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Ectodermal Differentiation of Wharton's Jelly Mesenchymal Stem Cells for Tissue Engineering and Regenerative Medicine Applications

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Mesenchymal stem cells (MSCs) from Wharton's jelly (WJ) of the human umbilical cord are perinatal stem cells that have self-renewal ability, extended proliferation potential, immunosuppressive properties, and are accordingly excellent candidates for tissue engineering. These MSCs are unique, easily accessible, and a noncontroversial cell source of regeneration in medicine. Wharton's jelly mesenchymal stem cells (WJMSCs) are multipotent and capable of multilineage differentiation into cells like adipocytes, bone, cartilage, and skeletal muscle upon exposure to appropriate conditions. The ectoderm is one of the three primary germ layers found in the very early embryo that differentiates into the epidermis, nervous system (spine, peripheral nerves, brain), and exocrine glands (mammary, sweat, salivary, and lacrimal glands). Accumulating evidence shows that MSCs obtained from WJ have an ectodermal differentiation potential. The current review examines this differentiation potential of WJMSC into the hair follicle, skin, neurons, and sweat glands along with discussing the potential utilization of such differentiation in regenerative medicine.

Introduction

WHARTON'S JELLY (WJ) is a mucoid, porous connective tissue that surrounds umbilical cord vessels and is derived from the extraembryonic mesoderm and partly from the embryonic mesoderm.[1,2] It protects umbilical cord vessels against damage from the compression of vessels and supports the cord in maintaining blood flow during fetal gasping, normal movements, and forces of labor.[3,4] The extracellular matrix (ECM) components of WJ are also known to be associated with a large number of growth factors like the insulin growth factor, fibroblast growth factor (FGF), and transforming growth factor-β, which control cellular proliferation, differentiation, synthesis, and remodeling of the ECM.[5–8] This mucoid connective tissue hosts some mesenchymal stem cells (MSCs) immersed in a ground substance called Wharton's jelly mesenchymal stem cells (WJMSCs) or umbilical cord MSCs.[1,2]

WJMSCs isolation, optimization, characterization, and scale-up have been extensively studied and described by several researchers[9–11] and therefore only discussed briefly here. WJMSCs can be extracted from three relatively indistinct regions of WJ called the perivascular, intervascular, and subamniotic region.[12] Significant differences *in vitro* have been noted between MSCs isolated from these three different regions structurally, by immunohistochemistry, and functional analysis.[13,14] WJMSCs are like fibroblasts or myofibroblasts[9,10,15] in appearance, are known to display MSC surface markers, and adhere to the plastic along with the capacity to self-renew and differentiate into various lineages like bone, cartilage, and adipose.[9,10] A single donor can provide $4–5 \times 10^9$ cells in five to six passages.[9] Because they have a high *ex vivo* proliferation index and low population doubling times, they can undergo a 300-fold expansion within six to seven passages without any abnormal karyotypes.[9,12,13,16,17] These cells are also known to be expanded in a complete xeno-free and serum-free media along with exhibiting superior growth kinetics and functional angiogenesis.[18] WJMSCs are known to have higher colony forming unit-fibroblast (CFU-F)[19] and produce some cytokines like the granulocyte-macrophage colony stimulation factor and granulocyte colony stimulating factor.[20] WJMSCs can be cryopreserved in 90% fetal bovine serum (FBS), 10% dimethyl sulfoxide for future applications, with 80% cell viability after a freeze thaw cycle as demonstrated by Puranik *et al.*[10]

The WJMSCs do not form teratomas and express a high percentage of tumor suppressor genes and secrete hematopoietic cytokines as compared to the other class of MSC.[21] WJMSCs are also known to provide a stromal supportive niche for several primitive stem cell populations like hematopoietic stem cells and spermatogonial stem cells among others.[22,23] Saito *et al.* also demonstrated that WJMSCs can support the growth of embryonic stem cells.[24]

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As described previously, MSCs obtained from WJ are multipotent and can successfully differentiate into diverse mature tissues favoring their use for regenerative medicine applications. WJMSCs share the same osteogenic differentiation pathway like the MSCs derived from bone marrow, but exhibit less mineralization in comparison to fetal bone marrow MSCs.[25] In comparison, WJMSCs also show higher collagen production and better compatibility with decreased expression of collagen II. They also express prochondrogenic markers like *Sox9* and *Runx2* when cocultured in the presence of microsphere-based scaffolds and polyglycolic acid scaffolds.[26] In addition, WJMSCs exhibit the potential to differentiate into cells of adipogenic lineage as shown by the robust oil droplet formation when stained with oil O red staining following exposure to proper inductive stimuli incubation times.[13] Due to their unique developmental position, these cells have an active growth potential, exhibit specific phenotype, possess fetal karyotype, express embryonic stem cell markers like *Sox 2*, *Nanog*, *Oct 3/4A*, MSC markers like CD73, CD90, CD105, and are also known to be hypoimmunogenic as they express molecules that can modulate natural killer cells and expand regulatory T-cell populations.[27,28]

This review focuses on the regenerative medicine applications that are related to the differentiation ability of WJMSCs into cells of ectodermal lineage.[12,29] The ectoderm is one of the three primary germ layers that can differentiate into structures of various shapes and sizes like the nervous system (peripheral nerves, brain, and spine), which develop from the neuroectoderm, and surface ectoderm that develops into the epidermis (skin and skin appendages), the lining of the mouth, anus, and nostrils, and exocrine glands (mammary, salivary, sweat, and lacrimal glands) (Fig. 1). The organogenesis of these organs and parts is initiated during the embryonic periods, while the morphogenesis continues postnatally. These organs also have limited ability for regeneration like the cyclical growth of hair and feathers, continuous growth of nails and the rodent incisor, and growth of the mammary gland during puberty and pregnancy.[30] Despite the diversity in form and function, all these organs originate from adjacent layers of the epithelial (ectodermal) and mesenchymal (mesodermal or neural crest-derived tissues). The development of these organs begins with the local thickening of the epithelial layers to form an ectodermal placode and is followed by a condensation of mesenchymal cells under the placode, which then buds into or out of the mesenchyme. Continued folding and branching of the epithelium follow growth of these epithelial and mesenchymal components, which result in the final shape and size of the organ. There has been a growing interest in studying the potential applications of WJMSC differentiation into cells of ectodermal origin. This review presents a comprehensive summary of these regenerative abilities of WJMSCs.

Surface Ectoderm-Related Regenerative Medicine Applications

Wound repair and healing

Oral mucosa and skin are made of epithelium and the underlying connective tissue and play a role as the primary defensive barriers and to maintain the physiological homeostasis.[31] Numerous diseases associated with trauma, cancer, burns, and infections among others affect the normal architecture of skin and oral mucosa. Keratinocytes form the major population of the epithelial layer, are tightly attached to each other by cell–cell junctions, and are arranged into a number of distinctive layers. The problems associated with autologous biopsies and long doubling times necessitate the need for an effective alternative to these areas affected by diseases.[32]

Skin cells are continuously renewed by the cells from the underlying epidermis, hair bulb, the melanocyte layer, and dermis.[33] Wound healing is characterized by complex biological and molecular events, which include inflammation, proliferation, and remodeling.[34,35] The major problems

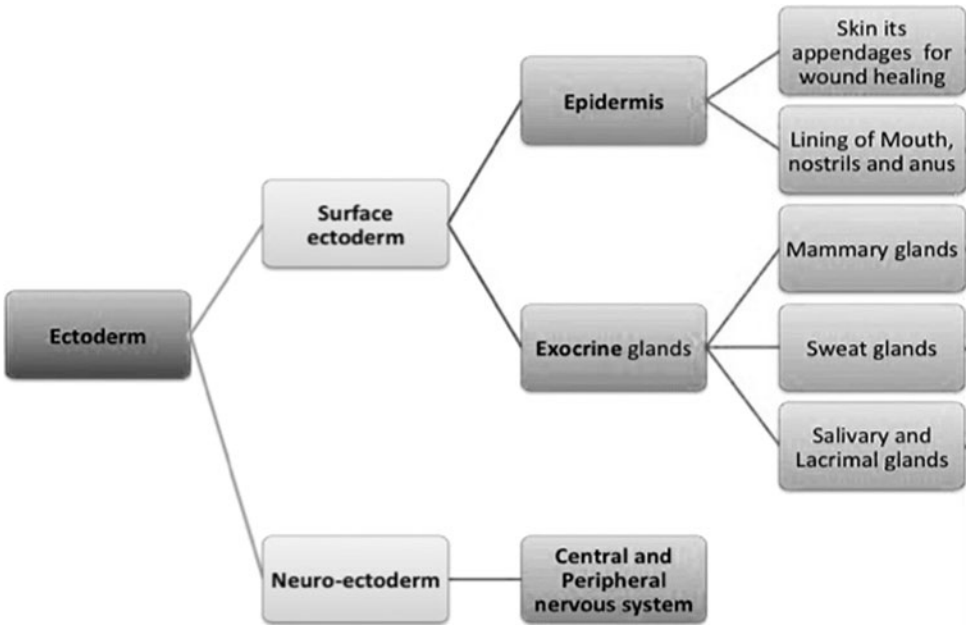

FIG. 1. Differentiation potential of ectoderm.

associated with prognosis of wound healing are scar formation, loss of normal function, and formation of skin appendages, and the hurdles with healing and repair of wounds are ischemia to the affected areas, patient mobility, advanced age, and related diseases.[36]

Currently, stem cell-based therapies are attractive candidates for treating skin injuries in regenerative medicine. For example, Arno *et al.* showed that wound healing of an excisional full-thickness skin murine model was enhanced by WJMSC by promoting normal skin fibroblast proliferation and migration.[37] In another example, Tam *et al.* used a nanoscaffold of polycaprolactone with aloe vera as an antibacterial agent, impregnated with green, fluorescent protein-labeled WJMSCs in its conditioned medium, and studied healing of excisional and diabetic wounds in rats. Using scratch wound assays, they found that skin fibroblasts migrated faster from scratches into vacant areas accompanied by an increased expression of collagen I and III, elastin, fibronectin, superoxide dismutase, and metalloproteinase-1 compared to their controls.[38] These scaffolds with WJMSCs also showed rapid wound closure, re-epithelization, increased number of sebaceous glands and hair follicles along with positive keratinocyte markers like cytokeratin, involucrin, filaggrin, and elevated expression of intercellular adhesion molecule-1 (ICAM-1), tissue inhibitor of metalloproteinase-1, and vascular endothelial growth factor-A when applied to excisional wounds in rats for 28 days.[38]

Zhang *et al.* studied the potential of a mixture of human umbilical cord MSCs, WJ pieces, and skin micro particles (composed of remnant tissue surrounding wounds made of cutaneous cells, transudate, inflammatory cytokines, inflammatory cell infiltration, fragments of hair follicles, sebaceous glands, sweat glands, and subcutaneous tissues) after transplantation into 10-mm, full-thickness, mid-dorsal, excisional skin wounds in mice. The transplanted MSCs and the other components demonstrated the development of new born skin and its appendages along with newly generated layers of the epidermis, sebaceous glands, hair follicles, and sweat glands after 7 days.[15] It is, therefore, possible that WJMSCs in combination with a three-dimensional (3D) scaffold mimicking the natural dermis and several paracrine and immunomodulatory factors could all act synergistically as wound dressings for slow healing and hard-to-heal chronic wounds.[15,38]

Similarly, WJMSCs have been investigated for oral mucosal healing applications. For example, Garzon *et al.* developed a 3D bioactive system composed of a stromal substitute from human fibrin and 0.1% agarose. Cultured oral mucosa and skin fibroblasts were added to this mixture before inducing polymerization. WJMSCs were seeded on the top of this 3D system and cultured for 7 days, followed by the air–liquid culture technique for a week to induce the final differentiation into multilayered oral mucosa and skin epithelium. When introduced into athymic nude mice, this bioactive 3D system mimicked the native epithelial mesenchymal transition and expressed typical markers of epithelial differentiation like cytokeratins 1, 4, 8, 13, plakoglobin, filaggrin, and involucrin and showed specific surface patterns. Epithelial-like cell layers and well-formed cell–cell junctions were also observed. These experiments show that WJMSCs can be a novel cell source for human oral mucosa and skin by forming epithelial keratinocytes.[32]

Hair regeneration

Dermal papilla-like tissue was developed from umbilical cord MSCs following exposure to a dermal papilla forming medium by Yoo et al.[39] These papilla when transplanted into an athymic mice, produce new hair follicles. In our experience, when WJMSCs were cultured on a decellularized Wharton's jelly matrix in an osteogenic differentiation medium, it was observed that hair-like structures (≤ 100 nm) were reproducibly protruding through the outer layer of the matrix material. However, even when WJMSCs were cultured on a nontissue culture-treated plate with osteogenic differentiation media, spheroids were formed, which also had similar hair-like structures and stained positive for alizarin red, indicating calcification. These differentiated WJMSCs exhibited increased expression of CK19 (a marker for hair follicle stem cells) with time in culture and also stained positive for CK15 (marker for bulge cells in the human hair follicle).[40] Alizarin red staining in spheroids revealed that the hair-like structures were localized in close proximity to areas of calcification and mineralization.[40]

Sweat glands

Xu *et al.* studied the potential of the human umbilical cord MSC to differentiate into sweat glands to help in their restoration after injury. When these MSCs were cultured in a medium of Dulbecco's modified Eagle's medium supplemented by 10% FBS, penicillin, streptomycin, glutamine, insulin/transferrin/sodium selenite solution, triiodothyreonine, hemisuccinate hydrocortisone, and human recombinant epidermal growth factor for 3 weeks differentiated into sweat gland-like cells. These cells maintained sweat gland-like morphology and expressed markers of sweat gland cells like carcinoembryonic antigen (CEA), CK14, CK19 along with expression of sweat gland developmental genes like ectodermal dysplasia and ectodermal dysplasia receptor. These results show that WJMSC can be used for sweat gland restoration after skin injury and to improve cutaneous regeneration.[41] Table 1 summarizes preclinical studies investigating the surface ectodermal differentiation potential of WJMSC.

Cornea

Corneal lattice dystrophy, congenital corneal stromal dystrophy, and pseuduophakic bullous keratopathy are examples of some diseases of the eye that require a corneal transplantation or keratoplasty.[42] The suboptimal medical conditions, deficiency of qualified people, and lack of donated cornea represent limitations to keratoplasty. Liu *et al.* investigated the use of WJMSC to treat thin cloudy corneas in lumican null mice and showed that the collagen lamellae were reorganized in the corneal stroma after transplantation with these cells. The keratocyte function was improved as shown by an enhanced expression of keratocan and aldehyde dehydrogenase class 3A1.[43] Coulson-Thomas *et al.* showed that when WJMSCs were intrastromally transplanted into corneas of mucopolysaccharide VII mice, the dendritic and hexagonal morphology of host keratocytes and endothelial cells, respectively, was restored. Corneal haze was reduced along with a decrease in glycosaminoglycan content, lysosomal number, and size of treated corneas.[44] These findings show that WJMSCs have the potential to

Table 1. WJMSCs as Candidates for Epithelial Differentiation

Epithelial organ	*Cell source and culture conditions*	*Potential application*	*Authors*
Skin repair and wound healing	WJMSC with skin microparticles from skin injury site	For wound healing	Zhang *et al.*[4,15,27,61]
	WJMSC on Aloe vera, PCL scaffolds	For wound healing	Tam *et al.*[39]
Hair	WJMSC with and without decelled Wharton's jelly matrix	Ectodermal differentiation for hair follicle	Aljitawi *et al.*[41]
Sweat glands	WJMSC	Sweat-gland restoration after skin injury for cutaneous regeneration	Xu *et al.*[42]
Cornea	WJMSC injected into cornea of mucopolysaccharide VII mice	Keratinocyte and endothelial cell morphology was restored	Coulson-Thomas *et al.*[45]
	WJMSC on fibrin agarose scaffolds	Corneal epithelial cells were formed	Garzon *et al.*[33]

PCL, polycaprolactone; WJMSC, Wharton's jelly mesenchymal stem cell.

treat corneal defects related to mucopolysaccharides. Garzon *et al.* used fibrin agarose scaffolds with a keratinocytic induction medium in a 3D system that allowed stromal and epithelial interactions and enabled *in vitro* differentiation of WJMSCs and corneal epithelial cells. They demonstrated that WJMSCs were able to differentiate into corneal epithelial-like cells with the expression of epithelial markers like cornea cytokeratin (CK3/12), plakoglobin (PKG), tight junctions zonula occludens-1 (Z01), and connexin 43 (CX43), along with other proteoglycans, collagen, elastic, and reticular fibers.[45] These findings suggest that WJMSC might play a role in cornea regeneration.

Neuroectoderm-Related Regenerative Medicine Applications

Cell-based therapies can be good strategies to create a favorable environment for nerve regeneration in both the central and peripheral nervous system and for treatment of nerve injury and nerve gap injuries. It has been demonstrated that MSCs from WJ can be differentiated into neuron-like cells, neuroglial cells, glial cells, axons, and Schwann cells (Table 1).[46] Fu *et al.* showed that WJMSCs could differentiate into neuron-like cells that express neurofilament and functional mRNAs responsible for the synthesis of subunits of the kainite receptor and glutamate decarboxylase, which generate an inward current in response to evocation by glutamate.[46,47]

Schwann cells are important components of the peripheral glia that forms myelin. These cells provide a favorable microenvironment for the repair of damaged nerve fibers, support axonal regeneration, construct myelin, and contribute to functional recovery in a spinal cord injury.[48] Due to the limitation associated with the isolation and expansion of Schwann cells from peripheral nerves, WJMSCs are an effective alternative with the potential to differentiate into Schwan cells capable of constructing myelin and regeneration of supporting nerves.[49,50] These WJ-derived Schwann cells maintained their differentiated phenotype and contributed to axonal regeneration and functional recovery even after transplantation into a rat-transected sciatic nerve.[49,50] Peng *et al.* treated undifferentiated WJMSCs with a mixture of glial growth factors like the basic FGF, platelet-derived growth factor, and forskolin and observed that these cells adopted a spindle-like morphology and were similar to Schwann cells by shape, phenotype, and function. Real time-polymerase chain reaction (RT-PCR), western blot, and immunocytochemistry revealed that these Schwann cells expressed the glial markers like the glial fibrillary acidic protein (GFAP), P75, S100, and P0. Upon coculture with dorsal root ganliga neurons, the differentiated WJMSCs improved the neurite length and the number of sprouting neurites in dorsal root ganglia neurons. These differentiated cells were found to secrete and express brain-derived neutrophic factors, nerve growth factor, and neutrofin-3.[41,49,51]

Koh *et al.* showed that human WJMSCs upon exposure to a neuronal progenitor differentiation medium comprised a neural progenitor basal medium, neural survival factor, supplemented with a brain-derived neutrophic factor, differentiate into cells that express the same morphological features of neurons and express neuronal cell markers like TU-20 (neuron specific beta III tubulin), Trk A (tyrosine kinase A), NeuN (neuronal nuclear protein), and NF-M (neurofilament M). Three weeks after implantation into the damaged hemisphere of immunosuppressed ischemic stroke rats, WJMSCs were present in the damaged hemisphere, expressed neuron-specific markers, and improved neurobehavioral functions and reduced infarct volume relative to control rats.[52] Cho *et al.* further showed that application of subsonic vibration to WJMSCs enabled them to differentiate into neural cells through extracellular regulated protein kinase (ERK) activation and expressing neuron-specific markers like microtubule-associated protein 2 (MAP2), NF-L, NeuroD1 along with increased expression of GFAP and O4.[53]

There are several neurological conditions that can potentially be treated with WJMSCs undergoing neuroectodermal differentiation. These conditions include:

Parkinson's disease

Parkinson's disease is a neurodegenerative disorder in the elderly characterized by tremors, rigidity, bradykinesia, and postural instability due to the degeneration of dopamine neurons.[54–57] Current pharmacological therapies for this disease mostly relieve symptoms, but do not restore the lesion's side loss of function.[58] Yan *et al.* transferred *Lmx1α*, neurturin genes into WJMSCs by a recombinant adenovirus and implanted the induced cells into the striatum and substantia nigra of MPTP

Table 2. WJMSCs as Candidates for Neuronal Differentiation

Author	*Cell source*	*Differentiated into*	*Conditions*	*Potential application*
Peng *et al.*[49]	WJMSCs	Schwann cell like	DMEM with glial growth factors (bFGF, PDGF, forskolin)	Suitable as Schwann cell substitutes for nerve repair
Zhang *et al.*[4,15,27,61]	WJMSCs	ChAT-positive cells	DMEM with neuronal induction media (BDNF in low serum media supplemented with HCNP and rDHE)	Potential for cell transplantation to treat Alzheimer's disease
Koh *et al.*[52]	WJMSCs	Neuronal cells	High glucose DMEM with neural progenitor differentiation media (NPBM, NSF-1, and supplemented with BDNF)	Neuroprotective therapy for ischemic strokes in rats
Cho *et al.*[53]	WJMSCs	Neural cells	Nonhematopoietic stem cell media followed by subsonic vibrations	Neural cells
Yan *et al.*[57]	WJMSCs transfected with Lmx1α, neurturin	Dopamine neurons	α-MEM with 15% FBS	Therapeutic application for Parkinson's disease in a rhesus monkey model
Yang *et al.*[61]	WJMSCs	Neuronal progenitor cells and immature neurons depending on time of exposure to medium	10% FBS-DMEM supplemented with NCM (media grown with brain cells from rats)	Promotes regeneration, wound healing, locomotor recovery, and provides neuroprotection around lesions in rats
Gartner *et al.*[62]	WJMSCs	Neuroglial-like cells	MSC neurogenic medium along with PCL membranes	To promote nerve regeneration in axonotmesis
Messerli *et al.*[63]	WJMSCs from preterm birth	Neuroglial-like cells	Neurobasal medium with 1×B27 supplement, basic fetal growth factor, and PDGF (for OPC lineage)	Potential as cellular graft in neuroregenerative therapy for peripartum brain therapy in preterm birth
Mitchell *et al.*[46]	WJMSCs	Neurons and glia	DMEM with bFGF, DMSO, BHA, KCl, valproic acid, forskolin, hydrocortisone, and insulin	Neurotherapy for neural diseases

bFGF, basic fibroblast growth factor; BDNF, brain-derived neutrophic factor; ChAT, choline acetyltransferase; HCNP, hippocampal cholinergic neurostimulating peptide; rDHE, rat denervated hippocampal extract; NPBM, neural progenitor basal medium; NSF-1, neural survival factor-1; NCM, neuronal conditioned medium; PCL, poly(DL-lactide-ε-caprolactone); OPC, oligodendrocyte progenitor cells; PDGF, platelet-derived growth factor; DMSO, dimethylsulfoxide; BHA, butylated hydroxyanisole; KCl, potassium chloride; DMEM, Dulbecco's modified Eagle's medium; MEM, modified Eagle's medium; FBS, fetal bovine serum; MSC, mesenchymal stem cell.

(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinehydrochloride)-lesioned hemi-parkinsonian rhesus monkeys. Behavioral tests performed on the transplanted monkeys for 6 months showed disease amelioration. Pathological and immunohistochemistry data showed that neuronal-like cells survived in the right brain of the diseased monkeys. These cells were thought to play a role as dopaminergic neurons.[57]

Alzheimer's disease

Alzheimer's disease is the most common neurodegenerative disease characterized by amyloid β peptide deposits in the brain leading to the loss of cholinergic neurons in the cortex and hippocampus. This loss reduces choline acetyltransferase, which leads to a lack of the neurotransmitter acetylcholine that is correlated with cognitive decline.[59] Ongoing efforts to reverse this process involved the use of WJMSCs. For example, after treating WJMSCs with the neuronal induction medium for 14 days, these cells were found to express a neuronal-specific marker, MAP2, and extended neurite-like processes. Also, upon treating WJMSC with neuronal induction media supplemented by hippocampal cholinergic neurostimulating peptide or rat enervated hippocampal extract, WJMSCs expressed choline acetyltransferase. These results were further confirmed by acetylcholine secretion measured by an enzyme-linked immunosorbent assay. These studies show the potential of WJMSC differentiation into choline acetyltransferase positive cells, therefore, showing a new candidate for the treatment of Alzheimer's disease.[60]

Spinal cord and peripheral nerve injuries

Therapeutic strategies involving exogenous cell replacement might be a good alternative for the treatment of spinal cord injuries as these injuries are associated with degeneration of axons, loss of neurons and glia, and demyelination around the lesion site.[61] To explore these potentials, Yang *et al.* examined the effects of WJMSC cultured for 3 and 6 days in the neuronal conditioned medium after their transplantation into complete spinal cord transection in rats. Three weeks after transplantation, they noticed significant improvements in locomotion, accompanied by increased numbers of regenerated axons in the corticospinal tract and the presence of neurofilament-positive fibers around the lesion site. It was also noticed that the transplanted WJMSCs survived for 16 weeks and produced large amounts of neutrophil activating protein-2, glucocorticoid-induced tumor necrosis factor receptor, vascular endothelial factor receptor 3, neutrotrophin-3, and basic fetal growth factor, thus, proving they could be candidates for the spinal cord repair.[61] Gartner *et al.* studied the therapeutic values of neuroglial differentiated WJMSC cultured with or without poly(DL-lactide-ε-caprolactone) membranes, on the rat sciatic nerve after axonotmesis injury. Twelve weeks after implantation, there was an enhanced recovery of motor and sensory function in animals accompanied by an increase in the myelin sheath showing their effectiveness for treating peripheral nerve lesions.[62] Table 2 summarizes preclinical studies investigating the neuroectodermal differentiation potential of WJMSC.

All the applications mentioned above in this review are for use in an allogeneic fashion, as WJMSCs are known to lack an immune response as they are CD45, CD34, and HLA class II antigen-negative.[12] Accordingly, WJMSCs represent an ideal stem cell source for allogeneic regenerative medicine applications. However, with our ability to cryopreserve and bank stem cells, these cells could potentially be used in an autologous fashion.

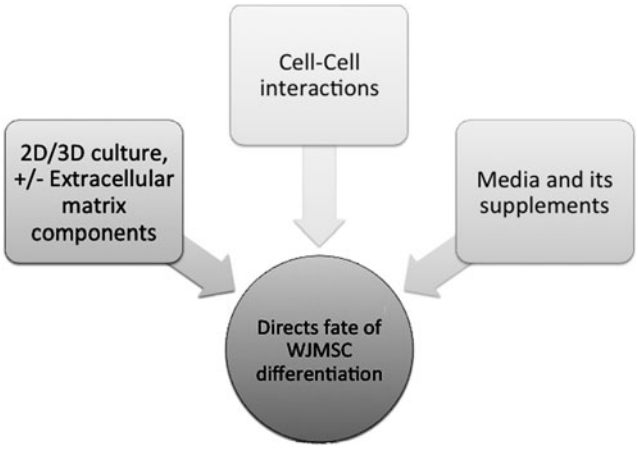

FIG. 2. Factors that govern the destiny of Wharton's jelly mesenchymal stem cell (WJMSC) differentiation.

Conclusion

WJMSC is an ideal and convenient source of cells that can potentially differentiate into ectodermal lineage. There has been a growing evidence to support the concept that upon exposure to the right conditioned medium, WJMSCs can form neurons, axons, Schwann cells, sweat glands, keratinocytes, hair follicles, and can help in wound repair and growth of skin, regeneration of the myelin sheath, and could potentially help in developing treatment options for diseases like Alzheimer's and Parkinson's. Factors related to culture conditions influence such differentiation potential (Fig. 2). There are many avenues that are yet to be explored, suggesting that there is a great potential for discovery and development in this field of tissue engineering and regenerative medicine using WJMSC.

Disclosure Statement

No competing financial interests exist.

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