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Therapeutic potential of stem cells in skin repair and regeneration

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Stem cells are defined by their capacity of self-renewal and multilineage differentiation, which make them uniquely situated to treat a broad spectrum of human diseases. Based on a series of remarkable studies in several fields of regenerative medicine, their application is not too far from the clinical practice. Full-thickness burns and severe traumas can injure skin and its appendages, which protect animals from water loss, temperature change, radiation, trauma and infection. In adults, the normal outcome of repair of massive full-thickness burns is fibrosis and scarring without any appendages, such as hair follicles, sweat and sebaceous glands. Perfect skin regeneration has been considered impossible due to the limited regenerative capacity of epidermal keratinocytes, which are generally thought to be the key source of the epidermis and skin appendages. Currently, researches on stem cells, such as epidermal stem cells, dermal stem cells, mesenchymal stem cells from bone marrow, and embryonic stem cells, bring promise to functional repair of skin after severe burn injury, namely, complete regeneration of skin and its appendages. In this study, we present an overview of the most recent advances in skin repair and regeneration by using stem cells.

Key words: ***Stem cells; Skin; Regeneration; Repair***

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Mammalian skin serves a number of vital physiological functions to maintain homeostasis. Skin provides a moisture barrier, regulates body temperature *via* hair follicles, sweat glands, and dermal capillaries, and provides lubrication *via* sebaceous glands. The functional properties of skin are often under-appreciated until substantial loss of the skin occurs. In adults, the normal outcome of repair of massive full-thickness burns is fibrosis and scarring without any appendages including hair follicles, sweat and sebaceous glands. To date, no effective therapies have been developed to prevent or reverse the fibrosis and scarring which are different from the original skin in appearance and function. Studies are made to evaluate the prospects of using stem cells for skin repair and regeneration.

Stem cells are found in embryonic and adult tissues, named independently embryonic stem cells (ESCs) and adult stem cet deep into the dermal layer such as mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), epithelial stem cells, and neural stem cells. These stem cells are defined by their capacity of self-renewal and multilineage differentiation (Fig. 1). For example, ESCs can give rise to cells of the three embryonic germ layers: endoderm, mesoderm and ectoderm.[1, 2] Similarly, MSCs and HSCs derived from bone marrow have the potential of differentiation into a variety of tissues, including endothelium, liver, muscle, bone, and skin. In addition, skin stem cells, including epidermal stem cells and dermal stem cells, are also multipotential. It is thought that they not only contribute to the production of their original tissues,[3, 4] but also can be stimulated into other cell types such as nerval and osteogenic lineages.[5-6] Because of the great differentiation potential, stem cells have been thought to be a powerful tool for treatment of a wide spectrum of dis-

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eases that are ineffectively treated by traditional approaches. In this review, we present an overview of the most recent advances in skin repair and regeneration by using stem cells.

Skin repair and regeneration after injury

Re-epithelialization Adult skin consists of two tissue layers: a keratinized stratified epidermis and an underlying thick layer of collagen-rich dermal connective tissues providing support and nourishment. Appendages, such as hair and glands, are derived from and linked to the epidermis but deep into the dermal layer (Fig. 2). Cutaneous wound repair is a multifaceted process involving clot formation, cell migration, extracellular matrix synthesis and deposition, and finally, dermal and epidermal reconstitution. During this process, the restoration of an intact epidermal barrier through wound epithelialization, also known as re-epithelialization, is an essential feature. Re-epithelialization of a wound primarily involves the migration of keratinocytes from the edges of the wound and hair follicles to an extracellular matrix, their proliferation, differentiation and stratification to form the neo-epithelium. Recent evidences from an organotypic model of wound healing in which keratinocytes were genetically labeled with retroviruses suggest that not only basal cells migrate toward wounds but also suprabasal cells may "leapfrog" over the basal cells.[7] Once the denuded wound surface has been covered by a monolayer of keratinocytes, epidermal migration ceases and a new stratified epidermis with underlying basal lamina is re-established from the margins of the wound. But the above natural repair process is slower compared with the need for rapid wound cover to reduce infection rates and the formed epidermis is different from the normal epidermis because the regenerated epidermis is thinner and with fewer and flatter epidermal ridges.

Regeneration of appendages In adult skin, superficial burns that destroy the interfollicular epidermis but leave hair follicles intact are healed rapidly and efficiently with the regeneration of epidermal appendages. But if a skin wound is deeper than the level of hair bulbs in the dermis so that no remnants of hair follicles remain, the repairing epithelium does not regenerate hairs, and the same is true for sweat and sebaceous glands lost at the site of injury. During embryogenesis, fibroblasts in the dermal connective tissues supply permissive and instructive signals that govern the positions and types of hairs and other cutaneous appendages that will be differentiated from the overlying epidermis. Adult wound epidermis fails to regenerate appendages, not only because it is unable to respond to appendage-inducing signals, but also because it does not receive such signals from the underlying wound dermis. To date, therefore, the regeneration of appendages, especially sweat glands, is a hard nut to crack in skin regeneration medicine.

Currently, researches on stem cells bring promise to functional repair of skin after severe burn injury, namely, complete regeneration of skin and its appendages. Application of stem cells can accelerate the re-epithelialization of skin wound and bring possibility of skin appendage regeneration (Fig.3). In essence, there are at least four different ways of using stem cells for skin repair and regeneration: local stem cell induction such as epidermal stem cells, transplantation of stem cells induced *in vitro*, combination of stem cell therapy with gene therapy, and the use of stem cells in tissue engineering protocols.

Epidermal stem cells and skin repair and regeneration

Significant advances have been made in identifying and locating the stem cells that inhabit the skin including epidermal stem cells (interfollicular and bulge stem cells), dermal stem cells,[8] sebaceous stem cells, hair follicle stem cells, sweat gland stem cells, melanocyte stem cells, MSCs, neural stem cells and endothelial stem cells. Epidermal stem cells first attract much attention because of their importance for cell and gene therapy in skin diseases. In the 1990s, researchers using ^{3}H-thymidine to evaluate the label retention to locate the stem cells in murine haired epidermis discovered that the majority of stem cells in the skin reside in the "bulge" region of the hair follicle (named bulge stem cells), with only a small fraction of stem cells residing in the basal layer of the interfollicular epidermis (named interfollicular stem cells). These stem cells ensure the maintenance of adult skin homeostasis and hair regeneration, but they also participate in the repair and regeneration of the epidermis after injuries.[9]

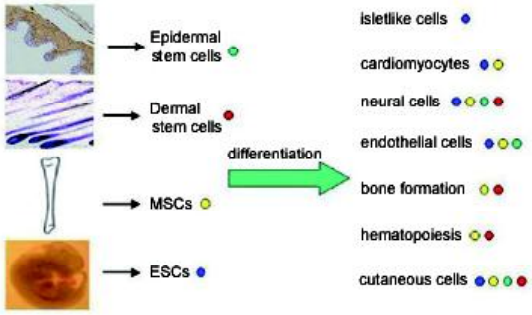

Fig.1. A schematic diagram showing the plasticity of epidermal stem cells, dermal stem cells, MSCs and ESCs in regenerative medicine.

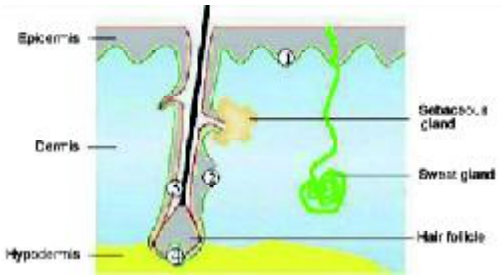

Fig.2. A schematic diagram of structure of skin. Location of epidermal stem cells including interfollicular stem cells (1) and bulge stem cells (2), dermal stem cells including dermal sheath stem cells (3) and dermal papillae stem cells (4).

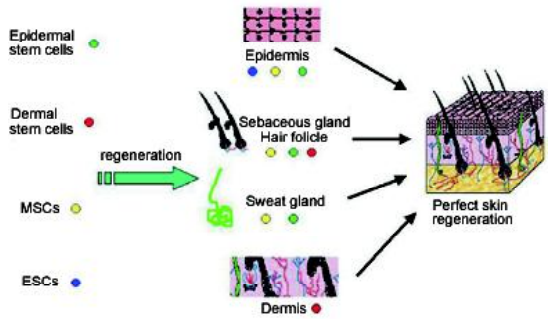

Fig.3. A schematic diagram of perfect skin regeneration by stem cells including epidermal stem cells, dermal stem cells, MSCs and ESCs.

Epidermal stem cells and re-epithelialization

(1) Induction of epidermal stem cells *in situ* Epidermal stem cells are generally thought to be the key source not only for development of epidermis but also for re-epithelialization of skin wound. Many factors, including cellular mediators, cytokines, and growth factors, induce and regulate the migration, proliferation and differentiation of local epidermal stem cells, which facilitate the repair and regeneration of skin after injury. El Ghalbzouri et al.[10] introduced a full-thickness wound in human skin equivalents (HSE) made with a scalpel, frozen by liquid nitrogen, and closed in the absence or presence of exogenous growth factors. Then the rate of re-epithelialization was monitored. The results obtained in this study demonstrate that the re-epithelialization process in full-thickness is accelerated by the presence of keratinocyte growth factor (KGF) and especially by epidermal growth factor (EGF), which support the migration and proliferation of keratinocytes. Intriguingly, the succedent research indicated that EGF promoted the motility of individual cell and monolayer wound closure, which was independent on an increase in cell number.[11] In addition, it's worthy of notice that when leg ulcers are treated with recombinant human epidermal growth factor (rhEGF), there are some stem cells or stem cells-like cells in the spinous and granular layers of regenerated epidermis and these cells bear no anatomic relation with the epidermal stem cells in the basal layer.[12-14] Therefore, it is possible that these undifferentiated cells come from the differentiated cells through dedifferentiation. Besides exogenous growth factors, recent reports proved that endogenous cytokines and growth factors secreted by local keratinocytes also have an important role in re-epithelialization of skin wounds. Simon et al.[15] addressed that the altered cytokines and growth factor expression in the wound resulted in the activation of follicular and epidermal progenitor keratinocytes, and ultimately mediated epidermal repair. From above researches, we conclude that growth factors, which may include KGF, EGF, TGF, bFGF, IL-6 and IL-1, play important roles in promoting re-epithelialization by inducing migration, proliferation, and differentiation of epidermal stem cells *in situ*.

(2) Tissue engineering Tissue engineering combines the principles of cell biology, engineering and material science to develop three-dimensional tissues in replacing or restoring tissue function. Skin tissue engineering emerges as an experimental regenerative therapy motivated primarily by the critical need for early permanent coverage of extensive burn injuries in patients with insufficient sources of autologous skin for grafting. Today, the most commonly used skin grafting technique employs the use of split-thickness grafts taken from unaffected skin. This method is effective, but it is limited by the available surface area of unaffected skin and creates some degrees of additional injuries. The technique of obtaining human skin with dermis and epidermis reconstructed from cells isolated from patients can enable autologous skin grafting in patients with few donor sites.[16] Indeed, the proliferative

capacity of just a small patch of adult skin is immense and the use of cultured keratinocytes allows a much greater surface to be covered. The initial clinical use of cultured keratinocytes is to create confluent epithelial sheets that can be gently removed from the culture dish and applied to reconstitute the epithelial portion of burns, chronic wounds, and ulcers.[17] At present, the use of cultured keratinocytes is limited by the length of time needed to grow epithelial sheets *in vitro*, during which time the patient is susceptible to infection. The epithelial sheets are also extremely fragile and do not adhere well to some burned surfaces. Skin substitutes that can function as dermal equivalents can hold the expanding keratinocytes, improve adhesiveness to the burn wound, and form a temporary wound cover to reduce infection rates.[18, 19] By seeding large-scale cultured epidermal stem cells onto a fibroblast-containing dermal substrate, tissue engineered skin (TES) composed of associated dermis and epidermis can be reconstructed *in vitro*.[20] The constructed TES is similar to the uninjured skin in morphological features and can satisfy the need of restoration of skin defects, yet it lacks several important functions including those provided by hair follicles, sebaceous glands, sweat glands and dendritic cells.

(3) Gene therapy Gene therapy has been described as a promising approach for treatment of skin diseases[21] and impaired wound healing[22] and cultured epidermal stem cells are used as delivery instruments for gene therapy[23]. Researchers had devised methods to use cultured human keratinocytes to correct inborn metabolic skin diseases.[24, 25] Keratinocytes were harvested from patients with recessive dystrophic epidermolysis bullosa, and the genetic defect was corrected either by genomic integration of the correct sequence using a bacteriophage integrase or by transgene expression using a lentivirus. The repaired keratinocytes were expanded in culture and grafted onto nude mice to produce healthy epithelia in which the defect was repaired. Although no study included grafting back onto the original patient suffering from skin diseases, these efforts represented a major advance toward the possibility of manipulating stem cells to treat human diseases. In the succedent research, epidermal stem cells were used to prepare genetically corrected and cultured epidermal grafts and were transplanted onto the surgically-prepared regions of the patient's legs. The results indicated that the regenerated epidermis was maintained by a defined repertoire of transduced stem cells.[26] In addition, gene therapy can also be used in wound healing to promote tissue regeneration, especially being combined with tissue engineering. Wound healing factors, such as platelet-derived growth factor (PDGF)[27] and vascular endothelial growth factor (VEGF)[28], can be transferred into epidermal stem cells that make up the skin tissues. Therefore, gene therapy has the potential to generate the next generation of three-dimensional skin substitutes with enhanced capacity for treatment of burns, chronic wounds and even systemic diseases.[29, 30]

Epidermal stem cells and regeneration of skin appendages Epidermal stem cells are the major resource of the epidermis and skin appendages such as hair follicles, sebaceous glands and sweat glands. At present, we know that when interfollicular epidermis is lost after injury hair follicle keratinocytes can repopulate the interfollicular epidermis and, conversely, interfollicular keratinocytes grafted into an empty hair follicle can produce normal hair. Hair cyclic regeneration by activation of hair stem cells located in the follicle bulge is regulated by Wnt/b-catenin, bone morphogenetic protein 2 (BMP-2) and BMP-4.[31, 32] But after injury, the nascent hair follicles arise from epithelial cells outside the stem cell niche of hair follicles, suggesting that epidermal cells in the wound assume a hair follicle stem cell phenotype. Further research shows that inhibition of Wnt signalling abrogates this wounding-induced folliculogenesis, whereas overexpression of Wnt ligand in the epidermis increases the number of regenerated hair follicles.[33] It is likely that epidermal stem cells give rise to sweat glands.[34] In view of the relationship between epidermal stem cells and sweat gland cell development, Fu et al.[35-36] ventured to suggest that there is a possibility to induce epidermal stem cells to directly differentiate into sweat gland cells and to establish a three-dimensional organization. Shikiji et al[37] attempted to regenerate eccrine sweat glands *in vitro* by inducing the keratinocytes which were derived from young donors. They first provided the evidence that reconstructed epithelia of skin equivalents invaded into a fibroblast-populated collagen gel and organized duct-like structures that resembled eccrine sweat ducts in the presence of EGF and FBS *in vitro*. These results indicate that some of the postnatal keratinocytes from young donors still possess pluripotent ability to be differentiated into eccrine sweat ducts/glands in response

to EGF and FBS. Although it is not a perfect model, this research provides hope that functional skin equipped with eccrine sweat glands can be generated *in vitro* for autologus transplantation in cases of extensive burns, for example.

Dermal stem cells and skin repair and regeneration

Hair follicle dermal stem cells Mammalian dermis contains a multipotent precursor cell population. These cells termed skin-derived precursors (SKPs) were isolated and expanded from rodent and human skin.[38] In recent years, it has become evident that the hair follicle represents an important stem cell niche in the skin. Hair follicle dermal stem cells reside in the dermal papillae (DP) at the base of the follicle and the dermal sheath (DS) that surrounds the outside of the hair follicle.[8, 39] These cells exhibit some properties of stem cells, including regenerative potential,[5, 39, 40] roles in wound healing and ability to produce a functional dermis. DP cells, which are the major components of a hair DP, produce and secrete several growth factors, molecules constructing extracellular matrix components, and cytokines such as basic fibroblast growth factor (bFGF), endothelin-1 (ET-1), stem cell factor (SCF), and so on.[41-43] So they are generally thought to play essential roles in the induction of new hair follicles and the maintenance of hair growth.[44, 45] DS cells can be transformed into DP cells under the influence of follicle germinative epithelium. If the base of a follicle is removed experimentally, the lower follicle dermal sheath (LDS) cells can replace the dermal papilla cells and restore functional hair growth. Similarly, DS cells can also be incorporated into dermal repair in a manner similar to skin fibroblasts, which results in qualitatively improved dermal repair.[46] Intriguingly, DS cells from the base and the upper follicle behave differently during this repair process: only the LDS cells are incorporated into follicle and interfollicular dermis.[47] In addition, there are many experiments about hair follicle induction in healing wounds by follicle dermal stem cells. Although DP cells do not induce the reformation of hair follicle structure *in vitro* due to some reasons such as that they cannot aggregate and reform the cell clump, that they successfully induce many hair follicles as well as the reformation of hair fibres when they are transplanted into the nude mice and that the reformed hair can be observed by naked eyes.[48] However, the number of regenerated hair follicles in above experiments is limited. So the remaining problem is how to enhance the follicle-inducing effects of follicle dermal stem cells. Osada et al.[49] aggregated the DP cells to form spheres and then injected them with epidermal cells. Unlike the dissociated DP cells, the spheres induced more new hair follicles. These results suggest that sphere formation partially models the intact DP, resulting in hair follicle induction. Moreover, the cell combination of intact papillae together with DS and outer root sheath (ORS) cell cultures can produce new follicles in wound sites and these follicles have sebaceous glands and are oriented in an irregular fashion.[47] Therefore, whether cultured DP cells induce follicle formation appears to depend, at least partly, on environmental criteria. Up to now, tissue-engineered skin equivalents have several deficiencies, including the absence of hair follicles. In some reports, the reformation of follicle-like structure is successfully induced *in vitro* by DP or DS cells in a three-dimensional mixture culture. Wu et al.[50] observed that hair follicle-like structure could be reformed in dermal sheath cell-populated collagen gel when combined with superior or inferior epithelial cells by organotypic (three-dimensional) culture. Therefore, we believe that the application of follicle dermal stem cells in the field of cell transplantation and tissue engineering will possibly achieve the hairy healing of the wound.

Non-follicle dermal stem cells Various cell populations can be obtained from dermis with different culture systems. Recently, Bartsch et al.[51] have isolated another multipotent cell population, termed dermal MSCs, from low-temperature preserved human foreskin biopsies with adherent culture. The isolated cells can be expanded for over 100 population doublings with retention of their chromosomal complement and multipotency. They reported that a single dermal MSC could differentiate into adipogenic, osteogenic and myogenic lineages. In addition, Perng et al.[52] reported that human dermis-derived MSCs (hDMSCs) possess differentiation potential of epidermis facilitating wound healing in skin-defect nude mice and the re-epithelialization marker of human pan-cytokeratin is significantly increased on day 14 and day 21 in the wound site of hDMSCs and gelatin/pNIPAAm/PP-treated group. Recently, multipotent fibroblasts expressing nestin$^-$ and vimentin$^+$ were also isolated from human dermis.[53] Dermal fibroblasts are commonly accepted as terminally differentiated cells, which are routinely used as a negative control for evaluation of cell multipotency in many

studies.[54] Yet, the researchers found that dermal fibroblasts isolated from human foreskin could differentiate into adipogenic, osteogenic or chondrogenic lineages in the presence of certain factors, suggesting that dermal fibroblasts might contain a pluripotent subpopulation. The relationship between multipotent dermal fibroblasts and dermal MSCs remains unclear and deserves further investigation. However, multipotent cells may also exist in other non-follicle zones of the dermis. Multipotent cells have been identified from marrow stroma and adipose tissues, which have a similar mesodermal origin from embryonic development with dermis.[55, 56] Although the potential physiological role of the non-follicle stem cells in dermis is unclear, the multipotency of these cells suggests that they might be a useful cell source for therapeutic purposes.

MSCs and skin repair and regeneration

MSCs are non-hematopoietic cells, which were first described by Fridenshtein et al.[57] in 1986 as the clonal and plastic adherent cells, being a source of osteoblastic, adipogenic and chondrogenic cell lines. The main source of MSCs is bone marrow. These cells constitute, however, only a small percentage of the total number of bone marrow populating cells. Apart from the bone marrow, MSCs are also located in other tissues of the human body. There is an increasing number of reports describing their presence in adipose tissues, umbilical cord blood, chorionic villi of the placenta,[58] amniotic fluid,[59] peripheral blood, fetal liver,[60] lungs,[61] and even in exfoliated deciduous teeth[62]. Human MSCs are known to constitute a heterogeneous population of cells. The culture of MSCs demonstrated that single-cell-derived colonies of human MSCs contain three morphologically distinct kinds of cells including spindle-shaped cells, large cuboidal or flattened cells and extremely small cells that are rapidly self-renewing (RS cells). The RS cells appear to be the earliest progenitors in the culture and have the greatest potential for multilineage differentiation.

MSCs and re-epithelialization Because they can be obtained easily, MSCs derived from bone marrow have received considerable attention. It has shown that a single bone marrow-derived stem cell is able to differentiate into epithelial cells of the liver, lungs, gastrointestinal tract, and skin.[63] Systemic transplantation and local implantation of MSCs are promising treatment methods for skin wounds,[64] especially for chronic wounds[65, 66]. Badiavas et al.[67] obtained bone marrow from green fluorescent protein (GFP)-expressing transgenic mice and then gave it to the recipient (non-GFP expressing) mice by tail vein injection. At last, they found that GFP-labeled cells differentiated into non-hematopoietic skin structures in wounded skin, implying that the transplanted MSCs have the ability to migrate to the damaged tissue sites and stimulate repairs by differentiating into skin-specific cells. In addition, experiments of influence of local application of MSCs on cutaneous wound regeneration showed that GFP-labeled MSCs could convert into the phenotypes of epidermal keratinocytes, sebaceous glands, follicular epithelial cells, and vascular endothelial cells by transdifferentiation.[68-71] Wu et al.[72] also proved that bone marrow derived MSCs-treated wounds exhibited significantly accelerated wound closure with increased re-epithelialization, cellularity and angiogenesis. In a human study of chronic nonhealing wounds, the results showed that direct application of bone marrow-derived cells can lead to wound closure and possible rebuilding of tissues.[65] This study, although interesting, was hampered by the lack of an efficient delivery system for the autologous bone marrow-derived cultured cells. More recently, Falanga et al.[66] have applied human autologous bone marrow-derived cultured cells to nonhealing and acute wounds, using a specialized fibrin spray system. This approach appears to be safe and may represent a rather ideal way of introducing cells into injury sites.

Despite the accumulating data of skin lineage differentiation from MSCs *in vivo*,[73] studies on differentiation of MSCs into epithelial lineage cells *in vitro* are also performed[74]. Wu et al.[75] proved that MSCs derived from the early human embryo have the ability to transform into epidermal cells *in vivo* and *in vitro*. Consistent with this, hMSCs derived from adult bone marrow also possess the potential of differentiating into epidermal-like cells with the production of keratin 19, P63, and β_1-integrin under certain conditions.[76, 77] Recently, our group reported that P38 route may play an active role in the differentiation of MSCs into epidermoid cells because enhancing the activation of P38 route by blocking the upstream signal Rho can promote the differentiation of MSCs into epidermoid cells.[78] All experimental data from above researches suggest that hMSCs have the potential of epidermal differentiation under a specific differentiation condition. But there are no experiments *in vivo* to verify that the induced hMSCs are the same as the epidermal cells. Therefore, we call them epider-

mal-like cells. However, we believe that epidermal-like cells differentiated from hMSCs can be used as a cell source for tissue engineering and cell therapy.

Skin tissue engineering is a possible solution for extensive skin defects. The ultimate goal of skin tissue engineering is to restore the complete functions of native skin, but until now the structure and function of skin are only partially restored. Recently, the suitability of hMSCs for soft tissue engineering was investigated.[79] These cells were seeded onto 1-ethyl-3-3-dimethylaminopropyl carbodiimide (EDC) cross-linked collagen sponges and implanted in immunodeficient mice. After 2 and 6 weeks *in vivo*, populated scaffolds showed a high density of vascularization and cellularity. Perng et al.[80] also demonstrated that the hBMSCs possess the differentiation potential of epidermis and the capability of healing skin wounds by culturing adult hBMSCs on gelatin scaffolds with poly N-isopropylacrylamide (pNIPAAm). Interestingly, BMSCs can be used to construct full-thickness tissue-engineered skin, because BMSCs have the potential to differentiate into epidermal cells and fibroblasts *in vitro* and show clinical feasibility when acting as epidermis-like and dermis-like seed cells in skin engineering.[81]

MSCs and regeneration of skin appendages In addition to transdifferentiation to epidermal-like cells *in vitro* and promotion of wound repair *in vivo*, regeneration of skin appendages may be another potential application of MSCs in regenerative medicine. The experiments of systemic transplantation and local implantation of MSCs for cutaneous wound regeneration demonstrate that the MSCs migrating into the wound sites can promote the repair and regeneration of skin wounds by transdifferentiation into phenotypes of epidermal keratinocytes, sebaceous glands, and follicular epithelial cells.[68-70] Wu et al.[72] transplanted GFP^+ BM-MSCs into wound healing model and determined the contribution of BM-MSCs in dermal keratinocytes and appendages. They found that some BM-MSCs positive for GFP and cytokeratin in the dermis of the wounded skin formed structures resembling developing sweat or sebaceous glands. In another research, porcine MSCs were isolated and engrafted into porcine skin. Results showed that transplanted MSCs lablled with BrdU were transdifferentiated into sebaceous duct cells.[82] Consistant with above results, we found that BrdU-labelled MSCs, which were collected from Wistar rats and infused from the penile vein, emerged in the hypodermia, the sebaceous glands, and the hair follicles of the wounds on the midback of the homogeneous male Wistar rats.[83] Investigation into the mechanism of the transdifferentiation shows that the wound microenvironment containing diverse growth factors or inflammatory factors may play an important role in this process. Among the skin appendages, sweat glands are most important because they possess the functions of temperature regulation and homeostasis maintenance. But they cannot be regenerated in full-thickness burn wounds *via* remnants of skin tissues.[35, 36] The recruitment of MSCs to skin wounds and the participation of MSCs in cutaneous repair imply that MSCs may play a potential role in sweat gland regeneration.[84] Despite the fact that the regeneration of sweat glands is a difficult and perplexing problem, we make an effort to explore the possibility of transdifferentiation of stem cells into sweat gland cells *in vitro*.[85, 86] In our experiment, adult MSCs were co-cultured directly or indirectly with heat-shocked sweat gland cells and the phenotypes of the co-cultured cells were examined. The results showed that 4% of the $BrdU^+$ MSCs added to the co-cultures were differentiated into sweat gland cells, which expressed the proteins of sweat gland cells: carcinoembryonic antigen (CEA) and cytokeratin 19, markers of the gland cells. In addition, the percentage of differentiation was enhanced by epidermal growth factor and the injured microenviroment.[87, 88] Further researches showed that the extracellular signal-regulated kinase (ERK) pathway, especially pERK, was involved in the phenotype conversion of human MSCs into human sweat gland cells.[87, 89] There are two possible mechanisms for the conversion of MSCs into other mature non-hematopoietic cells of multiple tissues: transdifferentiation and cell fusion. Transdifferentiation refers to the conversion of one cellular phenotype to another by changing the expression of a master regulatory (master switch) gene, whose normal function is to distinguish the two tissues in normal development and belongs to a wider class of cell-type switches termed metaplasias. Cell fusion means that stem cells or their progeny fuse with cells of other types, mixing cytoplasmic and even genetic materials of different (heterotypic) origins, thereby converting the gene expression pattern of stem cells to that of the fusion partner. However, which path should answer for the phenotype conversion of human MSCs into human sweat gland cells needs to be further investigated.

ESCs and skin repair and regeneration

Derivation of ESCs from reprogrammed human skin cells ESCs were first established from the inner cell mass (ICM) of mouse blastocysts in 1981,[90] and human ESCs were first derived in 1998[91]. However, the use of human embryos faces ethical controversies that hinder the application of human ESCs. In addition, it is difficult to generate patient- or disease-specific ESCs, which are required for their effective application. One way to circumvent these issues is to induce pluripotent status in somatic cells by direct reprogramming. Somatic cells can be reprogrammed to an embryonic-like state by injection of nucleus into enucleated oocyte[92-95] or by fusion with ESCs[96, 97]. Researchers reported the use of nuclei from hair follicle stem cells and other skin keratinocytes as nuclear transfer (NT) donors to clone mice, revealing skin as a source of readily accessible stem cells. And the nuclei of stem cells can be reprogrammed to the pluripotent state by exposure to the cytoplasm of unfertilized oocytes. In primates, the results of somatic cell nuclear transfer (SCNT) are characterized with inefficient reprogramming and poor embryonic development. Byrne et al.[98] used modified SCNT approach to produce rhesus macaque blastocysts from adult skin fibroblasts, and successfully isolated two ESCs lines from these embryos. The discovery that nuclei from mammalian differentiated cells can be reprogrammed to an undifferentiated state by transacting factors present in the oocytes leads to a search for factors that can mediate similar reprogramming without SCNT. Recently, four transcription factors (Oct4, Sox2, c-myc, and Klf4) were shown to be sufficient to reprogramme mouse fibroblasts to undifferentiated, pluripotent stem cells [termed induced pluripotent stem (iPS) cells].[99-103] Moreover, researchers have reprogrammed adult human dermal fibroblasts into so-called iPS cells with the same four factors and the human iPS cells were similar to human ESCs in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity.[104,105] Lowry et al.[106] also acquired the same result by ectopic expression of the same set of four genes with the addition of the NANOG transcription factor in dermal fibroblasts. At the same time, Yu et al.[107] also successfully reprogrammed human somatic cells into iPS cells that exhibited the essential characteristics of ESCs with four candidate reprogramming genes: OCT3 and SOX2, as Takahashi et al.[104] used, and two different genes, NANOG and LIN28. Nakagawa et al.[108] generated human iPS cells from adult dermal fibroblasts without c-Myc. Furthermore, the therapeutic potential of such iPS cells were investigated by using a humanized sickle cell anemia mouse model.[109] However, the human iPS cells are not identical to human ESCs. So further studies are essential to determine whether human iPS cells can replace human ESCs in medical applications.

ESCs and wound repair ESCs can be induced *in vitro* to differentiate into cutaneous cells, which can be applied to bioengineered skin to facilitate the wound repair and regeneration.[110] During embryonic development, skin forms as a result of reciprocal interactions between mesoderm and ectoderm. Epithelial cell differentiation from ESCs can be identified by the presence of cytokeratin intermediate filaments and keratinocyte-specific involucrin.[111, 112] After *in vitro* differentiation of mouse embryonic stem (mES) cells, enrichment of keratinocytes and seeding onto various extracellular matrix (ECM) proteins in the presence of BMP-4 and/or ascorbate can promote the formation of epidermal equivalents, which are composed of stratified epithelium (when cultured at the air-liquid interface on a collagen-coated acellular substratum). The resulted tissues display morphological patterns similar to normal embryonic skin. The cells express late differentiation markers of epidermis and markers of fibroblasts, which are consistent with those found in native skin. The data suggest that ESCs have the capacity to reconstitute *in vitro* fully differentiated skin.[113] In another report, Troy et al.[114] developed a two-step culture scheme in which pluripotent mouse ESCs were induced first to a surface ectoderm phenotype and then positively selected for putative epidermal stem cells. It showed that the earliest stage of epidermal development followed an ordered sequence that was similar to that observed *in vivo* (expression of Keratin 8, Keratin 19, Keratin 17, and Keratin 14). To date, however, the molecular mechanisms controlling epidermal commitment of ESCs are unclear. Further researches showed that p63 and BMP-4, which play pivotal roles in ectodermal and epidermal development, may be involved in this process.[115]

Other stem cells and skin repair and regeneration

Besides epidermal stem cells, marrow-derived stem cells and ESCs, other stem cells derived from adult tissues may also serve as cell therapy to enhance the

healing process in skin wounds. Muscle-derived stem cells obtained from the muscle of New Zealand white rabbits were cultured *in vitro* for two weeks and then seeded onto a circular 2-cm-diameter defect created on the dorsal side of the ear of the rabbit. Fourteen days later, re-epithelialized areas were significantly greater in the treatment group, but the wounds in the control group showed nonepithelialized areas.[116] Interestingly, there is another report that the sweat apparatus is capable of re-epithelializing the skin surface after a major cutaneous wound.[117] These data suggest that the use of autologous stem cells in skin wounds expedites and improves the organism's natural healing process.

Conclusion

In spite of recent advances from breakthroughs in recombinant growth factors and bioengineered skin, perfect skin regeneration remains a formidable challenge because of fibrosis and scarring without any appendages after full-thickness burns. Stem cells offer the possibility that some structures within the wound, such as epidermis, hair follicles, sebaceous glands and sweat glands, may be reconstituted. To what extent that it comes true remains uncertain and much work needs to be done before clinical application of a stem cell-based therapeutic approach in perfect skin regeneration. Firstly, we should explore more and more stem cells to solve the limited resource of stem cells. At present, there are three major ways by which scientists can acquire stem cells including direct isolation from embryonic and adult tissues, dedifferentiation from some differentiated cell types, and transdifferentiation from other cellular phenotypes. But all above methods are still not enough to provide sufficient source of stem cells for regenerative medicine. Secondly, despite recent progress in the lab and in clinic, there are still many gaps in our understandings of basic stem cell biology that must be addressed before stem cell therapy can be applied to its fullest potential in clinic. Therefore, if we can understand what regulates the fate of stem cells, we can potentially enhance the function of the graft, avoid potentially deleterious genes, and select target-specific differentiation pathways that lead to the formation of sebaceous and sweat glands, hair follicles, and interfollicular epidermis. Thirdly, three-dimensional reconstruction of appendages by using stem cells, such as sweat glands, has long been considered as a much greater challenge. Thus far, a few studies have offered some evidences that stem cells may play a potential role in sweat gland regeneration. But there is no report that intact sweat glands including secretion and duct sections are regenerated *in vivo* or *in vitro* by using stem cells. In conclusion, there is still a long way to go before we realize the full potential of stem cells in regenerative medicine. But with our great experimental efforts, the vision of perfect skin regeneration with stem cells will come true in the future.

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