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Review on human umbilical cord stem cells in periodontal regeneration- A step towards future.

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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 define a new subset of stem cells, termed 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 with or without the use of scaffolds.

An exhaustive search was undertaken to identify associated literature of human umbilical cord stem cells through MEDLINE/PubMed database using keywords "human umbilical cord stem cells," "periodontal regeneration," "mesenchymal stem cells," and "human umbilical cord" alone or in combination. Documented review of the relevant articles was performed for articles dated May 2013 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.

Key Words

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

I. INTRODUCTION

Stem cells are the most fascinating area of biology today and have been used clinically in the field of medicine to treat many incurable diseases. 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 (24). Periodontium also has limited capacity for regeneration in early phases of the disease (31). Most recent in the quest is the identification of putative dental stem cell populations and their potential use in stem cell based therapies to treat the damage caused by trauma, cancer, caries and periodontal disease.

In a diseased periodontal environment, tissue repair does not occur naturally because of the lack of robust stem cells (6). 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 many different tissue sources and also can differentiate into different lineages under appropriate induction conditions. Human bone-marrow-derived MSCs (BM-MSCs) are extensively used, their harvest involve a highly invasive procedure, and the frequency, proliferation efficiency, differentiation potential of BM-MSCs decline with age.

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, no induction of

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teratomas.(34) They are more primitive than MSCs derived from other tissue sources, additionally, the collection procedure is non-invasive and painless and ethically noncontroversial (34). Their intermediate state between adult and embryonic stem cells also makes them an ideal candidate for reprogramming to the pluripotent status. They are very attractive for a wide range of regenerative medicine applications.

Two outstanding features of MSCs are relevant to immunity: (1) immunosuppression, through specific interactions with immune cells that participate in both innate and adoptive responses, and (2) the so called immunoprivilege.(32) The mechanisms of immunoprivilege are largely unknown but are most probably due to low expression of MHC-I and MHC-II as well as the immunosuppressive functions, meaning that they do not challenge a response of allogeneic immune cells. Comparison of immunogenicity of human umbilical cord-derived MSCs (hUMSCs) and adult bone marrow-derived MSCs (bmMSCs) showed that hUMSCs had significantly lower HLA-I expression, higher production of tolerogenic TGF-b and IL-10, significantly higher proliferation activity, stronger in vitro activation of allogeneic lymphocytes, and delayed rejection in vivo (32).

Compartments of the Human Umbilical Cord

The human UC develops around the fifth week of gestation and at term has an average length of about 50 cm (37). 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 (17) 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 (12).

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 (12,1). 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 (21).These mesenchymal stromal cells become embedded in the Wharton's Jelly early in embryogenesis and remain there for the duration of gestation (21). 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 (38). (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 (39). (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 (21) To maximise the recovery of stem cells, Tsagias et al. (35) 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. 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 (17). (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) (33). (vi) Romanov et al. (28) 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. 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 (19).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 (26).

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 (18).

hWJSCs resemble hBMMSCs in terms of a short-fibroblast-like phenotype (13), nonhematopoietic surface markers (7), hypoimmunogenecity (40), 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 (22).

hWJSCs do not express CD45, CD14, CD56, CD31 and CD34 at high levels and are HLA DR+ (41) 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). 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.

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 et al. (40) 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.

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 et al. (11).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.

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

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).

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 (Maurya DK, Doi C, Kawabata A, et al. 2010)..hWJSCs were administered intravenously 8 days after tumor transplantation in a human mammary adenocarcinoma xenograft rat model, it was seen that they homed to metastatic tumor sites in the lungs and reduced tumor burden (25). The hypothesis suggested for the anticancer effect was

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thathWJSCs were first engulfed by mammary adenocarcinoma cells and then they disintegrated within the cancer cells leading to apoptosis of the mammary adenocarcinoma cells (9).

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 (27). Progression of periodontal disease leads to continuous destruction of alveolar bone resulting into tooth mobility and loss of tooth (3). 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 (42). 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) (42). 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 (19,38).

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 (34). 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-versushost disease (GvHD) (4,36), diabetes (2), Crohn's disease (36), heart disease (Boomsma RA et al 2007), and solid tumor cancers (29).

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 (20). 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 (30) 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 (30).

Future directions

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 suspention injections, cell sheet engineering, cell pellets/ microtissue, various biomaterial assisted cell delivery system (natural origin, synthetic polymers

or ceramics) can be harnessd for the inoculation of UCMSCs into the periodontal wound to facilitate periodontal regeneration.

II. 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 (16), Parkinson's (43), diabetes (10), Macular Degeneration (23) and spinal cord injuries (44). 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.

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Declaration of Interest
Authors report no declaration of interests.