Stem Cell Research and Therapeutics
SCRT, 7(1): 214-221
www.scitcentral.com

ISSN: 2474-4646

Review Article: Open Access

Sources and Clinical Applications of Human Umbilical Cord Stem Cells in Periodontal Regeneration: State of the Art Review

Pornika Chakravarty, Joann Pauline George* and Apeksha Kamble

*Department of Periodontology, Krishnadevaraya College of Dental Sciences & Hospital, Bangalore, India.

Received September 01, 2022; Revised September 12, 2022; Accepted September 15, 2022

ABSTRACT

This review focuses on the isolation and therapeutic potential of stem cells harvested from the Wharton's Jelly of the human umbilical cord. Recently, investigators have found that a potent stem cell population exists within the Wharton's Jelly. In this review, the authors discuss perinatal stem cells, and compare them to other sources of stem cells. Furthermore, cryopreservation of Wharton's Jelly stem cells is described for potential use in future cell-based therapies and/or regenerative medicine applications. Current evidence of the application of mesenchymal stem cells from various sources in both pre-clinical and clinical trials is reviewed in the context of potential indications of use for Wharton's Jelly derived mesenchymal stem cells. Such neonatal stem cells appear to be more primitive and have greater multi-potentiality than their adult counterparts and can be considered as an effective means for periodontal regeneration.

An exhaustive search was undertaken to identify associated literature of human umbilical cord stem cells through MEDLINE/PubMed database using keywords (Mesh) "human umbilical cord stem cells," "periodontal regeneration," "mesenchymal stem cells," and "human umbilical cord" alone or in combination for articles dated Aug 2022 and before. Review was done to assess the anatomy of umbilical cord, isolation of stem cells from umbilical cord and various applications of human umbilical cord stem cells (hUCMSCs) in medicine and periodontal regeneration. Articles that did not have English translation and book publications were excluded. In addition to the main literature search, other searches of additional publications from cross references were also undertaken.

Keywords: Mesenchymal stem cells, Umbilical cord tissue, Wharton's Jelly stem cells, Stem cells, Mesenchymal stromal cells, Periodontal regeneration

Key message: The pluripotent capacity of stem cells has tremendous opportunities for treatment of periodontal diseases. The ethical issues concerning the harvest and use of stem cells can be overcome when cells are harvested from umbilical cord. This review aims to highlight the properties and utility of human umbilical cord stem cells.

INTRODUCTION

Stem cell plasticity has resulted in a new field of medicine entitled regenerative medicine. Stem cells, directed to differentiate into specific cell types, offer the possibility of a renewable source of replacement cells and tissues to treat diseases [1] Periodontium also has limited capacity for regeneration in early phases of the disease [2].

In a diseased periodontal environment, tissue repair does not occur naturally because of the lack of robust stem cells [3]. Therefore, exogenous regenerative 'tools' such as ex vivo expanded/manipulated stem cells can be used to replenish the host cell niche and facilitate tissue regeneration. Mesenchymal stem cells (MSCs) are a rare population of multipotent precursors which can be isolated from dental or non-dental sources and can differentiate into different lineages under appropriate induction conditions. The dental sources are dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), stem cells from human exfoliated deciduous teeth (SHED) and stem cells from apical papilla (SCAP). These cells of origin are promising but include an invasive procedure. The sources of stem cells from non-dental tissues are human bone-marrow-derived MSCs (BM-MSCs), adipose tissue derived stem cells (ADSCs) and embryonic stem cells (ESCs). BM-MSCs are

Corresponding author: Joann Pauline George, MDS, Department of Periodontology, Krishnadevaraya College of Dental Sciences & Hospital, Krishnadevarayanagar, Bangalore India, Tel: +91-9448541637; E-mail: drjoannpaulinegeorge@gmail.com

Citation: Chakravarty P, George JP & Kamble A. (2022) Sources and Clinical Applications of Human Umbilical Cord Stem Cells in Periodontal Regeneration: State of the Art Review. Stem Cell Res Ther, 7(1): 214-221.

extensively used, their harvest involves a highly invasive procedure and the frequency, proliferation efficiency, differentiation potential of BM-MSCs decline with age. The ESCs show excellent regenerative potential but raises ethical issues and have reported unprecedented consequences such as tumors and undesired immunological reactions thus limiting their use for periodontal regeneration. An elaborate research is still mandated for its effective clinical applications. Induced pluripotent stem cells (iPSCs) are directly procured from somatic cells. The pluripotent nature and the immortalized replication capacity make iPSCs a desired option.

UMBILICAL CORD MESENCHYMAL STEM CELLS (UC- MSCs)

As an alternative source of MSCs, fetal or neonatal MSCs appear to be more primitive and have greater multi-potentiality than their adult counterparts. MSCs in Wharton's jelly (WJ) from umbilical cord, which is discarded at birth, can provide an inexhaustible source of stem cells for therapy. These have faster proliferation rates and greater expansion capability compared with adult MSCs, with wide multipotency and no induction of teratomas [4]. They are more primitive than MSCs derived from other tissue sources, additionally, the collection procedure is non-invasive and painless and ethically noncontroversial [4]. Their intermediate state between adult and embryonic stem cells also makes them an ideal candidate for reprogramming to the pluripotent status.

Two outstanding features of MSCs are:

1) Immunosuppression, through specific interactions with immune cells that participate in both innate and adoptive responses

2) The so called immunoprivilege, they do not challenge a response of allogeneic immune cells [5]. The mechanisms of immunoprivilege are largely unknown but are most probably due to low expression of major histocompatibility complex (MHC)-I and MHC-II as well as the immunosuppressive functions. Comparison of immunogenicity of human umbilical cord-derived MSCs (hUMSCs) and adult bone marrow-derived MSCs (bmMSCs) showed that hUMSCs had significantly lower human leucocyte antigen (HLA)-I expression, higher production of tolerogenic transforming growth factor (TGF)-b and interleukin (IL) -10, significantly higher proliferation activity, stronger *in vitro* activation of allogeneic lymphocytes and delayed rejection *in vivo* [5]. The reduced expression of HLA-DR reduces the risk of eliciting an allogenic immune response when used for clinical applications [6].

Compartments of the Human Umbilical Cord FIG

The human UC develops around the fifth week of gestation and at term has an average length of about 50 cm (37) [7]. The umbilical cord is made up of a vein and two arteries that are encircled by Wharton's jelly and coated by a basic epithelial membrane. Stem cells from UC can be derived in the amniotic compartment (outer epithelial layer and inner subamniotic mesenchymal layer), the Wharton's jelly (WJ) compartment, the perivascular compartment surrounding the blood vessels, the media and adventitia compartment of the walls of umbilical cord blood vessels, the endothelial compartment (inner lining of the vein) i.e.human umbilical vein epithelial cells (hUVEC), and the vascular compartment (blood lying within the umbilical cord blood vessels). All these compartments are described as distinct regions in the literature and have been termed such as 'cord lining', 'subamnion', 'intervascular', 'perivascular' and 'hUVEC' being used. Stem cell populations with varied stemness properties have been reported for each of these compartments [8,9].

Wharton's Jelly Stem cells-Origin

Wharton's Jelly prevents the compression, torsion, and bending of the umbilical vessels which provide the bi-directions flow of oxygen, glucose and amino acids to the developing fetus, while also depleting the fetus and placenta of carbon dioxide and other waste products [9,10]. Cells found in Wharton's Jelly are a primitive mesenchymal stem cell (MSC), trapped in the connective tissue matrix. Colonies of early hematopoietic cells and mesenchymal cells migrate through the early umbilical cord to the placenta between embryonic day 4 and 12 of embryogenesis [11]. These mesenchymal stromal cells become embedded in the Wharton's Jelly early in embryogenesis and remain there for the duration of gestation [11]. These primitive mesenchymal stromal cells have been derived for regenerative procedures in medicine.

Methods of Derivation of Stem Cells from the Human Umbilical Cord

Six different methods have been reported

i. UC pieces were first cut open, the umbilical blood vessels (which may carry with them the perivascular regions) removed and the remaining inner surface of the cord piece was scraped with forceps to retrieve the WJ from which stem cells were harvest [12].

ii. UC pieces were cut open, umbilical blood vessels retained and only the WJ was separated. The WJ was then either directly exposed to enzymatic solutions to release the cells or cut into small pieces and then enzymatically treated [12,13].

iii. Entire UC pieces with intact umbilical blood vessels were cut into smaller pieces and then grown as explants on plastic for a few days after which cell outgrowths from the explants were separated and cultured [11]. To

maximize the recovery of stem cells, Tsagias [14] first washed the entire length of the UC under sterile conditions to remove blood, then sterilized its surface, and with the umbilical blood vessels intact immersed the entire UC into a sterile bag containing an enzymatic solution of collagenase and hyaluronidase and incubated the bag at 37°C for 3 h with gentle agitation [14]. The UC was then exposed to trypsin for a further 30 min and the digested cell suspension collected by gravity.

iv. The subamnion region of the UC was removed with a razor blade, cut into small pieces and grown on plastic as explants from which the cell outgrowths were separated and cultured. These stem cell populations were called subamnion or cord lining MSCs [15].

v. The umbilical blood vessels were removed from cord pieces, tied at either end into loops and the loops placed into an enzymatic solution for a specific period of time to allow detachment of cells from the perivascular region which are then grown in culture. These were referred to as UC perivascular stem cells (UCPVSCs) [16].

vi. Romanov [17] isolated stem cells from the endothelial lining of the vein of the UC by first removing the vein and then passing through it an enzymatic solution to digest and remove the inner endothelial lining cells [17]. The cell suspension was centrifuged to remove the enzymes and the cell pellet washed and seeded into culture medium in plastic dishes to grow the endothelial cells. The stem cells from such endothelial linings have been commonly referred to as human umbilical vein epithelial cells (hUVECs).

Different Stem Cell Populations in the Various Human Umbilical Cord

Compartments with Different Stem Cell Characteristics

MSCs derived from arterial (UCA), venous (UCV) and Wharton's jelly (UCWJ) explants of the human UC were compared for osteogenic, UCWJ were the least effective while UCA-derived cells developed alkaline phosphatase activity with or without an osteogenic stimulus [17]. MSCs isolated from the cord blood, WJ and perivascular regions of the UC, those from the WJ (which they included as intravascular and subamnion) offered better clinical utility because isolation frequency of colony forming unit-fibroblasts (CFU-Fs) were extremely high and delays in processing did not impact isolation [18]. UC- MSCs have shown the propensity to differentiate into hematopoietic stem cells, epithelial stem cells, mesenchymal stem cells, endothelial progenitors and induced pluripotent stem cells.

Umbilical Cord versus Bone Marrow-Derived Mesenchymal Stromal Cells

Even though hWJSCs and hBMMSCs may have common origins during human embryonic and fetal development, hWJSCs appear to be distinctly different and have advantages over hBMMSCs when it comes to clinical application [19]. hWJSCs resemble hBMMSCs in terms of a short-fibroblast-like phenotype, nonhematopoietic surface markers, hypoimmunogenecity, multipotent plasticity and expression of some markers such as CD90, CD105, CD13, CD73, CD10, CD29 CD51, CD166, CD44 and the HLS antigens HLA-A, B, C and G which implies that hUCMSCs have multipotent feature of adult stem cells [20-22]. hWJSCs do not express CD45, CD14, CD56, CD31 and CD34 at high levels and are HLA DR+ unlike hBMMSCs, have higher proliferation rates, increased colony forming unit (CFU) formation and stemness characteristics that last for longer periods of time after serial passaging (14) [23,24]. hWJSCs also express several ESC markers at different levels of expression such as the members of the OCT family, embryonic surface marker antigens (SSEA-4, Tra-1-60 and Tra-1-81), alkaline phosphatase (ALP), DNMT3B and GABRB3 and the genomic markers (SOX2, NANOG, REX2) (8) which suggests that along with few adult stem cell markers these cells also have the quality of embryonic stem cells [25].

Immunogenecity of hWJSCs

Stem cells harvested directly from the Wharton's jelly compartment of the human UC have been shown to possess hypoimmunogenic properties that have been characterized both *in vitro* and *in vivo*. Weiss [22] showed that hWJSCs express mRNA for pan-HLA-G and do not express the costimulatory surface antigens CD40, CD80, and CD86. There was no evidence of frank immunorejection of undifferentiated hWJSCs and that they would be tolerated in allogeneic transplantation settings [22].

The hypoimmunogenecity of MSCs from other compartments of the human UC derived by different methods have also been reported. The immunogenecity and immunomodulatory properties of umbilical cord lining (subamnion) MSCs were studied by Deuse [26]. When these workers compared the immunogenecity of hBMMSCs and their subamniotic MSCs in immunocompetent mice the hBMMSCs exhibited a faster immunorejection response whereas in immunodeficient mice cell survival was prolonged and similar for both hBMMSCs and subamniotic MSCs.

Differentiation: UC-MSCs have a faster rate of differentiation as compared to PDL and adipose tissue. But GMSCs (MSCs derived from gingiva) have a higher doubling time than UC-MSCs.

CLINICAL APPLICATIONS

Stems cells have been investigated in the treatment of hematological diseases such as Sickle cell anemia , Aplastic anemia, Thalassemia, Leukemia; Treatment of cardiovascular diseases such as Buerger's disease, Dilated cardiomyopathy and Stroke; Bone regeneration -

Osteoporosis, Congenital abnormalities, trauma, tumor resections, fractures as well as disorders such as arthritis; Treatment of eyesight diseases - Diabetic retinopathy-associated neurodegeneration, Traumatic optic neuropathy; Treatment of metabolic disorders - Type 1 diabetes, Type 2 diabetes; Treatment of neurodegenerative and neurodevelopmental disorders - Parkinson's disease, Huntington's disease, Alzheimer's disease, Amyotrophic lateral sclerosis, Autism and Wound healing.

Clinical Applications of hWJSCs and Its Extracts (Conditioned Medium and Cell-free Lysate)

Wharton's jelly MSCs have much lower differentiation capacity than PDLSCs, and unmodified Wharton's jelly MSCs may not be effective seeding cells for periodontal regeneration [27]. Human UC-MSCs have the ability to develop into cells that can bind to tooth root surfaces. Incubating UC-MSCs with FGF improved their proliferation and adhesion to root surfaces. These findings by George [28] suggest that involvement of UC-MSCs in periodontal regeneration can be investigated further. PDLSCs were shown to have more osteogenic differentiation capability than human UC-MSCs. Meanwhile, UC-MSCs secreted more extracellular matrix-related genes, including fibronectin, integrin β, and collagen type I, and had better anti-inflammatory properties, i.e., higher expression of TGF-β than PDLSCs [29]. Bone collagen particles coupled with UC-MSCs were much more effective than bone collagen particles only in promoting bone repair and regeneration; thereby providing a simple, quick, and effective strategy for filling a bone defect area and treating AB alveolar cleft lesions in rabbits [30]. UC-MSCs have the ability to develop into odontoblast-like cells with typical dentin-like matrix deposition *in vivo*, indicating the use of UC-MSCs as a therapeutic source of cells for tooth regeneration [31].

Cell-based Therapies

UC-MSCs differentiated and engrafted with successful functional outcome *in vivo* in rat models for cerebral ischemia, intracerebral hemorrhage, spinal cord injury, Parkinson's disease, retinal disease, Type 1 diabetes and myogenic disease (15) [32].

Anticancer Effects

Many groups have reported that hWJSCs, its conditioned medium (hWJSC-CM) and cell-free lysate (hWJSC-CL) exhibit anticancer effects on solid tumors and are therefore attractive candidates for future cancer therapies [33,34]. hWJSCs were administered intravenously 8 days after tumor transplantation in a human mammary adenocarcinoma xenograft rat model, it was seen that they hoped to metastatic tumor sites in the lungs and reduced tumor burden [33]. The hypothesis suggested for the anticancer effect was that hWJSCs were first engulfed by mammary adenocarcinoma cells and then they disintegrated within the cancer cells leading to apoptosis of the mammary adenocarcinoma cells [34].

Regeneration of periodontal tissues by UC-MSCs

Chronic periodontitis is an infectious disease resulting in inflammation of the supporting tissues of the teeth leading to progressive attachment and bone loss and is characterized by pocket formation and/or recession of the gingiva. Progression of periodontal disease leads to continuous destruction of alveolar bone resulting into tooth mobility and loss of tooth [35]. Treatment of periodontal disease aims not only at arresting the disease process but also strives for regeneration of the structures that are lost in the course of the disease. Substantial progress has been made in understanding the basis of regeneration and various studies have proven that regenerative cells dwell in bone and periodontal ligament (PDL) [36]. PLFs play a crucial role in periodontal healing and regeneration. They are essential for the formation of new attachment on the root surfaces exposed due to disease process.

Cell implantation with human umbilical cord stem cells can be considered useful as implantation of dental derived stem cells with a collagen scaffold has resulted in optimal bone repair and complete osseous regeneration [37,38]. hUCMSC can differentiate into hPDLC under certain conditions *in vitro* and hUCMSC may hopefully become the stem cells in periodontal tissue engineering. When the effects of demineralized bone matrix on proliferation and osteogenic differentiation of mesenchymal stem cells from human umbilical cord was analyzed it was concluded that mesenchymal progenitor cells derived from umbilical cord could differentiate along an osteogenic lineage and thus provide an alternative source for cell-based therapies and tissue engineering strategies [39]. When human Umbilical cord Wharton's jelly-derived mesenchymal stem cells (hUCMSCs) in combination with poly ε-caprolactone (PCL)-graphene oxide (GO)-Cissus quadrangularis (CQ) (PCL-GO-CQ) scaffold for the treatment of multiple gingival recessions was assessed 80% success rate was reported [40].

Regenerative medicine applications of MSCs

Additional properties of MSCs make them useful stem cell candidates for use in various cell-based therapies, beyond umbilical cord blood hematopoietic engraftment. For instance, UC-MSCs share the natural homing capabilities of BM-MSCs. For MSCs, an injury serves as a homing beacon, as they home to sites of inflammation and to locally effect the inflammatory/immune mediated tissue damage with subsequent ability to support tissue healing. They shift the spectrum of local cytokines from proinflammatory to anti-inflammatory [41]. Thus this property of these stem cells makes them potent candidates for treating periodontal inflammation. Studies are currently ongoing to take full advantage of these unique properties for specific indications.

The immunosuppressive ability of these cells has the potential to treat many disorders including graft-versus host disease (GvHD), diabetes, Crohn's disease, heart disease, and solid tumor cancers (29) [42-46].

GROWTH CHARACTERISTICS OF hWJSCs

Growth factors in the culture medium may have a positive influence on the proliferation rates of these cells *in vitro*. Culture media that have been used to propagate UC-MSCs have ranged from simple un-supplemented basal media to supplemented super-complex media containing a multitude of supplements. The super-complex media contain additional nutrients such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), ascorbic acid and dexamethasone [47]. Ascorbic acid and dexamethasone are traditionally used as differentiation agents to drive stem cells towards osteogenic or chondrogenic lineages and as such it is not known whether the use of such agents will compromise the differentiation of hWJSCs into other desirable lineages. Most of the culture media that is used to grow UC-MSCs have ingredients of animal origin (xenoproteins) such as bovine brain extracts, fetal bovine serum and animal sources of insulin and FGF.

The important role played by bFGF as an inductive media in propelling hUCMSCs into a more committed lineage of differentiation has been evaluated by Ramasamy. This role of bFGF in inducing the proliferation of fibroblast or fibroblast like cells has also been noted in various studies. As fibroblasts are the most crucial cells in periodontal regeneration. This characteristic feature of hUCMSCs can be essential in formation of new attachment. Ramasmamy [48] in his study showed that bFGF drives UC-MSC into S phase, as indicated by elevated levels of cyclin D1 and D3 proteins and cyclin-dependent kinase, which means that under the influence of FGF cells are driven into the active phase of cell cycle. He also mentioned that bFGF does not alter the osteogenic differentiation potential and immunosuppressive activity of UCMSCs and preserves the primitive status of UCMSC by increasing expression of NANOG, Sox2 and Rex1 transcription factors [48].

Among MSCs isolated from the three different tissues periodontal ligament, umbilical cord (UC-MSCs) and adipose tissue, UC-MSCs grew the fastest *in vitro*. When the regenerative potentials , multi-differentiation ability and anti-inflammatory capability of hUCMSCs was compared with hPDLSCs it was observed that the osteogenic potential of hPDLSCs was better, but hUCMSCs demonstrated better anti-inflammatory properties and also laid down more extracellular matrix; however both were similar in the regenerative potential [49].

ADVANTAGES

1. Painless collection procedure and faster self-renewal properties.
2. Their collection does not necessitate the invasive method required for PDLSCs and does not include the concerns associated with human. Furthermore, human UC-MSCs were found to be primordial MSCs with significant plasticity and developmental flexibility. Furthermore, human UC-MSCs have shown little immunorejection *in vivo* and are not tumorigenic [22]. Because of these benefits, human UC-MSCs are a promising choice for periodontal regeneration treatment.

FUTURE DIRECTIONS

This treatment option is yet to be approved by the US Food and Drug Administration (FDA). The implantation of UCMSCs into periodontal wound via either biomaterials-free or biomaterial based approaches is a possibility that may enhance periodontal regeneration. *Ex- vivo* expansion and reimplantation of stem cells derived from other sources may have already shown remarkable results. Bioengineered cell constructs delivering seeding cells; scaffold free systems to prevent immunologic reactions, cell suspension injections, cell sheet engineering, cell pellets/ microtissue, various biomaterials assisted cell delivery system (natural origin, synthetic polymers or ceramics) can be harnessed for the inoculation of UCMSCs into the periodontal wound to facilitate periodontal regeneration. One of the major challenges faced in clinical applications of stem cells is the limited cell availability. Donor age is another factor. The proliferation and migration capacity and differentiation ability of these cells decrease as donor age advances. Contamination of MSCs with other cells such as hematopoietic cells, fibroblasts or endothelial cells during isolation is also a factor to be considered. Potential long-term associated risks of MSC transplantation have not been explored fully and there is need for the same. Plants have also shown to contain hormones or functional molecules. Numbers of preclinical studies have revealed that plant products can be successfully applied in modulating proliferation and differentiation of human mesenchymal stem cells. Plant-derived substances can induce stem cells osteogenic differentiation, and they also possess angiogenic potency.

CONCLUSION

Although there are no current clinical trials ongoing with WJSCs or UC-MSCs, several pre-clinical trials have been conducted to suggest the possible clinical benefits of this cell source. Several indications have been investigated in animals including hematopoietic reconstitution, Parkinson's, diabetes, macular degeneration and spinal cord injuries. Before WJSCs can be safely translated into human clinical trials for periodontal regeneration, further investigation and characterization in animals must be completed to ensure safety and efficacy in inoculating these cells in periodontal defects. WJSCs are immuno-privileged, immunosuppressive, have a multipotent/pluripotent differentiation capacity and are readily available as a cell source; WJSCs may be an

important cell therapy source for periodontal regeneration in the near future to treat several diseases and improve the quality of life in many patients.

REFERENCES

1. Ercal P, Pekozer GG, Kose GT (2018) Dental stem cells in bone tissue engineering: Current overview and challenges. Adv Exp Med Biol 1107: 113-127.
2. Shi S, Bartold PM, Miura M, Seo BM, Robey PG, et al. (2005) The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthod Craniofac Res 8: 191-199.
3. Chen FM, Sun HH, Lu H, Yu Q (2012) Stem cell-delivery therapeutics for periodontal tissue regeneration. Biomaterials 33: 6320-6344.
4. Taghizadeh RR, Cetrulo KJ, Cetrulo CL (2011) Wharton's Jelly stem cells: Future clinical applications. Placenta 32 Suppl 4: S311-S315.
5. Liu S, Yuan M, Hou K, Zhang L, Zheng X, et al. (2012) Immune characterization of mesenchymal stem cells in human umbilical cord Wharton's jelly and derived cartilage cells. Cell Immunol 278: 35-44.
6. Kim JH, Jo CH, Kim HR, Hwang YI (2018) Comparison of immunological characteristics of mesenchymal stem cells from the periodontal ligament, umbilical cord, and adipose tissue. Stem Cells Int 2018: 8429042.
7. Tyndall A, Uccelli A (2009) Multipotent mesenchymal stromal cells for autoimmune diseases: Teaching new dogs old tricks. Bone Marrow Transplant 43(11): 821-828.
8. Gauglitz GG, Phan TT, Herndon DN, Kita K (2011) Umbilical cord lining membrane and Wharton's jelly-derived mesenchymal stem cells: the similarities and differences. Open Tissue Eng Regen Med J 4: 21-27.
9. Fong CY, Gauthaman K, Bongso A (2009) Reproductive stem cells of embryonic origin: Comparative properties and potential benefits of human embryonic stem cells and Wharton's jelly stem cells. In C. Simon & A. Pellicer (Eds.), A Stem cells in human reproduction. New York: Informa Healthcare 2nd ed., pp: 136-149.
10. Angelucci S, Marchisio M, Giuseppe FD, Pierdomenico L, Sulpizio M, et al. (2010) Proteome analysis of human Wharton's jelly cells during *in vitro* expansion. Proteome Sci 8: 18-25.
11. Lu LL, Liu YJ, Yang SG, Yang S-G, Zhao Q-J, et al. (2006) Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis-supportive function and other potentials. Haematologica 91: 1017-1026.
12. Tsumanuma Y, Iwata T, Washio K, Yoshida T, Yamada A, et al. (2012) Comparison of different tissue-derived stem cell sheets for periodontal regeneration in a canine 1-wall defect model. Biomaterials 32(25): 5819-5825.
13. William PL, Banister LH, Berry MM (1995) Gray's anatomy 38th ed. London: ELBS Churchill Livingstone.
14. Tsagias N, Koliakos I, Karagiannis V, Eleftheriadou M, Koliakos GG (2011) Isolation of mesenchymal stem cells using the total length of umbilical cord for transplantation purposes. Transfus Med 21: 253-261.
15. Romanov YA, Svintsitskaya VA, Smirnov VN (2003) Searching for alternative sources of postnatal human mesenchymal stem cells: Candidate MSC-like cells from umbilical cord. Stem Cells 21: 105-110.
16. Hynes K, Menicanin D, Gronthos S, Bartold PM (2012) Clinical utility of stem cells for periodontal regeneration. Periodontol 59(1): 203-227.
17. Huang YC, Parolini O, La Rocca G, Deng L (2012) Umbilical cord versus bone marrow derived mesenchymal stromal cells. Stem Cells Dev 21: 2900-2903.
18. Fong CY, Richards M, Manasi N, Biswas A, Bongso A (2007) Comparative growth behavior and characterization of stem cells from human Wharton's jelly. Reprod Biomed Online 15: 708-718.
19. Can A, Karahuseyinoglu S (2007) Concise review: Human umbilical cord stroma with regard to the source of fetus-derived stem cells. Stem Cells 25: 2886-2895.
20. Weiss ML, Medicetty S, Bledsoe AR, Rachakatla RS, Choi M, et al. (2006) Human umbilical cord matrix stem cells: Preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. Stem Cells 24: 781-792.
21. Wang H-S, Hung S-C, Peng S-T, Huang C-C, Wei H-M, et al. (2004) Mesenchymal stem cells in the Wharton's Jelly of the human umbilical cord. Stem Cells 22: 1330-1337.
22. Fong CY, Subramanian A, Biswas A, Gauthaman K, Srikanth P, et al. (2010) Derivation efficiency, cell proliferation, frozen-thaw survival, 'stemness' properties, and differentiation of human Wharton's jelly stem cells: Their potential for concurrent banking with cord blood for regenerative medicine purposes. Reprod Biomed Online 21: 391-401.
23. Conconi MT, Di Liddo R, Tommasini M, Calore C, Parnigotto PP (2011) Phenotype and differentiation potential of stromal populations obtained from various zones of human umbilical cord: An overview. Open Tissue Eng Regen Med J 4: 6-20.

24. Deuse T, Stubbendorff M, Tang-Quan K, Phillips N, Kay MA, et al. (2011) Immunogenecity and immunomodulatory properties of umbilical cord lining mesenchymal stem cells. Cell Transplant 20: 655-667.

25. Shaikh MS, Shahzad Z, Tash EA, Janjua OS, Khan MI, et al. (2022) Human Umbilical Cord Mesenchymal Stem Cells: Current Literature and Role in Periodontal Regeneration. Cells 11(7): 1168.

26. George JP, Chakravarty P, Chowdhary KY, Purushothama H, Rao JA (2015) Attachment and differentiation of human umbilical cord stem cells on to the tooth root surface with and without the use of fibroblast growth factor-an *in vitro* study. Int J Stem Cells 8: 90.

27. Shang F, Liu S, Ming L, Tian R, Jin F, et al. (2017) Human umbilical cord MSCs as new cell sources for promoting periodontal regeneration in inflammatory periodontal defect. Theranostics 7: 4370.

28. Sun X-C, Wang H, Li J-H, Zhang D, Yin L-Q, et al. (2020) Repair of alveolar cleft bone defects by bone collagen particles combined with human umbilical cord mesenchymal stem cells in rabbit. Biomed Eng Online 19: 1-19.

29. Chen Y, Yu Y, Chen L, Ye L, Cui J, et al. (2015) Human umbilical cord mesenchymal stem cells: A new therapeutic option for tooth regeneration. Stem Cells Int 2015: 549432.

30. Fan CG, Zhang Q, Zhou J (2011) Therapeutic potentials of mesenchymal stem cells derived from human umbilical cord. Stem Cell Rev Rep 7: 195-207.

31. Maurya DK, Doi C, Kawabata A, Pyle MM, King C, et al. (2010) Therapy with un-engineered naïve rat umbilical cord matrix stem cells markedly inhibits growth of murine lung adenocarcinoma. BMC Cancer 10: 590-601.

32. Chao KC, Yang HT, Chen MT (2011) Human umbilical cord mesenchymal stem cells suppress breast cancer tumorogenesis through direct cell-cell contact and internalization. J Cell Mol Med16: 1803-1815.

33. Burt B (2005) Position paper: Epidemiology of periodontal diseases. J Periodontol 76: 1406-1419.

34. Wang HL, Greenwell H, Fiorellini J, Giannobile W, Offenbacher S, et al. (2005) Periodontal regeneration. J Periodontol 76: 1601-1622.

35. Honsawek S, Dhitiseith D, Phupong V (2006) Effects of demineralized bone matrix on proliferation and osteogenic differentiation of mesenchymal stem cells from human umbilical cord. J Med Assoc Thai 89 Suppl 3: S189-S195.

36. Kadam S, Gautam S, Dwivedi A, Jain V (2019) Treatment of gingival recession defect using human umbilical cord mesenchymal stem cells cultured on PCL based bone regenerating scaffold: A randomized controlled clinical study. Cytotherapy 21(5): S51.

37. Sarugaser R, Lickorish D, Baksh D, Hosseini MM, Davies JE (2005) Human umbilical cord perivascular (HUCPV) cells: A source of mesenchymal progenitors. Stem Cells 23: 220-229.

38. Bernardo ME, Pagliara D, Locatelli F (2011) Mesenchymal stromal cell therapy: A revolution in regenerative medicine. Bone Marrow Transplant 47(2): 164-171.

39. Anzalone R, Lo Iacono M, Loria T, Di Stefano A, Giannuzzi P, et al. (2011) Wharton's jelly mesenchymal stem cells as candidates for beta cells regeneration: extending the differentiative and immunomodulatory benefits of adult mesenchymal stem cells for the treatment of type 1 diabetes. Stem Cell Rev Rep 7(2): 342-363.

40. Boomsma RA, Swaminathan PD, Geenen DL (2007) Intravenously injected mesenchymal stem cells home to viable myocardium after coronary occlusion and preserve systolic function without altering infarct size. Int J Cardiol 122(1): 17-28.

41. Rachakatla RS, Pyle MM, Ayuzawa R, Edwards SM, Marini FC, et al. (2008) Combination treatment of human umbilical cord matrix stem cell-based interferon-beta gene therapy and 5-fluorouracil significantly reduces growth of metastatic human breast cancer in SCID mouse lungs. Cancer Invest 26(7): 662-670.

42. Ishige I, Nagamura-Inoue T, Honda MJ, Harnprasopwat R, Kido M, et al. (2009) Comparison of mesenchymal stem cells derived from arterial, venous and Whartons' jelly explants of human umbilical cord. Int J Hematol 90: 261-269.

43. Ramasamy R, Tong CK, Yip WK, Vellasamy S, Tan BC, et al. (2012) Basic fibroblast growth factor modulates cell cycle of human umbilical cord-derived mesenchymal stem cells. Cell Prolif 45: 132-139.

44. Ding DC, Chang YH, Shyu WC, Lin SZ (2015) Human umbilical cord mesenchymal stem cells: A new era for stem cell therapy. Cell Transplant 24(3): 339-347.

45. Friedman R, Betancur M, Boissel L, Tuncer H, Cetrulo C, et al. (2007) Umbilical cord mesenchymal stem cells: Adjuvants for human cell transplantation. Biol Blood Marrow Transplant 13(12): 1477-1486.

46. Watt SM, Su CC, Chan JY (2010) The therapeutic potential of stem cells in umbilical cord and umbilical cord blood. J Med Sci 30: 177-187.

47. Chao KC, Chao KF, Fu YS, Liu SH (2008) Islet-like clusters derived from mesenchymal stem cells in Wharton's jelly of the human umbilical cord for transplantation to control type 1 diabetes. PLoS One 3(1): 1451.

48. Lund RD, Wang S, Lu B, Girman S, Holmes T, et al. (2007) Cells isolated from umbilical cord tissue rescue photoreceptors and visual functions in a rodent model of retinal disease . Stem Cells 25(3): 602-611.

49. Yang CC, Shih YH, Ko MH, Hsu SY, Cheng H, et al. (2008) Transplantation of human umbilical mesenchymal stem cells from Wharton's jelly after complete transection of the rat spinal cord. PLoS One 3(10): 3336.