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Review Article

Stem cell therapy on skin: Mechanisms, recent advances and drug reviewing issues

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ABSTRACT

Stem cell products and its clinical applications have been widely discussed in recent years, particularly when the Japanese “induced pluripotent stem cells” founder Dr. Yamanaka was awarded as Nobel Prize laureate in 2013. For decades, major progresses have been achieved in the stem cell biology field, and more and more evidence showed that skin stem cells are involved in the process of skin repair. Stem/progenitor cells of the epidermis are recognized to play the most essential role in the tissue regeneration of skin. In this review, we first illustrated basic stem cell characteristics and various stem cell subtypes resided in the skin. Second, we provided several literatures to elucidate how stem/progenitor cells collaborate in the process of skin repair with the evidence from animal model studies and *in vitro* experiments. Third, we also introduced several examples of skin cell products on the pharmaceutical market and the ongoing clinical trials aiming for unmet medical difficulties of skin. Last but not least, we summarized general reviewing concerns and some disputatious issues on dermatological cell products. With this concise review, we hope to provide further beneficial suggestions for the development of more effective and safer dermatological stem/progenitor cell products in the future.

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1. Skin stem cells and the mechanism of skin repair

Stem cells generally have two major characteristics that they can give rise to specialized cell lineages or cells and are

capable of self-renewing for long periods [1,2]. Traditionally, stem cells can be categorized into two different groups, embryonic stem cells and somatic stem cells. Embryonic stem cells are obtained from the inner cell mass of blastocyst in mammalian embryos. Embryonic stem cells are pluripotent;

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therefore, they have the potential to derive progeny cells belonged to all three germ layers including ectoderm, endoderm and mesoderm [3]. Unlike embryonic stem cells, somatic stem cells are typically found in mature organs or tissues. Some somatic stem cells might be multipotent but majority of them are lineage limited, i.e. hematopoietic stem cells can only give rise to mature blood cells [4], whereas neural stem cells can only divide into neuronal and glial cells [5]. With the huge success of Professor Yamanaka's lab in Kyoto, differentiated, adult somatic cells can be reprogrammed to generate induced pluripotent stem cells (iPSCs), and now iPSCs become a new emerging group of stem cells. The reprogramming is achieved by exogenous addition of four transcription factors (Oct-3/4, Sox2, c-Myc, and Klf4) using retroviral transduction. iPSCs have been shown to be pluripotent and can give rise to a wide range of mature cell types [6].

Skin stem cells as well fall into the classification as somatic stem cells, however, due to the cellular heterogeneity of skin, various types of skin stem cells were found in past decades [7]. Recently, significant advances have been made in identifying different types of skin stem cells with the aid of molecular tools. Subgroups of skin stem cells are listed as below.

1.1. Epidermal stem cells

Most resided in the basal layer of epidermis, can derive into transient amplifying cells and terminal-differentiated epidermal cells. Specified cell markers are p63, $\beta 1^{\text{high}}$ /melanoma chondroitin sulfate proteoglycan + (MCSP+), $\alpha 6^{\text{high}}$ /CD71^{dim} [8–10].

1.2. Follicular stem cells

Located at the follicle bulge region, can derive into hair follicle epithelium, including outer root sheath, inner root sheath, and hair shaft. Specified cell markers are K15, CD34, Lgr5, Sox9, Lhx2, NFATC1, NFIB, K15, PHLDA1, CD200, K19, etc. [11–17].

1.3. Melanocyte stem cells

Located at the follicle bulge region and hair germ. Specified cell markers are Dct, Sox, and Pax3 [18–21].

1.4. Sebaceous gland stem cells

Resided around sebaceous glands and infundibulum. The unique cell marker is Blimp1 [22].

1.5. Mesenchymal stem-cell-like cells

Located at dermis, might divide into Mesodermal derivatives and some neural cell types. Specified cell markers are CD70, CD90, and CD105 whereas negative for CD34 [23].

1.6. Neural progenitor cells

Located at the follicle dermal papillae, might divide into neural and glial lineages, shared similar cell markers as counterparts in other organs or tissues.

1.7. Hematopoietic stem cells

Located at the follicle dermal papillae, might divide into erythroid and myeloid lineages, shared similar cell markers as counterparts in other organs or tissues.

Among all these distinct skin stem cell subgroups, epidermal stem cells are the most deeply correlated to tissue repair and skin regeneration. Scientific reports supported that stem cells of epidermis are rare, infrequently dividing, and generate short-lived, rapidly dividing cells that carry out the regeneration of the epidermis. The same infrequently dividing stem cells of epidermis are assumed to be the major epidermal cell population responsible for repairing skin injury. Most epidermal stem cells reside in the basal layer of epidermis, some might also be found in the bulge region of the hair follicle and the base of the sebaceous glands [24,25]. Throughout its whole life cycle, epidermal stem cells are circulated between two different cell phases. Under the slow cell phase, epidermal stem cells are quiescent. While entering transit amplifying cell phase, they are quickly divided and the number of skin cells is amplified for the replenishment of skin tissue. Finally, they undergo numerous cell divisions before becoming terminally differentiated to accomplish skin regeneration.

Toward skin injury, both epidermal stem cells and follicular stem cells contribute to the re-epithelialization of wounds [26–28]. In the full-thickness wound, epidermal stem cells and progenitor cells from the hair follicle initially migrate toward the wound site. Epidermal stem cells have been reported to be reactivated in response to skin injury and contribute to skin regeneration on the cellular level [29]. Further clinical evidence also suggested that epidermal stem cells and follicular stem cells participate in the re-epithelialization of wounds by evaluating the potential healing capacity of autologous scalp follicle grafts transplanted into chronic leg ulcerations. This pilot study reported that the size of ulcer areas reduced (27.1% vs. 6.5%, compared to the control group) by the end of eighteen weeks' engraftment in totally ten patients [30]. Epithelialization, neovascularization, and dermal reorganization were also enhanced within these wound areas. One interesting finding to note is that hair follicular progenitor cells were largely replaced by epidermal progeny following repair in a long-term follow-up. This accidental finding might indicate that nevertheless epidermal stem cells and hair follicular stem cells collaborate in the early phase of skin healing, however, the hair follicular stem cells might not be essential for the long-term maintenance after skin repair.

With the major advances of molecular biology, the role of small molecules involved in skin repairing has been well documented, ex. the miRNAs. MiRNAs are central regulators of gene expressions and are capable of tuning genes with either upregulations or downregulations. Therefore, miRNAs play key roles in various biological processes including cell survival, homeostasis, and differentiation. Several miRNAs were identified to be expressed exclusively in epidermal stem cells in animal models compared with other skin cells, including miR-200, miR-141, miR-429, miR-19 and miR-20 [31,32]. Upregulation of several miRNAs was also reported

(miR-23b, miR-95, miR-210, miR-224, miR-26a, miR-200a, miR-27b, and miR-328) under the terminal differentiating process of epidermal stem cells to keratinocytes. Besides, the skin regenerative mechanism is precisely regulated by the balance of both activating factors (e.g., COL17A1, Wnts, etc.) and inhibiting factors (e.g., DKK1, Bmps, Sfrp4, etc.) for skin stem cells. In the skin of aged mice, inhibiting factors are present in a wider dermal region and persist for long time; however, once the dermal microenvironment of a young mouse was transplanted into the skin of an old mouse, the skin phenotype can be partially reversed and rescued [33]. The essential role of paracrine molecules in skin repair can also be illustrated by the following study. Scientists also discovered that the distressed hair follicle secretes the cytokine Ccl2; therefore M1 macrophages will be attracted to the distressed follicle. Subsequently, M1 macrophages will secrete Tnf- α to activate regeneration of both distressed and healthy follicles. Only high density of distressed follicles can recruit effective numbers of M1 macrophages to the follicles for skin regeneration [34]. To our understanding, several mesenchymal stem cells or progenitor cells have the capacity to release immunomodulation factors, and this might be the reason that different engrafted cell types have different efficacy upon skin repair. Although the precise molecular mechanism of skin repair is still not clear, those studies already depicted an outline how epidermal stem cells participating in tissue regeneration and provided future strategies for skin cell products development.

2. Cell products aiming for skin repair

Major skin injuries, resulting from extensive burns, infection or trauma, require medical interventions to heal properly and are always huge challenges for clinicians. Skin loss from thermal injuries (burns) represents about 1 million people each year in America [35]. In 2015, a fiery explosion that occurred at the Formosa Fun Coast Water Park in New Taipei left 499 people injured, including more than 300 who were severely burned. Those numbers reveal the importance of this clinical problem and the need for further research in the treatment of skin injuries. Traditionally, autologous skin grafting is the most feasible and esthetic technique for the treatment of extensive skin injuries. However, with whole-body severe burn victims, only limited fraction of the skin can be repaired. In 1980s, scientists already developed methods to proliferate human keratinocyte *in vitro* with fibroblast feeder cells [36,37]. Cultured human keratinocyte can differentiate and reform a functional skin barrier that can be transplanted into patients suffering from severe burn injuries. Nowadays, cultured human keratinocyte became the most widely used cell products in the world.

On the pharmaceutical market, various types of cell products aiming skin repair were developed, in the forms of confluent or pre-confluent autologous or allogeneic keratinocytes. For example, EpiDex autologous cellular sheet, which is comprised of outer root sheath keratinocytes, has been applied for chronic leg ulcers [38,39]. Cryoskin is another example of confluent but allogeneic keratinocyte sheet [40]. Different delivery systems for keratinocyte sheets or

suspension to wounds such as fibrin glue in conjunction with the aerosol were also been reported (BioSeed-S, CellSpray) [41,42]. These epidermal substitutes can be applied not only for the treatment of severe burns, but also venous or diabetic ulcer cases.

Since epidermal stem cells have the potential to regenerate skin, several clinical trials have focused on it to offer an alternative treatment option. A phase I clinical trial, which aimed for patients with recessive dystrophic epidermolysis bullosa, using autologous epidermal cellular sheets with type VII collagen is currently ongoing [43,44]. Researchers also applied genetic modified keratinocytes (might have the potential as epidermal stem cells) from patients of junctional epidermolysis bullosa on severe combined immune deficient (SCID) mouse model for effective skin proliferation and regeneration [45]. Recently, the concept of somatic stem cells transdifferentiation [46] (i.e. somatic stem cells might have the potential to cross lineage boundaries under specific environmental niches) has been emphasized in regenerative medicine; therefore, not only epidermal stem cells but also plenty of somatic stem cells are involved in clinical trials for skin and soft tissue repair. As shown in Table 1, several global clinical trials using different sources of somatic stem cells targeting on critical limb ischemia (CLI), known as an advanced stage of peripheral artery occlusive disease, are currently ongoing. In Taiwan, two investigational new drug trials also applied autologous bone marrow mononuclear cells or angiogenic cell precursors for treating CLI within the past two years.

3. Drug reviewing issues of cell products for skin repair

Generally, all the cell products are facing similar reviewing challenges as below:

- A How to ensure stem/progenitor cells to differentiate into desired cell phenotypes? Are there potential karyotype changes during long-term cell culture?
- B What are the best biomarkers to identify the cellular purity before cell products finalized?
- C What are the possible impacts of adjuvants and/or biomaterials for building suitable cellular microenvironment? Will these adjuvants and/or biomaterials induce unpredicted immunological responses?
- D What types of grafting are planned for the patients? How to choose the appropriate method for delivering these grafting to patients?
- E How long will these cells persist to survive in patients' bodies? Are there any none- or minimal-invasive methods for tracing cells *in vivo*?
- F How to assess the benefit/risk of proposed cell products when comparing to standard treatments? What will be the clinical evaluation methods (end points) to investigate the functionality of the grafts?

With remarkable advances of cellular biology, some challenges now might be easier to overcome; still some remained to be solved for better qualification control. On the other hand,

Table 1 – Listing of global and Taiwan somatic stem/progenitor cell trials on critical limb ischemia and/or skin ulcerations.

ClinicalTrial.gov/ TaiwanTrial No.	Clinical trial phases	Somatic stem/progenitor cell types	Indications	Autologous/Allogeneic
Global trials				
NCT02304588	I	Mesenchymal stem cells	Diabetic foot ulcers	Autologous
NCT00955669	I	Bone marrow mesenchymal stem cells	Critical limb ischemia	Autologous
NCT02394886	I	Adipose mesenchymal stem cells	Diabetic foot ulcers	Allogeneic
NCT00951210	I	Placental-derived adherent stromal cell	Critical limb ischemia	Allogeneic
NCT00919958	I	Placental-derived adherent stromal cell	Critical limb ischemia	Allogeneic
NCT02336646	I	Mesenchymal stem cells	Critical limb ischemia	Allogeneic
NCT02863926	I	Bone marrow cells	Critical limb ischemia	Allogeneic
NCT01686139	I/II	Bone marrow mesenchymal stem cells	Diabetic foot ulcers	Allogeneic
NCT01903044	I/II	Bone marrow mononuclear cell	Lower extremity ischemia & ulcer	Autologous
NCT00922389	I/II	Peripheral blood mononuclear cell	Critical limb ischemia	Autologous
NCT00883870	I/II	Mesenchymal stem cells	Critical limb ischemia	Allogeneic
NCT01558908	I/II	Endometrial regenerative cells	Critical limb ischemia	Allogeneic
NCT03239535	I/II	Mesenchymal stem cells	Critical limb ischemia	Allogeneic
NCT01750749	II	Bone marrow mesenchymal stem cells	Venous ulcer	Autologous
NCT01232673	II	Bone marrow mesenchymal stem cells	Critical limb ischemia	Autologous
NCT01065337	II	Bone marrow mesenchymal stem cells	Critical limb ischemia	Autologous
NCT01232673	II	Bone marrow mesenchymal stem cells	Critical limb ischemia	Autologous
NCT01484574	II	Bone marrow mesenchymal stem cells	Critical limb ischemia	Allogeneic
NCT03056742	II	Bone marrow mesenchymal stem cells	Critical limb ischemia due to Buerger's disease	Allogeneic
NCT03042572	II/III	Mesenchymal stromal cells	No-option ischemic limbs	Allogeneic
Taiwan trials				
PH01	I/II	Bone marrow mononuclear cell	Critical limb ischemia	Autologous
NCT02551679	II	Angiogenic cell precursor	Critical limb ischemia	Autologous

for dermatological topical products, several controversial concerns have been raised in recent years. Since skin stem/progenitor cell products are belonged to the same pharmaceutical category, we first have to go through these disputatious reviewing issues on dermatological topical products.

Many new mechanisms underlying the development of dermatosis have recently been found; therefore, advances in the development of topical dermatological medication have been rapid. In the new era, some issues are still easily ignored, such as ethnic difference. For convenience, we refer to differences in race as 'white' or 'black' according to the most apparent appearance. However, melanin is only one component of skin. In fact, the compositions of this organ are more complicated. For the development of new drugs, some companies classify patients according to the Fitzpatrick skin phototype, claiming that types I and II represent Caucasians, whereas types III and IV represent Asians. This concept might be controversial. Thus, there are approximately 100% Caucasians volunteers in many pivotal studies initiated from European and American regions. The relative lack of efficacy and safety data for Asians is a concern in the development of new drugs.

The Fitzpatrick skin phototype was first described by Thomas B. Fitzpatrick in 1975 based on a person's natural color and responses to sun exposure in terms of degree of burning and tanning. It has most commonly been used to analyze sun sensitivity in studies related to the cause of skin cancer, exposure to ultraviolet radiation, tanning and protective behaviors. Skin phototype typing is also widely used for estimating UV, PUVA and laser treatment doses [47]. Thus, people who have the same Fitzpatrick skin phototype may have similar responses to light exposure. However, the Fitzpatrick

skin phototype is unable to indicate similar responses to all drugs with various mechanisms and extrapolate ethnicity.

Second, misinterpretation of Fitzpatrick skin phototype is sometimes noted. The Fitzpatrick skin phototype classification includes six different skin types that range from very fair (skin type I) to very dark (skin type VI). The two main factors that influence skin type are genetic disposition and reaction to sun exposure and tanning habits. Skin phototype is genetically determined and is one of the many aspects of overall appearance. It also comprises eye and hair color. The Fitzpatrick scale is a numerical classification scheme for determining skin color based on a questionnaire related to an individual's genetic constitution, reaction to sun exposure and tanning habits. Response to each question is measured on a scale of 0–4. The responses to all the questions are totaled to obtain a final score corresponding to the Fitzpatrick skin type [47]. The score is not decided based on current skin color alone. Human skin color can be changed by several factors, including sun exposure, post-inflammatory hyperpigmentation, etc. The core determinant of the scores is the natural color of the skin, eyes and hair, and the Fitzpatrick skin phototype is almost genetically determined. Therefore, it is a useful tool to evaluate photoaging and risk of skin cancer, minimal erythema dose for UV phototherapy and cosmetic dermatology. However, if we observe skin color only, the Fitzpatrick skin types become changeable.

Final, pigmentation is the most obvious morphology but not the only difference between different racial groups. Four chromophores are responsible for the varying colors found in human skin: hemoglobin, oxyhemoglobin, melanin and

carotenoids. Skin hues are the result of a combination of all pigments. Melanin is the most apparent contributor to skin color. Melanin is synthesized in melanocytes and packaged into melanosomes that are found dispersed throughout the epidermis. Variations in melanosome distribution, together with the quantity and type of melanin present, are responsible for differences in skin pigmentation. Differences in racial skin pigmentation may be due to differences in the production of melanin. This indicates that there may be structural differences in the melanogenic enzymes. Besides, intracellular pH may also influence melanogenesis and differ between different ethnic skin cells [48]. These findings indicate that the amount of melanin is determined using multiple factors.

There remain some differences in skin composition between ethnic groups, e.g. the stratum corneum structure. Investigations on transepidermal water loss in patients of different races have unfortunately reported conflicting results. However, when collectively interpreting all available data, most studies indicate differences between African American, Caucasian and Asian skin. These reports may demonstrate that Asian skin has the poorest barrier function upon mechanical challenge. These data emphasize racial differences in skin barrier function as measured by transepidermal water loss. The findings have important implications for the ability of different skin types to endure and recover from exogenous insults, absorb topical therapeutic agents and maintain moisture under various physiological conditions [49].

Topical dermatological formulations aim to deliver therapeutically effective concentration of drugs to the skin layers, which are also the target site. The barrier function of skin is mostly mediated by the stratum corneum. The stratum corneum consists of 15–20 layers of acutely flattened, metabolically inactive, polygonal cells. The process of drug or chemical absorption into the skin is influenced by several factors. These include molecular size, lipophilicity, pH of formulation, penetrant concentration, temperature and formulation compositions among others [50]. Although differences in morphology and physiology do not fully determine differences in efficacy and safety, variability between ethnic groups warrants further study.

Besides, skin contains all the major enzymes found in the liver and other tissues capable of catalyzing a number of metabolic reactions. Metabolism of topically applied compounds results in altered pharmacological and toxicological effects. There are a number of chemical groups that are particularly susceptible to skin metabolism, including alcohols, acids, primary amines and esters, among others [50]. Thus, the skin has unique and complicated dermatokinetics similar to pharmacokinetics in plasma. Assessment of the dermatokinetics of topical dermatological formulations is of utmost importance in assessing the safety and efficacy of dermatological products. Numerous approaches are reportedly being used to determine the real-time measurement of molecules in the skin layers. Regulatory agencies, such as the U.S. FDA, are still exploring different techniques for characterizing drug dermatopharmacokinetics. Certain dermatological products applied to the skin surface may penetrate into deeper tissue layers and reach the systemic circulation [50]. The issue of efficacy must also be considered.

As to the stem cell therapy on skin, although initially most clinical trials were mainly designed as autologous engraftment, nowadays already some of them aimed for allogeneic indications (see also Table 1). Similar to the topical formulations applied in the dermatological fields, reviewing policies should be more dedicated on potential safety concerns, especially on ethnic bridging issues as mentioned above. Based upon the differences in morphology and physiology between different races, the possible variation in efficacy and/or safety of allogeneic skin cell products should not be ignored. Moreover, allogeneic skin cell/tissue engraftment might be in high demand under specific occasions with mechanic explosions or accidents. In 2015, there were massive burn victims in the Formosa Fun Coast Water Park event in Taiwan, and we are deeply appreciated to introduce the advanced technique of autologous transplantation of human keratinocyte cultivation (JACE®) by J-TEC (Japan Tissue Engineering Co., Ltd., now had been merged by Fuji). This technology was originally developed by Professor Howard Green of Harvard Medical School in the 1970s, and later been transferred to J-TEC by Professor Minoru Ueda of Nagoya University. By isolating keratinocytes from a 1-cm² skin sample from the patient and culturing them on the fibroblast feeder, a sheet of cultured epidermis measuring around 1000 cm² can be produced in around two weeks. In the event mentioned above, total five patients were benefited by JACE®, however, facing massive amount of burn patients, both autologous and allogeneic skin products will be considered to facilitate skin repair under highly demanding circumstances. Therefore, both regulatory bodies and pharmaceutical companies should work together to set the standard bridging criterion for skin stem cell products, especially those of allogeneic indications. The best solution will be always to enroll adequate numbers of non-Caucasian subjects into future clinical trials. For developing ideal medications, we definitely have to verify the characteristics of proposed skin stem cell products and clarify the differences in efficacy and safety across different races, hence to actually promote public health.

Conflict of interest statement

The authors report no conflict of interest.

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