryopreservative	Pharmaceutical acceptable carriers

MCB /WCB components	FBS/HSA/Cord serum / pooled human AB serum	DMSO / CryoStor™CS10 / CryoStor™CS5	-
MCB /WCB composition	• FBS+DMSO + MCB /WCB • HSA +DMSO + MCB /WCB		
MCB /WCB components	FBS/HSA/CS/ pooled human AB serum	DMSO/CS10/CS5	Plamalyste A and HBBS
MCB /WCB composition	• PL/HBBS + HSA+DMSO+ MCB /WCB • PL/HBBS + CS+DMSO+ MCB /WCB • PL/HBBS + pooled HS+DMSO+ MCB /WCB • CryoStor™CS10 + MCB /WCB • CryoStor™CS5+ MCB /WCB		
Final Composition*	HSA/Cord serum S/Pooled Human AB serum	DMSO/ CryoStor™CS5	Plamalyste A and HBBS
Pooled WJ-MSC's	HSA	DMSO	Plamalyste A/HBBS
Pooled WJ-MSC's	Cord serum	DMSO	Plamalyste A/HBBS
Pooled WJ-MSC's	Pooled Human AB Serum	DMSO	Plamalyste A/HBBS
Pooled WJ-MSC's	-	CryoStor™CS5	-
Pooled WJ-MSC's	-	CryoStor™CS5	-
Pooled WJ-MSC's	-	CryoStor™CS5	-
Note : Cord serum used for MCB cryopreservation can be autologous cord serum or pooled allogeneic cord serum. While in case of WCB and final composition it is only pooled allogeneic cord serum.			

NOTE: WCB – Working Cell Bank

MCB- Master Cell Bank

HSA- Human Serum Albumin
Pooled HS- Pooled Human AB Serum
CS- Cord Serum
DMSO- Dimethyl Sulfoxide
5 FBS- Fetal Bovine Serum
CS5- CryoStorTMCS5 [DMSO 5%]
CS10- CryoStorTMCS5 [DMSO 10%]
HBBS- Hank's balanced Salt Solution
PL- Plasmalyte A

10

The cell numbers differed considerably between the different donor samples. Pooling of WJ-MSCs from multiple donors average out the variability existing in growth kinetics between individual donor WJ-MSCs samples.

15 **Example 2**

Comparison of proliferation kinetics of human Wharton's Jelly –derived MSCs from multiple donors:

Figure 1 shows the comparison of proliferation kinetics of human Wharton's jelly-derived MSCs from multiple donors in the child bearing age group. After
20 collagenase/trypsin digestion of multiple human umbilical cords, cells are plated on T75 flasks for primary culture. In the next step, P0 cells are harvested and large-scale expansions of WJ-MSCs is initiated in 2 Cell STACK chambers (1272cm²) at a seeding density of 1000 cells/cm². The total cell yield at the end of each passage is calculated and plotted for each of the WJ-MSCs samples. There is a considerable difference in the cell
25 yield, both at P0 and P1, between the five different donor samples. Pooling WJ-MSCs from different donors overcomes the donor-to-donor variability in cell numbers and provides a consistent and averaged MSCs product for therapy.

Example 3

30 Immunosuppressive Capacity:

In yet another embodiment, immunosuppressive capacity of WJ-MSCs from single versus pooled is compared. Figure 2 shows comparison of immunosuppressive capacity of individual WJ-MSC samples and between individual and pooled samples of WJ-MSCs. To evaluate and compare the inhibitory effect of individual and pooled samples of WJMSCs on proliferation of T cells in peripheral blood mononuclear cell (PBMCs), mitogen activated PBMCs are incubated with and without a layer of mitomycin-C arrested WJ-MSCs and results are demonstrated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assays. MTT assays were performed in triplicate and the results were averaged. The data demonstrated that the pooled sample of WJ-MSCs could suppress T cell proliferation equally if not more efficiently than the individual WJ-MSCs samples.

Example 4

Immunophenotype of WJ-MSCs:

Figure 3 shows immunophenotype of individual and pooled samples of WJ-MSCs by flow cytometric analysis. To detect and compare the surface marker expression profile, individual and pooled samples of WJ-MSCs are subjected to flow cytometric analyses. Pooled vs individual samples of WJ-MSCs are positive for mesenchymal markers CD 44, CD 73, CD 90, CD 105 and negative for CD 34, HLA-DR. No statistically significant difference is observed between pooled and the individual samples.

Example 5

Flow Cytometry:

Figure 4 shows that WJ-MSCs are known to be immune privileged and they have a low immunogenicity profile. Human MSCs express low levels of MHC class I but are negative for MHC class II and co-stimulatory molecules CD 40, CD 80 and CD 86. This could render the T cells anergic and explain the immune privileged status of MSCs. Expression of co-stimulatory molecules (CD 40, CD 80 and CD 86), on pooled sample of WJ-MSCs is analyzed by flow cytometry and found to be negative. Thus even the pooled sample of WJ-MSCs evades local immune surveillance and therefore, are regarded as hypoinmunogenic cells.

Example 6**Phase-contrast microscopy:**

Figure 5 shows Morphology of individual and pooled samples of WJ-MSCs.

- 5 Representative phase-contrast images of individual and pooled samples of WJ-MSCs are displayed. There is no difference in morphology between the two populations with respect to the cell morphology.

Example 7

10 **Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis:**

In figure 6, relative mRNA expression levels of c-myc, p-53, p-21 and p16 is compared between P0 and P1 cultures of three different samples of WJ-MSCs by RT-PCR. Variation in gene expression levels between the different WJ-MSCs samples is noted (A). RT-PCR analysis of chemokine related genes: Relative mRNA expression levels of some
 15 chemokine-related genes, as analyzed by semi-quantitative RT-PCR, is compared between individual and pooled samples of WJ-MSCs. Pooling of WJ-MSCs from several donors diminishes intra-sample variability and leads to more stable expression of genes (B).

- 20 The Data presented in the figure suggest difference between WJ-MSCs samples in terms of gene expression and therefore justify pooling of samples from different cord to minimize individual variability.

18s rRNA is used as an internal control. The primer sequences are as follows, CXCL2
 25 forward primer 5'- AACCGAAGTCATAGCCACAC-3', reverse primer 5'-
 CAGGAACAGCCACCAATAAG-3'; CXCL5 forward primer 5'-
 CTGTGTTGAGAGAGCTGCGT-3', reverse primer 5'-
 GTTTTCCTTGTTTCCACCGTC-3'; CXCL6 forward primer 5'-
 CCTGAAGAACGGGAAGC-3', reverse primer 5'- GACTGGGCAATTTTATGATG-
 30 3'; IL-1A forward primer 5'- CTGCATGGATCAATCTGT-3', reverse primer 5'-
 CCCATGTCAAATTTCACTGC; P21 forward primer 5'-

GAGGCCGGGATGAGTTGGGAGGAG-3', reverse primer 5'-
 CAGCCGGCGTTTGGAGTGGTAGAA-3'; P53 forward primer 5'-
 TTGGATCCATGTTTTGCCAACTGGCC-3', reverse primer 5'-
 TTGAATTCAGGCTCCCCTTCTTGCG-3'; cMYC forward primer 5'-
 5 AAGACTCCAGCGCCTTCTCTC-3', reverse primer 5'-
 GTTTTCCAACCTCCGGGATCTG-3'; p16 forward primer 5'-
 TTATTTGAGCTTTGGTTCTG-3', reverse primer 5'- CCGGCT TTCGTAGTTTTCAT-
 3'; 18S forward primer 5'- CGGCTACCACATCCAAGGAA-3', reverse primer 5'-
 GCTGGAATTACCGCGGCT-3'.

10

Pooling of WJ-MSCs from several donors diminishes intra-sample variability and leads to more stable expression of genes.

Example 8

15 Reversal of STZ-induced hyperglycemia by WJ-MSC composition in Swiss albino mice:
 All experiments were conducted in accordance with the institutional animal ethics
 committee guidelines of Manipal University. 8 week old male Swiss albino mice were
 used in this study. Experimental diabetes is induced by intraperitoneal injection of
 streptozotocin (STZ) 40 mg/Kg/day for 5 days and diabetic condition is allowed to
 20 stabilize for 3 days before WJ-MSC comprising composition transplantation. Blood
 drawn from tail vein is collected to monitor blood glucose levels before STZ injection,
 after STZ injection and also once in every three days after WJ-MSC transplantation.
 Approximately 25×10^6 cells/Kg body weights in 200 μ l of saline were injected into the
 tail vein of STZ-induced diabetic mice. Figure 8 shows the blood glucose level (mg/ml)
 25 in the mice before induction of diabetic condition using STZ, after development of STZ-
 induced diabetic condition and finally the blood glucose level after administration of WJ-
 MSCs.

We Claim:

1. A composition comprising pooled Wharton's Jelly derived Mesenchymal stem cells, optionally along with pharmaceutically acceptable carrier, cryopreservant, serum, excipient or any combination thereof.
- 5 2. The composition as claimed in claim 1, wherein the Mesenchymal Stem Cells are at an amount ranging from about 25 to about 200 million cells; and wherein the pharmaceutically acceptable carrier is selected from a group comprising Plasmalyte A and Hank's balanced Salt Solution (HBSS) or a combination thereof, at concentration ranging from about 80% to about 95%; the
10 cryopreservant is Dimethyl Sulfoxide (DMSO) at concentration ranging from about 2% to about 5% or animal protein-free, defined cryopreservation medium having 2% or 5% DMSO; serum selected from a group comprising Human serum albumin (HSA), pooled cord serum and allogeneic pooled human AB serum or any combination thereof at concentration ranging from about 1% to about 10%.
- 15 3. A method of obtaining composition comprising pooled Wharton's Jelly derived Mesenchymal stem cells, optionally along with pharmaceutically acceptable carrier, cryopreservant, serum, excipient or any combination thereof, said method comprising acts of:
 - a. exposing Wharton's Jelly in plurality of mammalian umbilical cords and
20 treating each individual cord matrix enzymatically or non-enzymatically in presence of a culture medium to obtain a cell suspension;
 - b. straining and centrifuging the cell suspension to obtain a pellet and resuspending the pellet in the culture medium optionally along with serum, amino acids and growth factors, followed by passaging the cells;
 - 25 c. harvesting the passaged cells and establishing Master Cell Bank (MCB) of each individual umbilical cord, optionally followed by cryopreserving the established MCB in first cryopreservation medium to obtain Master Cell Bank (MCB) composition;
 - d. pooling mesenchymal stem cells from plurality of established or optionally
30 cryopreserved Master Cell Bank and establishing a Working Cell Bank

(WCB) comprising pooled Mesenchymal stem cells optionally followed by cryopreserving the established WCB in the first cryopreservation medium to obtain Working Cell Bank (WCB) composition; and

- 5 e. resuspending the WCB cells as carried out in step (b) and passaging the cells for one or more generations followed by harvesting the passaged cells and adding the second cryopreservation medium for obtaining the said composition.

4. The method as claimed in claim 3(a), wherein the exposing comprises acts of:

- 10 a. rinsing the umbilical cord with normal saline and clearing blood clots followed by cutting the rinsed cord into small pieces of about 1 centimeter to about 5 centimeters each and storing the pieces in normal saline containing antibiotic for time period ranging from about 2 hours to about 4 hours;
- 15 b. washing the stored pieces of cord with buffered saline and treating with iso-propyl alcohol for time period ranging from about 20 seconds to about 45 seconds, followed by rewashing with the buffered saline to remove the alcohol; and
- c. cutting the small pieces of cord longitudinally to expose the Wharton's Jelly.

20 5. The method as claimed in claim 4, wherein the buffered saline is selected from a group comprising Dulbeccos Phosphate-Buffered Saline (DPBS), phosphate-buffered saline (PBS) and Hank's balanced Salt Solution (HBSS) or any combination thereof.

25 6. The method as claimed in claim 3, wherein the culture medium is selected from a group comprising Dulbecco's modified Eagle's medium- Knock out (DMEM-KO), Dulbecco's modified Eagle's medium- F12 (DMEM-F12), Dulbecco's modified Eagle's medium- High glucose (DMEM-HG) and Dulbecco's modified Eagle's medium- Low glucose (DMEM-LG) or any combination thereof.

30 7. The method as claimed in claim 3, wherein the serum is selected from a group comprising Fetal Bovine serum (FBS), allogeneic pooled human AB serum, Human serum albumin (HSA) and cord serum or any combination thereof at

concentration ranging from about 5% to about 15%; the amino-acid is L-Glutamine at concentration ranging from about 1mM to about 3mM and the growth factor is basic fibroblast growth factor (bFGF) at amount ranging from about 1 ng/ml to about 4 ng/ml.

- 5 8. The method as claimed in claim 3, wherein the enzymatic treatment is carried out in presence of collagenase and trypsin-EDTA; and the non-enzymatic treatment is carried out by explant culturing.
9. The method as claimed in claim 8, wherein the enzymatic treatment comprises contacting the cord matrix with collagenase dissolved in the culture medium,
10 preferably DMEM-KO medium, having concentration ranging from about 400 ug/ml to about 600 ug/ml, for time period ranging from about 14 hours to about 16 hours, at temperature ranging from about 36°C to about 38°C.
10. The method as claimed in claim 9, wherein the treated cord matrix is digested with trypsin-EDTA at concentration ranging from about 0.01% to about 0.1%, for
15 time period ranging from about 7 minutes to about 13 minutes, at temperature ranging from about 36°C to about 38°C.
11. The method as claimed in claim 10, wherein the digested cord matrix is neutralized with the culture medium, preferably DMEM-KO medium containing Fetal Bovine Serum having concentration ranging from about 5% to about 15%.
- 20 12. The method as claimed in claim 3, wherein the straining is carried out by a strainer having pore size ranging from about 0.15µm to about 0.30µm; and wherein the centrifugation is carried out at about 1000rpm to about 1500rpm for time period ranging from about 4 minutes to about 6 minutes.
13. The method as claimed in claim 3(c), wherein the passaged cells have a
25 confluency ranging from about 70% to about 80%.
14. The method as claimed in claim 3, wherein the first cryopreservation medium:
 - a. comprises animal protein-free, defined cryopreservation medium having 5% or 10% DMSO; or
 - b. comprises serum selected from a group comprising fetal bovine serum
30 (FBS), Human serum albumin (HSA), cord serum and allogeneic human AB serum or any combination thereof at concentration ranging from about

85% to about 95% and DMSO at concentration ranging from about 5% to about 10%; and

- c. optionally along with pharmaceutically acceptable carriers selected from a group comprising Plasmalyte A and Hank's balanced Salt Solution (HBSS) or a combination thereof, at concentration ranging from about 80% to about 95%.

15. The method as claimed in claim 3, wherein the Master Cell Bank composition and the Working Cell Bank composition comprises Mesenchymal Stem Cells at an amount ranging from about 1 million to about 5 million cells, along with:

- a. either animal protein-free, defined cryopreservation medium having 5% or 10% DMSO; or
- b. serum selected from a group comprising fetal bovine serum (FBS), Human serum albumin (HSA), cord serum and allogeneic pooled human AB serum or any combination thereof at concentration ranging from about 85% to about 95% and DMSO at concentration ranging from about 5% to about 10%; and
- c. optionally along with pharmaceutically acceptable carriers selected from a group comprising Plasmalyte A and Hank's balanced Salt Solution (HBSS) or a combination thereof, at concentration ranging from about 80% to about 95%.

16. The method as claimed in claim 3, wherein the second cryopreservation medium:

- a. comprises animal protein-free, defined cryopreservation medium having 5% or 10% DMSO; or
- b. comprises pharmaceutically acceptable carriers selected from a group comprising Plasmalyte A and Hank's balanced Salt Solution (HBSS) or a combination thereof, at concentration ranging from about 80% to about 95%, serum selected from a group comprising Human serum albumin (HSA), cord serum and allogeneic human AB serum or any combination thereof at concentration ranging from about 1% to about 10%, and DMSO at concentration ranging from about 2% to about 5%.

17. A Master Cell Bank composition or a Working Cell Bank composition comprising Mesenchymal Stem Cells at an amount ranging from about 1 million to about 5 million cells, along with:
- a. either animal protein-free, defined cryopreservation medium having 5% or 10% DMSO; or
 - b. serum selected from a group comprising fetal bovine serum (FBS), Human serum albumin (HSA), cord serum and allogeneic human AB serum or any combination thereof at concentration ranging from about 85% to about 95% and DMSO at concentration ranging from about 5% to about 10%; and
 - c. optionally along with pharmaceutically acceptable carriers selected from a group comprising Plasmalyte A and Hank's balanced Salt Solution (HBSS) or a combination thereof, at concentration ranging from about 80% to about 95% or excipients or a combination thereof.
18. A method of managing immune-mediated disorders, said method comprising step of administering a composition comprising pooled Wharton's Jelly derived Mesenchymal stem cells, optionally along with pharmaceutically acceptable carrier, cryopreservant, serum, excipient or any combination thereof, to a subject in need thereof.
19. The method as claimed in claim 18, wherein, the immune mediated disorder is selected from a group comprising diabetes type 1, multiple sclerosis, Rheumatoid arthritis and lupus or any combination thereof.
20. The method as claimed in claim 18, wherein the composition is administered through modes selected from a group comprising intravenous administration, intra articular administration, pancreatic duodenal artery administration, intraperitoneal administration, hepatportal administration and intramuscular administration or any combination thereof.
21. The method as claimed in claim 18, wherein the composition is formulated into dosage forms selected from a group comprising tablet, troches, lozenges, aqueous or oily suspensions, ointment, patch, gel, lotion, dentifrice, capsule, emulsion, creams, spray, drops, dispersible powders or granules, emulsion in hard or soft gel

capsules, syrups, elixirs, phytoceuticals, nutraceuticals and food stuffs or any combination thereof.

22. The composition as claimed in claim 1 and the methods as claimed in claims 3 and 18, wherein the excipient is selected from a group comprising granulating agents, binding agents, lubricating agents, disintegrating agents, sweetening agents, glidants, anti-adherents, anti-static agents, surfactants, anti-oxidants, gums, coating agents, coloring agents, flavouring agents, coating agents, plasticizers, preservatives, suspending agents, emulsifying agents, plant cellulosic material and spheronization agents or any combination thereof.

5

10

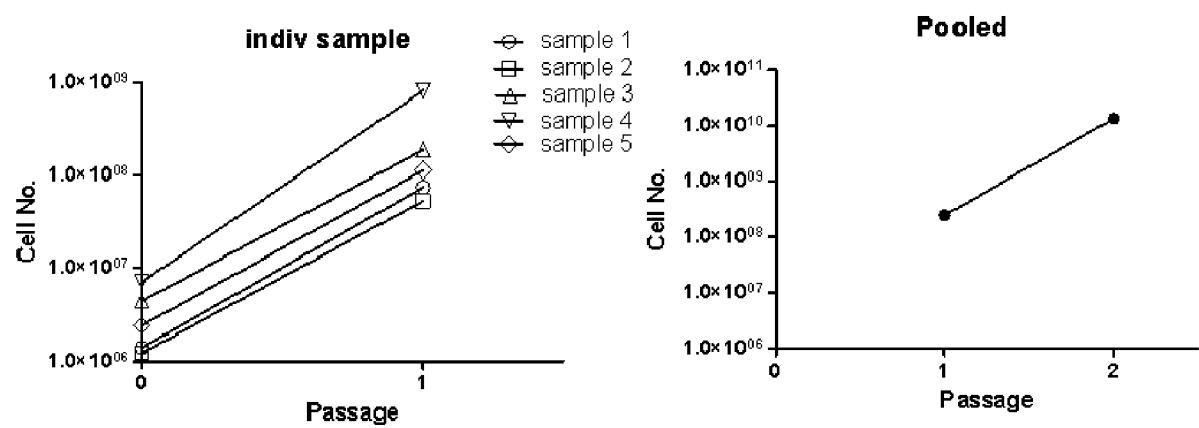


Figure 1

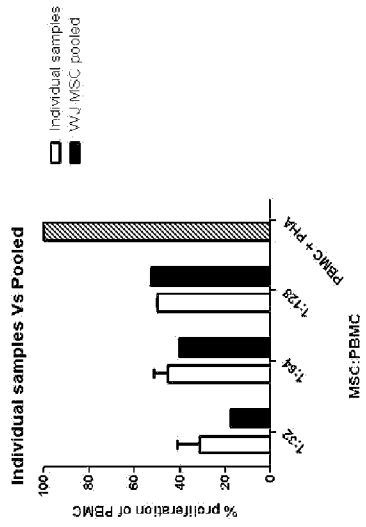
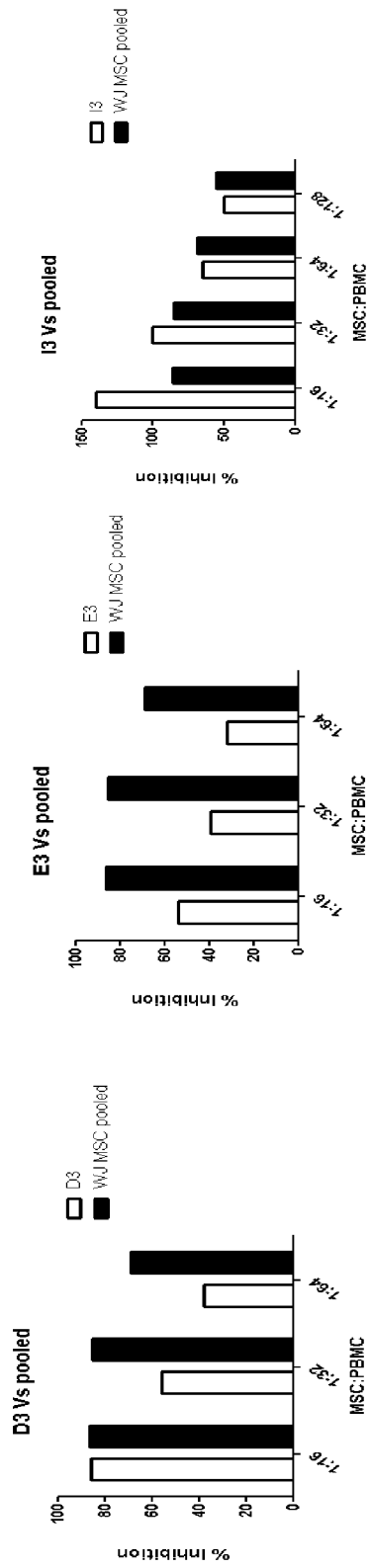


Figure 2

3/7

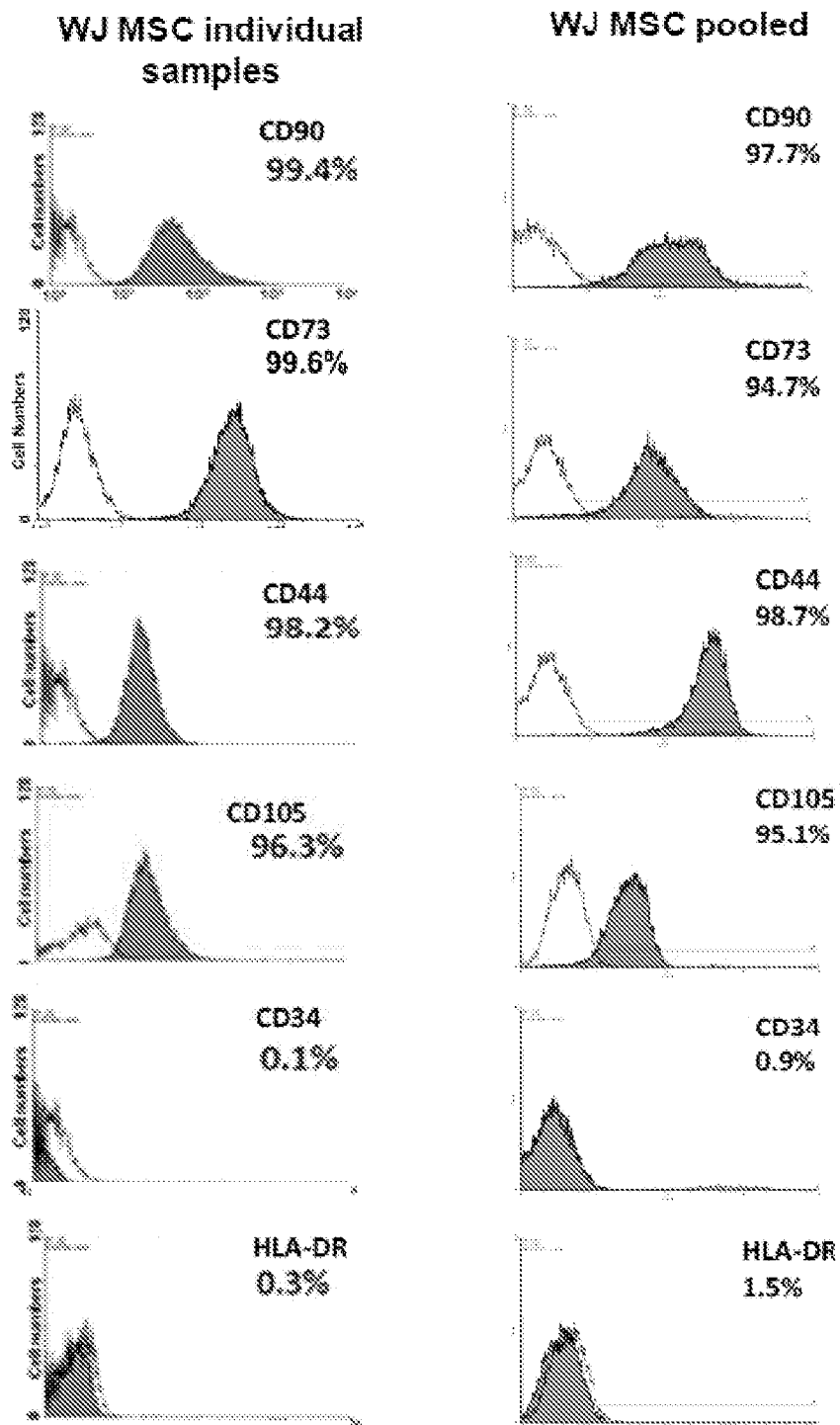


Figure 3

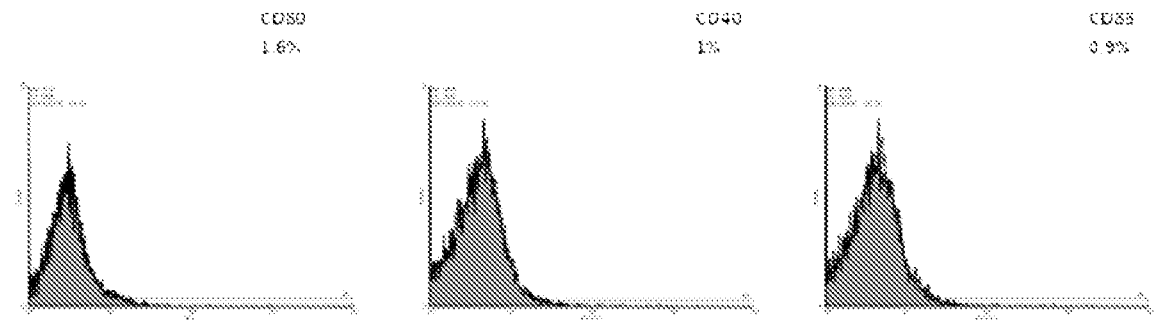


Figure 4

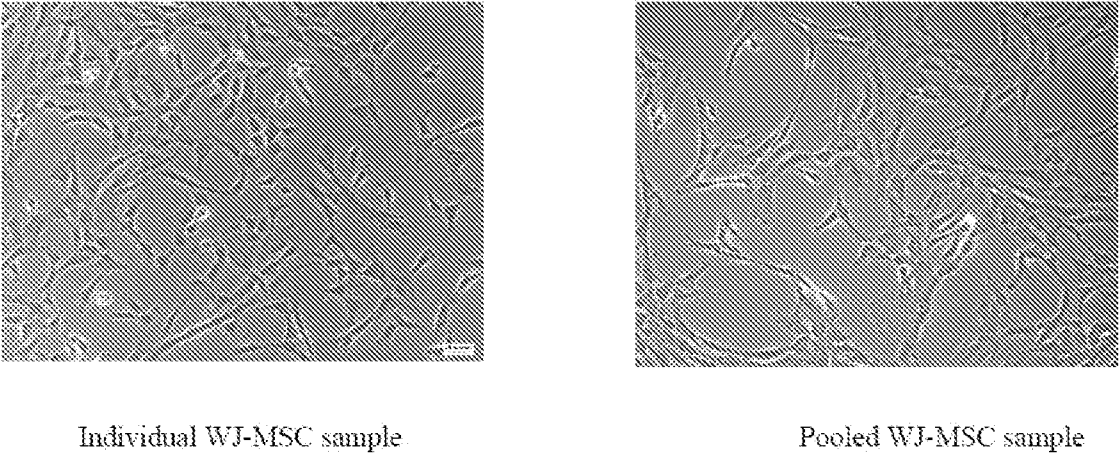


Figure 5

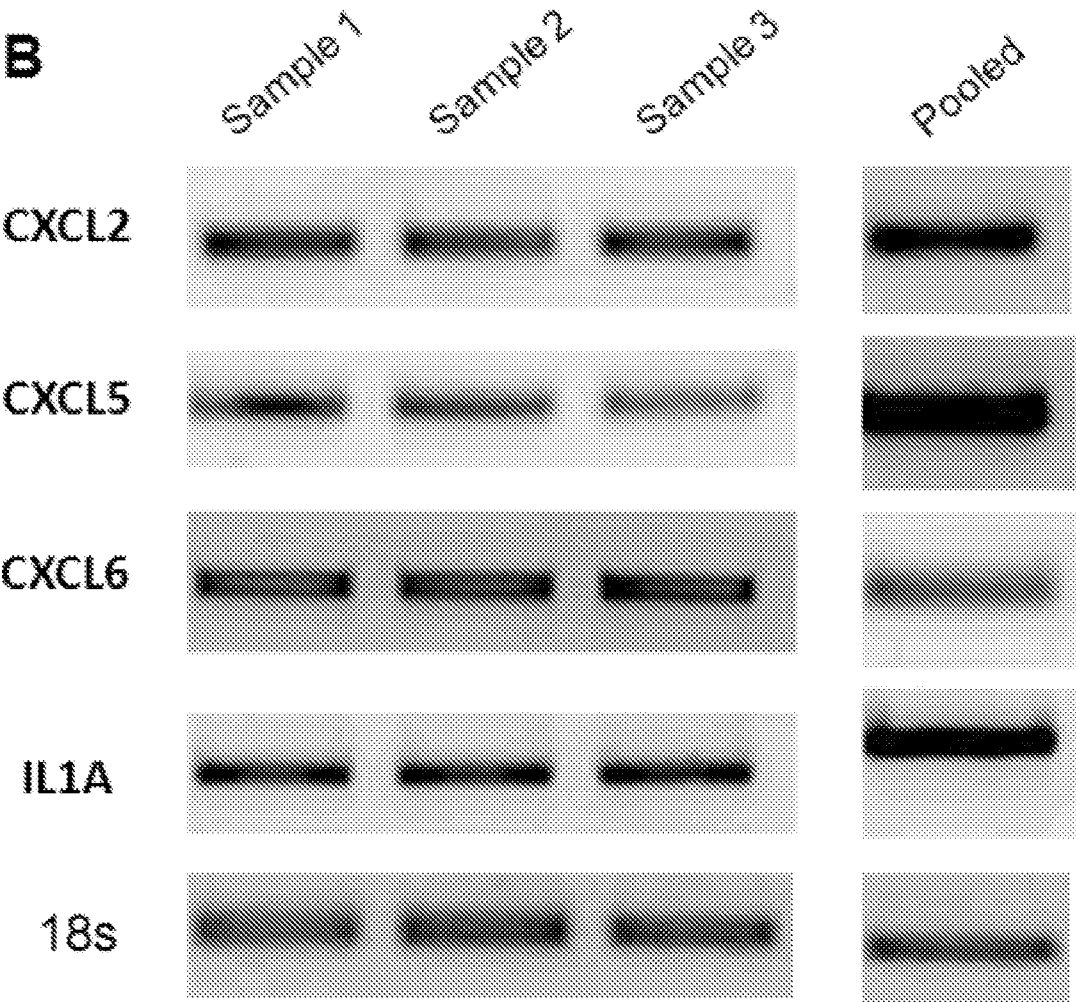
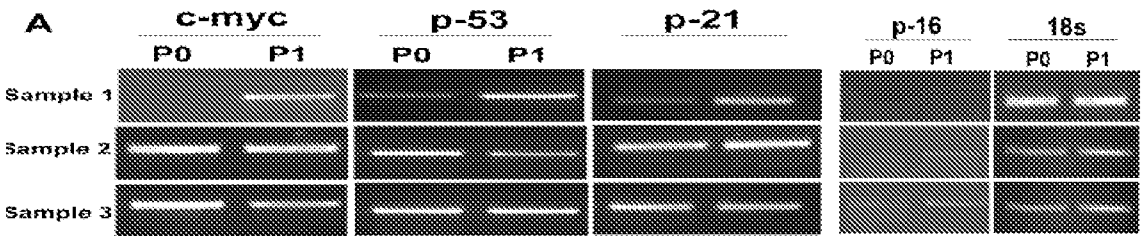


Figure 6

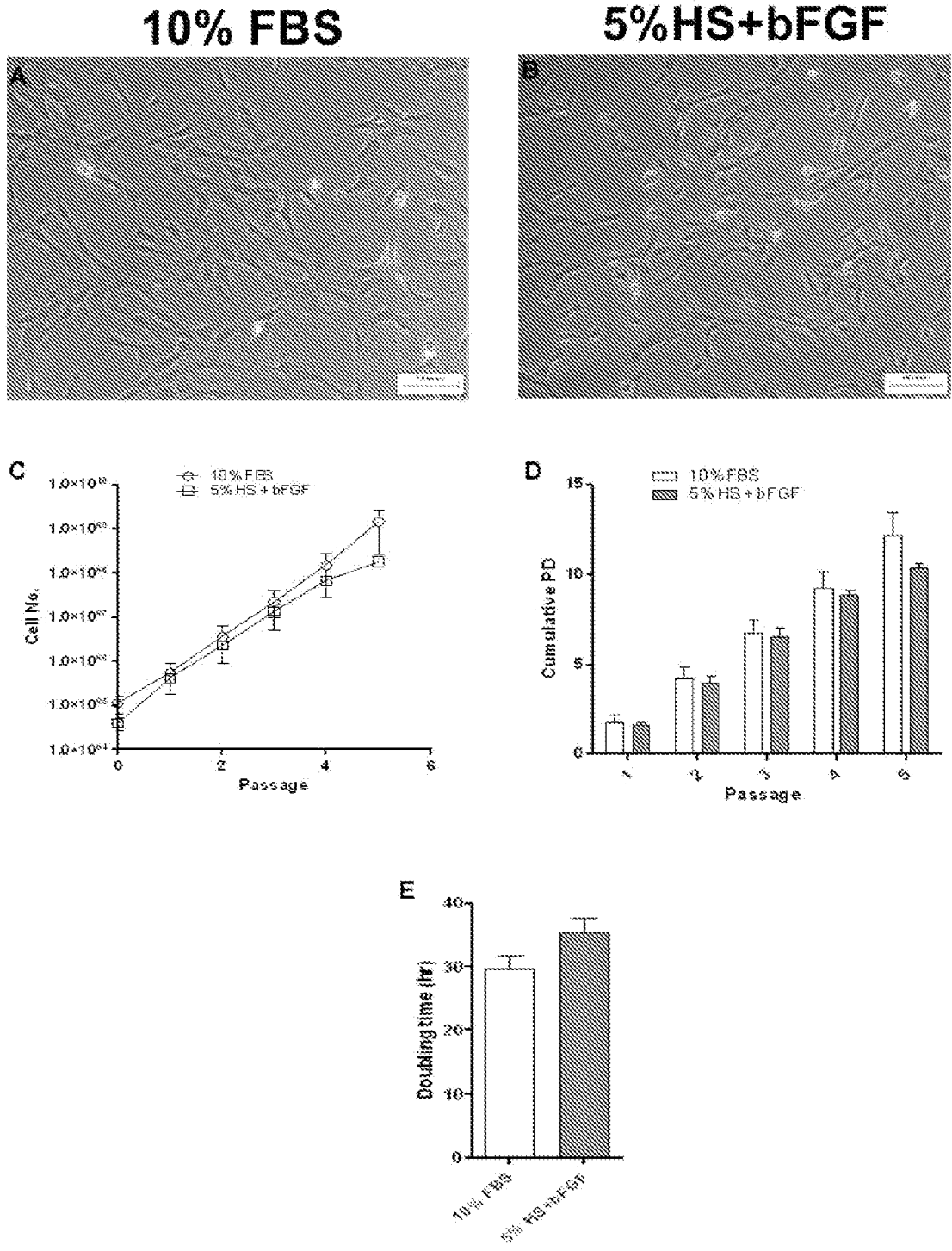


Figure 7

Reversal of STZ-induced hyperglycemia by WJ-MSCs

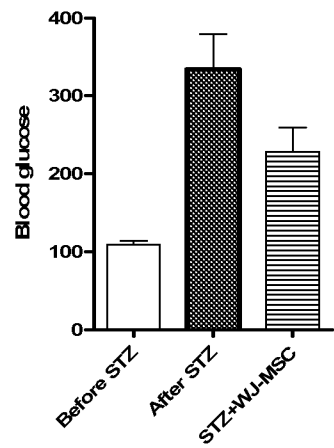


Figure 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2012/051519

A. CLASSIFICATION OF SUBJECT MATTER

C12N 5/0775 (2010.01) A61K 35/44 (2006.01) A61P 37/00 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI & EPODOC for Mesenchymal stem cells, Wharton's Jelly and like terms; MEDLINE, HCAPLUS, BIOSIS & EMBASE for Mesenchymal stem cells, Wharton's Jelly, cryo, plasmalyte, DMSO, HBSS, DMEM, FCS, cell bank and like terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
02 August 2012Date of mailing of the international search report
20 August 2012

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
Email address: pct@ipaustalia.gov.au
Facsimile No.: +61 2 6283 7999

Authorized officer

Vishal Desai
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No. 0262833180

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	PCT/IB2012/051519
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NEKANTI, U. et al. 'Optimization and scale-up of Wharton's jelly-derived mesenchymal stem cells for clinical applications'. Stem Cell Research. November 2010, Vol.5, No.3, pages 244-254, ISSN 1873-5061. Abstract; Figures 1, 2A & 4A-F; Materials and Methods; Table 1; p.248, left col., para.2;	1-22
X	FONG, C.-Y. et al. 'Derivation efficiency, cell proliferation, freeze-thaw survival, stem-cell properties and differentiation of human Wharton's jelly stem cells'. Reproductive BioMedicine Online. September 2010, Vol.21, No.3, pages 391-401, ISSN 1472-6483. Abstract; 'Derivation, growth and population doubling times of human WJSC'; 'Cryopreservation of human WJSC';	1-22
X	PETSA, A. et al. 'Effectiveness of protocol for the isolation of Wharton's Jelly stem cells in large-scale applications'. In Vitro Cellular & Developmental Biology-Animal. December 2009, Vol.45, No.10, pages 573-576, ISSN 1071-2690. 'Materials and Methods';	1-22
X	SUN, L. et al. 'Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus'. Arthritis & Rheumatism. August 2010, Vol.62, No.8, pages 2467-2475, ISSN 1529-0131. p.2469, left col., para.1; Table 1; p.2469, left col., para.3-right col., para.2; Figures 1 & 2;	1-22
X	NEKANTI, U. et al. 'Long-term expansion and pluripotent marker array analysis of Wharton's jelly-derived mesenchymal stem cells'. Stem Cells and Development. January 2010, Vol.19, No.1, pages 117-130, ISSN 1547-3287. 'Isolation and culture of WJMSCs'; Figures 1 & 2;	1-22
X	CAVALLO, C. et al. 'Comparison of alternative mesenchymal stem cell sources for cell banking and musculoskeletal advanced therapies'. May 2011 [e-Pub: 14 February 2011], Vol.112, No.5, pages 1418-1430, ISSN 0730-2312. Title; Abstract; p.1420, left col., para.1-para.2;	1-22
A	HUNT, CJ. 'Cryopreservation of human stem cells for clinical application: a review'. April 2011 [e-Pub: 16 March 2011], Vol.38, No.2, pages 107-123, ISSN 1660-3796. see whole citation;	
A	LIANG, J. et al. 'Allogeneic mesenchymal stem cells transplantation in treatment of multiple sclerosis'. Multiple Sclerosis. May 2009, Vol.15, No.5, pages 644-646, ISSN 1352-4585. see whole citation;	
P,X	WO 2011/101834 A1 (ADVANCED NEURO-SCIENCE ALLIES PRIVATE LIMITED) 25 August 2011 p.1, para.4-p.3, para.2; p.4, para.2; Examples 1 & 2;	1-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/IB2012/051519

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report**Patent Family Member/s****Publication Number****Publication Date****Publication Number****Publication Date**

WO 2011/101834 A1

25 Aug 2011

WO 2011101834 A1

25 Aug 2011

End of Annex