Recent Progress on Skin-Derived Mesenchymal Stem Cells in Pigs

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ABSTRACT

Skin serves as an easily accessible source of multipotent stem cells with potential for cellular therapies. In pigs, stem cells from skin tissues of fetal and adult origins have been demonstrated as either floating spheres (cell aggregates) or adherent spindle-shaped mesenchymal stem cell (MSC)-like cells depending on culture conditions. The cells isolated from the epidermis and dermis of porcine skin showed plastic adherent growth in the presence of serum and positively expressed a range of surface and intracellular markers that are considered to be specific for MSCs. The properties of primitive stem cells have been observed with the expression of alkaline phosphatase and markers related to pluripotency. Further, studies have shown the ability of skin-derived MSCs to differentiate *in vitro* along mesodermal, neuronal and germ-line lineages. Moreover, preclinical studies have also been performed to assess their *in vivo* potential, and the findings appear to be effective in tissue regeneration at the defected site after transplantation. The present review describes the recent progress on the biological features of porcine skin-derived MSCs as adherent cells, and summarizes their potential in advancing stem cell based therapies.

(Key words : Skin, Mesenchymal stem cells, Adherent cells, Differentiation, Pig)

INTRODUCTION

Mesenchymal stem cells (MSCs) are adult stem cells with a potential for self-renewal and differentiation toward multiple mesodermal as well as non-mesodermal lineages. In pigs, MSCs have been isolated and characterized from different fetal and adult tissues (Ringe *et al.,* 2002; Bosch *et al.,* 2006; Carlin *et al.,* 2006; Faast *et al.,* 2006; Huang *et al.,* 2007; Kumar *et al.,* 2007, 2012; Ock *et al.,* 2010; Chen *et al.,* 2011; Kang *et al.,* 2011). Porcine MSCs exhibited a spindle-like morphology and were able to differentiate *in vitro* into osteocytes, adipocytes and chondrocytes under specific culture conditions (Ringe *et al.,* 2002; Bosch *et al.,* 2006; Kumar *et al.,* 2012). Recent studies have also demonstrated the plasticity of porcine MSCs to form neuron-like cells, smooth muscle cells, cardiomyocytes and germ cell-like cells (Moscoso *et al.,* 2005; Huang *et al.,* 2007; Shukla *et al.,* 2008; Song *et al.,* 2011; Kumar *et al.,* 2012). In addition to *in vitro* characterization and lineage differentiation, several attempts have been made to evaluate the therapeutic potential of MSCs in a porcine model (reviewed by Rho *et al.,* 2009). Although bone marrow has been found to be the most commonly employed source, recent studies have also investigated more easily accessible tissues, such as adipose or skin as alternative tissue sources to isolate MSCs in pigs (Dyce *et al.,* 2006; Huang *et al.,* 2007; Kang *et al.,* 2010; Song *et al.,* 2011; Park *et al.,* 2012).

Skin has been considered as an ideal source of multipotent stem cells comprising a population of most primitive or progenitor cells with a potential for regenerative medicine (reviewed by Vishnubalaji *et al.,* 2012). However, studies have demonstrated significant differences in cell morphology, phenotypic properties and differentiation potential depending on isolation methods and culture conditions (Zhao and Prather, 2011; Vishnubalaji *et al.,* 2012). Quite similar to the characteristics of rodent and human skin-derived precursors or progenitors (SKPs) (Toma *et al.,* 2001, 2005), stem cells have been isolated from porcine skin as floating spheres (cell aggregates) (Dyce *et al.,* 2004, 2006, 2011; Zhao *et al.,* 2009). These porcine skin-derived stem cells when allowed to culture in serum free medium in the presence of growth factors generated floating spheres or sphere cells (Dyce *et al.,* 2004; Linher *et al.,* 2009; Zhao *et al.,* 2009). Alternatively, if cultured in the presence of serum with or without supplementation of growth factors, the cells became plastic adherent and exhibited fibroblast-like morphology by sharing the features of MSC-like cells (Kang *et al.,* 2010; Lermen *et al.,*

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2010; Ock *et al.*, 2010; Song *et al.*, 2011; Park *et al.*, 2012). Stem cells isolated from porcine skin tissues of both fetal and adult origins, whether expanded to either floating spheres or adherent cells, have revealed pluripotent or multipotent characteristics with an ability to differentiate along mesodermal, neural and germ-line lineages. In addition, preclinical studies have also been performed to assess the *in vivo* potential of skin-derived MSCs using a porcine model.

The main purpose of this present review is to summarize the recent progress regarding the biological features of porcine skin-derived MSCs as adherent cells, and to discuss their potential in advancing stem cell therapy applications.

ISOLATION, CULTURE AND EXPANSION POTENTIAL

The porcine skin-derived adherent MSCs have been isolated based on the characteristic property of adherence to plastic culture dish (Kang *et al.*, 2010). Attached fibroblast-like stromal cells appeared as single, stretched cells and in most cases, leading to the formation of cell clusters or colonies (Dominici *et al.*, 2006). In order to isolate non-floating MSCs, skin tissues were sliced into 1~3 mm^2 pieces which contained epidermis and dermis. Without subjecting for any enzyme digestion, sliced porcine ear tissue explants were directly cultured in Dulbecco's Modified Eagle Medium (DMEM)-F12 (1:1) supplemented with 10% fetal bovine serum (FBS), 10 ng/ml each of basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF), 100 U/ml penicillin, and 100 μg/ml streptomycin at 38.5℃ in a humidified atmosphere containing 5% CO_2 in air for 2 to 3 days (Kang *et al.*, 2010; Park *et al.*, 2012). In another study by Lermen *et al.* (2010), the adult porcine skin explants (1~3 mm^2) were digested overnight at 4℃ in 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA). Following incubation in 0.25% trypsin/EDTA for 30 min at 37℃, skin pieces were mechanically dissociated by vortexing and vigorous pipetting. Then, the cell suspensions were prepared after filtration, and allowed for culture in DMEM/ F12 (1:1) containing 10% fetal calf serum (FCS), 2% B27, 40 ng/ml bFGF, 20 ng/ml EGF and antibiotics at 37℃ in a 5% CO_2 incubator. The culture of MSCs has also been performed by using advanced DMEM with no addition of growth factors, such as bFGF and EGF (Ock *et al.*, 2010; Song *et al.*, 2011). Attached skin tissues and non-adherent cells were gently removed off and the attached cells were allowed to culture for 1 to 2 weeks. Once reached confluence, cells were dissociated using 0.1% or 0.25% (w/v) trypsin-EDTA solution and centrifuged at 500 ×g for 5 min. Afterwards, the cell pellet was gently re-suspended in culture medium and re-grown for further expansion. During the primary culture, cells exhibited a mixture of round (epithelial-like), spindle or elongated (fibroblast-like) shape. After 3~4 passages of culture, only the homogeneous fibroblast-like cells maintained proliferation and employed for further analysis. Interestingly, the morphological characteristics of porcine skin-derived MSCs were quite uniform when cultured in the presence of serum with or without growth factors (Kang *et al.*, 2010; Lermen *et al.*, 2010; Ock *et al.*, 2010; Song *et al.*, 2011; Park *et al.*, 2012). Despite the differences in culture methods, both the cells appeared to possess similar morphology and expansion potential.

MSCs have been known to display subtle changes during prolonged culture and may become senescent after a few population doublings (Rho *et al.*, 2009). The culture environment affected the growth and differentiation of MSCs with varying serum levels and presence or absence of growth factors (Bosch *et al.*, 2006). Analysis of doubling time or colony-forming unit fibroblasts assay generally indicated the proliferation ability of MSCs. The analyzed skin-derived MSCs showed a shorter doubling time than that of MSCs from bone marrow (Ock *et al.*, 2010). Further, a tendency for the delay in doubling time was observed in both MSCs at later passage than in early passage. Porcine skin-derived stem cell-like cells (SSCs), with features similar to MSCs, were expanded under slightly different conditions subpassaged at least 18 times during 126 days of culture and showed a population doubling time of ~47 hr without undergoing any significant morphological or proliferative changes (Lermen *et al.*, 2010). In addition, isolated SSCs were able to form colonies of larger than 1 mm size after 11 days of culture. This data supported the notion that *in vitro* culture system influenced the expansion potential of MSCs.

The proliferation ability of MSCs was also assessed by evaluating the cell cycle status. Cell cycle analysis of skin-derived MSCs demonstrated the majority of cells (58~68%) at G0/G1 phase (Ock *et al.*, 2010; Song *et al.*, 2011), and illustrated the fact that most circulating cells reside in resting phase (Kumar *et al.*, 2007). The results also indicated that skin-derived MSCs had more number of cells at S-phase with greater proliferation ability compared to MSCs from adipose tissue (Song *et al.*, 2011). In contrast, the number of skin-derived MSCs at S-phase was observed to be lower to that of MSCs from bone marrow (Ock *et al.*, 2010). Despite the differences between cells from various tissue origins, the findings showed that skin tissue supports the existence of MSCs representing at both quiescent and proliferating phases.

PHENOTYPIC CHARACTERIZATION, ALKALINE PHOSPHATASE (AP) ACTIVITY AND EXPRESSION OF PLURIPOTENT MARKERS

General consensus drafted by International Society for Cellular Therapy (ISCT) for human MSCs is also widely followed for porcine MSCs derived from different tissue origins (Dominici *et al.*, 2006; Rho *et al.*, 2009). Accordingly, the phenotypic characterization of MSCs relies primarily on plastic adherence under standard culture conditions; secondly, MSCs are positive for typical cell surface molecular markers, such as CD29, CD105, CD73, CD44 CD90 and CD116 and negative for CD11b, CD14, CD31, CD34 and CD45 (which are commonly expressed by haematopoietic and endothelial cells). Thirdly, these MSCs are able to differentiate into osteogenic, adipogenic and chondrogenic cell types *in vitro*. Skin-derived MSCs have been characterized for specific surface antigen expression by flow cytometry analysis. They were positive for specific surface antigen markers of MSCs including CD29, CD44 and CD90, but largely negative for CD45 (hematopoietic cell marker), and immunophenotype markers like major histocompatibility complex II (MHCII) and swine leukocyte antigen (SLA) (Kang *et al.*, 2010; Ock *et al.*, 2010; Song *et al.*, 2011: Park *et al.*, 2012). Further, these cells strongly expressed the mesenchymal cell marker, vimentin, but not cytokeratin, an epithelial cell marker (Song *et al.*, 2011). As reported previously, vimentin is an intermediate filament protein of all cells of mesenchymal origin and in combination with negative staining for cytokeratin, isolated MSCs from skin tissue were shown to be from mesenchymal origin (Lorenz *et al.*, 2008). Like MSCs, porcine SSCs also expressed CD29, CD44 and CD105, but CD90 was expressed at a very low level indicating the features of adult stem cell populations (Lermen *et al.*, 2010). Collectively, in porcine, the expression profile of majority of specific markers in skin-derived MSCs was almost identical to that of MSCs from other sources (Rho *et al.*, 2009).

In porcine, the positive expression of alkaline phosphatase (AP) activity in multipotent stem cells has been demonstrated to illustrate the existence of primitive stem cell or precursor cell population in optimal culture conditions (Kues *et al.*, 2005: Carlin *et al.*, 2006; Chen *et al.*, 2011). The colonies formed by porcine umbilical cord (PUC) matrix cells (Carlin *et al.*, 2006) and amniotic fluid derived multipotent stem cells (AF-MSCs) (Chen *et al.*, 2011) exhibited AP activity in culture, which is consistent with primitive stem cell identity. Similar to these observations, skin-derived MSCs were AP positive and its intensity of expression was strongly associated with the colony-forming ability of cells (Ock *et al.*, 2010; Song *et al.*, 2011). The reaction of endogenous AP staining was more intense in colony-formed cells than in non-colony-formed individual cells. Observation of colony growth and AP expression in skin-derived MSCs indicated the presence of a subpopulation of primitive or pluripotent stem cells in culture.

Several reports have demonstrated that Oct4, Sox2 and Nanog are the master transcription factors that regulate the self renewal and differentiation of pluripotent stem cells and hence, considered as important markers of primitive stem cells (Kashyap *et al.*, 2009; Kellner and Kikyo, 2010). In porcine, previous studies on multipotent stem cells showed the expression of pluripotency factors Oct4, Sox2 and Nanog, but reported the tissue dependent differences in the intensity of expression levels (Carlin *et al.*, 2006; Ock *et al.*, 2010; Song *et al.*, 2011). In addition, the expression of Oct4, Sox2 and Nanog has been demonstrated in skin-derived MSCs at both the protein and mRNA levels (Kang *et al.*, 2010; Ock *et al.*, 2010; Song *et al.*, 2011: Park *et al.*, 2012). Apart from Oct4 and Sox2, porcine SSCs also expressed progenitor marker nestin, the side population stem cells associated gene Bcrp1/ABCG2, the polycomb group repressor Bmi1 and the transcription factor Stat3 (Lermen *et al.*, 2010). However, conflicting results have been reported as regards to the functional role of all the three transcription factors Oct4, Sox2 and Nanog in adult stem cells like MSCs (Wang and Dai, 2010). Nonetheless, the expressions of AP activity and pluripotency markers have been consistent with the notion that skin-derived MSCs contain a subpopulation of cells with primitive status than other sources. Furthermore, it was supported by a presumption that the presence of a rare population of small quiescent pluripotent stem cells in adult tissues might be involved in tissue maintenance or regeneration (Kang *et al.*, 2011). Thus, it is assumed that such cells could restore the population of progenitors to facilitate in tissue repair.

IN VITRO MULTILINEAGE DIFFERENTIATION POTENTIAL

Following successful isolation and expansion of skin-derived MSCs, further analyses have also been performed to determine their ability for multilineage differentiation. Generally, MSCs are defined based on their trilineage multipotency of forming osteogenic, adipogenic and chondrogenic cells. However, some reports have indicated that the cell source largely influences their multipotent characteristics (Rho *et al.*, 2009). Under *in vitro* culture conditions, specific external stimuli induced these multipotent stem cells to differentiate along specific cell lineage that is not only restricted to mesodermal tissues but also into cells of non-mesenchymal lineages.

External stimuli could be in the form of cytokines, growth factors, extracellular matrix proteins and other soluble factors which are responsible for initiating and maintaining the desired differentiation process with appropriate culture conditions (Vishnubalaji *et al.*, 2012). Thus, the multipotency of porcine skin-derived MSCs provide an ideal *in vitro* culture model to further understand the cellular and molecular pathways essential for designing the effective strategies for cell based therapies.

Osteogenic Differentiation

The differentiation of skin-derived MSCs into osteocytes that produce mineralized matrices has been assessed using osteocyte-specific induction medium (Ock *et al.*, 2010; Song *et al.*, 2011: Park *et al.*, 2012). The common procedure for osteogenesis involved the culture of 70~80% confluent cells in induction medium supplemented with 10% FBS, 0.1 μM dexamethasone, 50 μM ascorbate-2-phosphate and 10 mM of glycerol-2-phosphate for 3 to 4 weeks. Aggregates of round calcium producing cells were formed on the monolayer culture and mineralization was demonstrated at the end of treatment by staining with von Kossa and Alizarin red S dyes. In addition, the expression of selected gene transcripts, such as osteonectin (ON), osteocalcin (OC) and runt-related transcription factor-2 (RUNX2) confirmed the osteocyte commitment of induced cells as analyzed by reverse transcription-polymerase chain reaction (RT-PCR) (Song *et al.*, 2011: Park *et al.*, 2012). It was also reported that bone marrow derived MSCs had stronger expression of Alizarin red S staining indicating the calcium deposition than skin-derived MSCs (Ock *et al.*, 2010). However, in another study, the intensity of osteogenesis in skin-derived MSCs was greater than stem cells derived from adipose and ovarian stromal tissues (Song *et al.*, 2011). These reports further confirmed the differences in the osteogenic potential of MSCs based on the tissue origins. Besides this basic characterization of induced osteocytes, no attempt has been made to explore the temporal transcriptional control mechanisms associated with the differentiation pathway of osteogenesis in skin-derived MSCs.

Adipogenic Differentiation

Skin-derived MSCs have been shown to form adipocytes following treatment with 10% FBS or FCS, 0.5 mM of 3-isobutylmethylxanthine (IBMX), 1 μM dexamethasone, 10 μM insulin, 100 or 200 μM indomethacin for 3 to 4 weeks (Lermen *et al.*, 2010; Ock *et al.*, 2010; Song *et al.*, 2011: Park *et al.*, 2012). The signaling molecules in culture media promoted the conversion of fibroblast-like cells into adipocytes with no effect on cell proliferation, and actively involved in adipogenic commitment (Gregory *et al.*, 2005). The formation of adipocytes consisting of neutral lipid droplets was demonstrated by Oil red O staining. Furthermore, the expression of adipocyte markers, including adipocyte fatty acid-binding protein (aP2) and peroxisome proliferator activated receptor gamma 2 (PPARγ2) was detected by RT-PCR analysis (Song *et al.*, 2011: Park *et al.*, 2012). In another report, porcine SSCs differentiated along the adipogenic lineage as demonstrated by the formation of lipid droplets in the cytoplasm and the expression of leptin, a proteohormone released mainly by adipocytes (Lermen *et al.*, 2010). Differentiation into adipose precursor cells or pre-adipocytes could occur after 10~12 days of induction, but the treatment is maintained up to 3 to 4 weeks till the formation of intracellular lipid vacuoles covering the entire adipocytes. Quite similarity with osteogenic differentiation, skin-derived MSCs showed lesser ability to form adipocytes than bone marrow derived MSCs (Ock *et al.*, 2010), but possessed a greater potential for adipogenesis than adipose and ovarian stroma derived multipotent stem cells (Song *et al.*, 2011). The findings showed that skin-derived MSCs reciprocate efficiently to closely related processes of osteo- and adipogenic differentiation, in spite of differences observed while comparing to the cells from different tissue origins.

Chondrogenic Differentiation

Chondrogenic differentiation of porcine MSCs has been performed in the presence of dexamethasone, ascorbic acid phosphate, and ITS^{+} supplement consisting of insulin, transferrin and selenium for 3 to 4 weeks (Kumar *et al.*, 2007). Supplementation of transforming growth factor-β1 (TGF-β1) and sodium pyruvate also promoted MSCs to differentiate into chondrocytic cells in a monolayer culture. Chondrogenic potential of porcine skin-derived MSCs was demonstrated by using commercially available chondrocyte differentiation medium (HyClone AdvanceSTEM^{TM}) for 4 weeks (Park *et al.*, 2012). Synthesis of proteoglycans by induced chondrocytes was confirmed by Alcian blue staining. Upregulation of specific gene transcripts associated with chondrogenesis has also been reported for porcine MSCs of different tissue origins (Rho *et al.*, 2009), but not for skin-derived MSCs. Nevertheless, the results showed that skin-derived MSCs were able to differentiate along chondrocytic cell lineage.

Neural Differentiation

The plasticity of skin-derived stem cells to undergo transdifferentiation along the ectodermal lineage of neuronal cells has been demonstrated by using the combined stimuli generated from chemicals, cytokines and growth factors (Lermen *et al.*, 2010; Park *et al.*, 2012). The process of neuronal *in vitro* differentiation from porcine SSCs was carried out up to 4 weeks using neuro-

nal induction medium of DMEM-F12 (1:1) containing 2 mM L-glutamine, 2% B27, growth factors, such as 20 ng/ml EGF, 40 ng/ml bFGF, and antibiotics (Lermen *et al.,* 2010). Over the 2 weeks of incubation, a subpopulation of cells showed morphology similar to neurons and stained positive for β-III-tubulin, a neuron specific marker, and glial fibrillary acidic protein (GFAP), a protein biomarker almost exclusively expressed in astrocytes. Quite surprisingly, besides the expression of neurofilament-medium (NF-M), Lermen *et al.* (2010) also observed the expression of alpha-smooth muscle actin (α-SMA), a marker of smooth muscle cell lineage. Flow cytometry analysis further revealed a large population of SSCs expressing nestin and a subpopulation of the cells expressing β-III-tubulin, GFAP and α-SMA prior to neuronal differentiation. However, after neural induction, nestin expressing cells were drastically decreased with simultaneous increase in the expression of β-III-tubulin and GFAP positive cells. Additionally, the number of α-SMA positive cells was also increased in neural culture medium, which concluded the fact that SSCs were able to undergo neuro-muscular differentiation (Lermen *et al.,* 2010). The co-expression of nestin and α-SMA further indicated the progressive differentiation of neuronal cells.

Porcine skin-derived MSCs were differentiated *in vitro* into neuronal cells by Park *et al.* (2012) using previously published 2-step protocol (Woodbury *et al.,* 2002). Cells were initially exposed to neuronal pre-induction medium containing DMEM with 20% FBS and 10 ng/ml bFGF for 24 hr. To initiate neural differentiation, the cells were then allowed to culture in DMEM supplemented with 2% dimethylsulfoxide (DMSO), 200 μM butylated hydroxyanisole (BHA), 25 mM KCl, 2 mM valproic acid, 10 μM forskolin, 1 μM hydrocortisone, 5 μg/ml insulin and 2 mM L-glutamine without FBS for up to 48 hr. Following induction, MSCs were changed into neuron-like cells by exhibiting the morphologies of multipolar, retracted cell bodies with a network like structure. However, no spontaneous neuronal potential was exhibited in uninduced cells. Further, the induced cells expressed the neural markers, including nestin, β-III-tubulin, neurofilament (NF), tyrosine kinase receptor A (TrkA), nerve growth factor receptor (NGFR), tyrosine hydroxylase (TH) and S-100 protein (S100). Interestingly, neuronal features of differentiated cells disappeared as induction time passed over 48 hr and returned to their pre-inducted shape with increased cell number, when the medium was changed to DMEM with FBS at 72 hr. Therefore, authors opined that the chemical induction protocol was not a suitable choice for the neural differentiation of skin-derived MSCs (Park *et al.,* 2012). Similar contrasting view has also been indicated that the chemical protocol resulted in rapid changes of cell morphology in a short induction period (Barnabe *et al.,* 2009). Observed morphological changes have been speculated merely as stress related artifact with no electrophysiological features. Despite these results, the above method is being commonly employed for differentiating MSCs into neuron-like cells with varying levels of efficiency (Kumar *et al.,* 2012). But the electrophysiological properties of neuronal cells induced from porcine skin-derived MSCs have not been evaluated till date.

Differentiation into Oocyte-Like Cells (OLCs)

Studies over the last few years have shown the ability of somatic stem cells derived from non-gonadal origin, such as bone marrow, peripheral blood and pancreatic tissue to generate oocytes or germ cells under optimal culture conditions (Johnson *et al.,* 2005; Danner *et al.,* 2007). Supporting the germ line potential of stem cells derived from adult tissue sources, recent works demonstrated the capability of porcine skin-derived stem cells (floating spheres) to form primordial germ cell- like cells and oocyte-like cells (OLCs) using a culture medium consisting of porcine follicular fluid and FBS (Dyce *et al.,* 2006, 2011; Linher *et al.,* 2009). Furthermore, we also observed that plastic adherent stem cells derived from skin, adipose and ovarian stromal tissues were capable of differentiating *in vitro* into OLCs under similar culture conditions (Song *et al.,* 2011). *In vitro* differentiation of skin-derived MSCs into OLCs was carried out by employing the protocol described earlier (Dyce *et al.,* 2006). Induction was performed in DMEM- F12 (DMEM/F12, 1:1) supplemented with 5% FBS, 5% porcine follicular fluid, 0.23 mM sodium pyruvate, 0.1 mM non-essential amino acids, 2 mM L-glutamine and 0.1 mM β-mercaptoethanol for 40 days. Inclusion of follicular fluid, which contains several endocrine-paracrine factors, was found to be a favourable factor for *in vitro* oogenesis and only the medium containing follicular fluid supported the expression of markers consistent with germ cell differentiation (Dyce *et al.,* 2006). The key events of induced differentiation were evaluated at different time points, and sequential morphological changes involving the formation of OLCs from follicular cell aggregates were clearly recorded (Song *et al.,* 2011). Additionally, the formation of OLCs was confirmed by the time dependent expression of specific marker genes related to germ cell formation and folliculogenesis, such as Oct4, growth differentiation factor 9b (GDF9b), c-Mos, Vasa, deleted in azoospermia-like gene (DAZL), zona pellucida C (ZPC), follicle stimulating hormone receptor (FSHR) genes. Thus, the collective findings from porcine skin-derived stem cells of both fetal and adult tissue origins showed their ability to generate OLCs with associated phenotypical changes and consistent expression of markers (Dyce *et al.,* 2006, 2011; Linher *et al.,* 2009; Song *et al.,* 2011). However, at this point, none of the reports have demonstrated the capacity of *in vitro* gen-

erated OLCs to undergo complete development like nuclear/cytoplasmic maturation or *in vitro* fertilization with sperm, as observed in naturally competent oocytes. Despite this it can be opined that the greater plasticity of stem cells offer an opportunity to better understand the fundamentals of germ cell development and potential of treating reproductive disorders.

Potential of Skin-Derived MSCs for Therapeutic Applications

MSCs have been considered as ideal sources for cell therapy due to their ability to expand *in vitro*, differentiation potential into various specialized cell types and modulation activity of a number of immune cells (Rho *et al.*, 2009). These properties allowed the use of MSCs in a number of pre-clinical and clinical studies for the treatment of various degenerative conditions. In this view, pre-clinical animal model like pig is highly valuable for exploring the cell regenerative medicine as it provides the tissue or organ and the type of desired condition to be investigated, the option of assessing the host response to different treatments and techniques, the choice of comparative analysis with other animal models and lastly, the effective translation of therapeutic findings into human applications (Carpenter and Hankenson, 2004).

Studies in our lab have demonstrated the therapeutic potential of skin-derived MSCs after transplantation into a pig model (Kang *et al.*, 2010; Park *et al.*, 2012). In general, MSCs in combination with natural or synthetic biomaterials offer the most efficient way to perform regeneration in bone tissue engineering applications. More recently, *in vitro* and *in vivo* osteogenic ability of skin-derived MSCs with a demineralized bone (DMB) and fibrin glue scaffold was evaluated (Kang *et al.*, 2010). Cells co-cultured with a DMB and fibrin glue scaffold resulted in the expression of osteocyte specific marker genes ON, OC and RUNX2, indicating *in vitro* osteogenic potential. Moreover, grafting of autogenous skin-derived MSCs (1×10^7 cells, PKH26 labeled) with the DMB and fibrin glue scaffold in the maxillary sinus floor stimulated the trabecular bone synthesis and enhanced the OC expression. New bone formation was also observed from the periphery to the center of the grafted material, and the number of proliferating cells increased over the time reaching maximum at 4 weeks after graft placement (Kang *et al.*, 2010). These promising results are potentially useful in the bone graft replacement techniques.

Additionally, pre-clinical application of skin-derived MSCs to induce neural tissue regeneration has been investigated in a minipig model (Park *et al.*, 2012). A concentration of 5×10^6 autologous skin-derived MSCs labeled with PKH26, supplemented with fibrin glue scaffold and collagen tubulization, was transplanted into the miniature pigs carrying peripheral nerve defects. *In vivo* peripheral nerve regeneration potential was evaluated at 2 and 4 weeks after cell transplantation. Features with histologically remarkable nerve regeneration were clearly evident at transplanted sites and supported by the substantial expression of neuronal mar-kers S-100 and p75NGFR in the regenerated nerve fibres or bundles (Park *et al.*, 2012). The intensity of expression was higher in regenerated tissues compared to non- cell grafted control specimen. It was, therefore, concluded that autogenous skin-derived MSCs transplanted with fibrin glue scaffold possess nerve regeneration abilities and thus act as an available substitute for nerve conduit material in the peripheral nerve defect areas. Collectively, the above findings demonstrated that skin serves as an excellent autogenous tissue source for obtaining the population of primitive or progenitor cells, that have the potential for regeneration of bone and neural tissues (Kang *et al.*, 2010; Park *et al.*, 2012).

Conclusions and Future Research

Skin-derived plastic-adhered MSCs derived from both fetal and adult origins represent attractive cells with self-renewal ability and multi-lineage differentiation, and also offer a great potential for the regenerative medicine. With its easy accessibility and the prospect of autologous transplantation, skin tissue has been an important source of MSC-based therapies. Moreover, a few recent studies have indicated the broader differentiation potency of skin-derived MSCs by forming primordial germ-like cells and OLCs. However, research at both basic and preclinical stages is necessary to be continued for addressing various issues related to the isolation, expansion, phenotypic characterization, regulation of lineage pathways, immune modulation and *in vivo* functionalities of skin-derived MSCs. Furthermore, safety evaluations of stem cell therapies in pig as a relevant model are essential for translational biomedical research.

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