Review

Mesenchymal Stem Cells for the Treatment of Skin Diseases

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Abstract: Mesenchymal stem cell (MSC)-based therapy involving both autologous and allogeneic MSCs shows great promise in treating several conditions. MSCs promote wound healing, and can differentiate into multiple cell lineages, including keratinocytes. Therefore, MSCs can be used for the treatment of congenital or acquired skin defects. Because of their immunomodulatory properties, MSCs may be useful for the treatment of inflammatory and autoimmune skin diseases. In particular, MSCs might be effective for the treatment of large vitiligo lesions as immunosuppressant or cultured grafts. MSCs can also be a novel cell source for regenerating hair in the treatment of scarring alopecia and androgenic alopecia. MSCs might also be an effective treatment for alopecia areata, which is associated with autoimmunity. Stem cell therapies with topical administration of MSCs and bone marrow transplantation were shown to alleviate recessive dystrophic epidermolysis bullosa in both animal models and human subjects. In addition to cell transplantation, the mobilization of endogenous MSCs has been attempted for skin regeneration. Overall, this review highlights the great potential of MSCs for the treatment of skin diseases in the near future.

Abbreviations: ESCs, embryonic stem cells; MSCs, mesenchymal stem cells; BM-MSCs, bone marrow-derived mesenchymal stem cells; UC-MSCs, umbilical cord tissue-derived mesenchymal stem cells; UCB-MSCs, umbilical cord blood-derived mesenchymal stem cells; P-MSCs, placenta-derived mesenchymal stem cells; AF-MSCs, amniotic fluid-derived mesenchymal stem cells; ADSCs, adipose tissue-derived stem cells; HLA, human leukocyte antigen; ATRA, all-trans retinoic acid; BMP4, bone-morphogenetic protein-4; KSFM, keratinocyte serum-free medium; Muse, multilineage-differentiating stress enduring; UV, ultraviolet; EB, epidermolysis bullosa; RDEB, recessive dystrophic epidermolysis bullosa; Col7, type VII collagen; JEB, junctional EB; HMGB1, high-mobility group box 1.

1. Introduction

Regenerative medicine is a novel approach for the treatments of various diseases. Stem cells are a unique population of undifferentiated cells that can self-renew and differentiate into various cell lineages. These cells are expected to play a central role in the treatment of skin diseases as well as diseases of other organs. Stem cells are classified into embryonic stem cells (ESCs), induced pluripotent stem cells, and adult stem cells. The use of adult stem cells circumvents the ethical issues associated with the use of ESCs, and their generation does not require the introduction of exogenous transcription factors using viral vectors or other *ex vivo* manipulations into cells. Therefore, adult stem cells are thought to be very useful in regenerative medicine.

Adult stem cells include hematopoietic stem cells, mesenchymal stem cells (MSCs), and endothelial stem cells, among others. MSCs can be isolated from different tissues such as the bone marrow (BM-MSCs), umbilical cord (UC-MSCs), placenta (P-MSCs), umbilical cord blood (UCB-MSCs), amniotic fluid (AF-MSCs), fetal tissues, skin (dermal MSCs), and adipose tissues (ADSCs) [1–5]. MSCs are multipotent stromal cells that can differentiate into various cell types such as adipocytes, osteoblasts, chondrocytes, fibroblasts, and myoblasts [6,7]. MSCs were first discovered from the bone marrow, which showed the potential to differentiate into bone cells [8,9]; since then, these BM-MSCs have been the major source of MSCs for clinical use. However, UC-MSCs, UCB-MSCs, P-MSCs, and AF-MSCs can be collected in relatively larger quantities using less invasive techniques [10]. Although dermal MSCs can be easily harvested, dermal stem cells only account for 0.3% of human dermal foreskin fibroblasts [2,11]. ADSCs can be easily harvested in large quantities, and produce 500-times more colony-forming units than BM-MSCs [12]. Therefore, ADSCs will be a practical and promising tool for regenerative medicine [13].

2. Biological Characteristics of MSCs

Development of stem cells for tissue engineering has greatly progressed in recent years. MSC-based therapy involving autologous and allogeneic MSCs isolated from related or unrelated

donors with various matching levels of human leukocyte antigen (HLA) is a promising therapy. MSCs are immune-evasive cells because they do not express MHC class II antigens and co-stimulatory molecules such as CD40, CD80, and CD86, and minimally express MHC class I antigens [14]. This lack of immunogenicity facilitates the clinical use of MSCs including for allogeneic cell transplantation [15].

In addition to their differentiation potential, MSCs secrete immunomodulatory, antiapoptotic, anti-inflammatory, proangiogenic, promitogenic, and antibacterial factors such as transforming growth factor β-1, hepatocyte growth factor, haemoxgenase-1, prostaglandin E2, interleukin-10, and HLA-G5 [16–19]. MSCs also decrease T-cell proliferation, inhibit cytotoxic T-cell production, and suppress the T-cell response to their cognate peptides. In addition, MSCs may activate or inhibit immunoglobulin G secretion by B-cells and enhance the proliferation and differentiation of plasma cells from memory B-cells [17]. MSCs also inhibit the differentiation of dendritic cells from CD34+ progenitor cells and monocytes, and decrease the secretion of proinflammatory cytokines [20]. Based on these facts, MSCs are thought to be effective for inflammatory and autoimmune disorders. Among the different MSCs available, ADSCs show low immunogenicity and high immunosuppressive potential [21–27].

MSCs were reported to promote wound healing in both animal model and human studies [28–35]. The clinical utility of MSCs in wound healing is based on repairing and replacing cellular substrates, attenuation of inflammation, increasing angiogenesis, and enhancing the migration of reparative cells by cytokine/chemokine production.

3. Differentiation of MSCs into Keratinocyte-like Cells

The ability of MSCs to differentiate into keratinocytes and melanocytes and to be bioengineered into hair follicles is important for their use in organ replacement therapy in dermatology. In particular, with respect to the differentiation potential of MSCs into keratinocytes, the cells responsible for wound re-epithelialization after wound formation are needed for the use of MSCs in skin regeneration. In addition, the differentiation of MSCs into ectoderm cells has been considered to be difficult. However, BM-MSCs were reported to have the ability to transdifferentiate into keratinocyte-like cells [36–39].

ADSCs can also differentiate into keratinocyte-like cells [40,41]. In a previous study, ADSCs were co-cultured with normal human dermal fibroblasts on type IV collagen in Dulbecco's modified Eagle's medium containing all-trans retinoic acid (ATRA) and bone morphogenetic protein 4 (BMP4). Next, the cells were co-cultured with fibroblasts in keratinocyte serum-free medium (KSFM) lacking ATRA and BMP4, followed by culturing in KSFM on type IV collagen in the absence of other cells. These differentiated cells showed high expression of the keratinocyte markers desmoglein 3 and cytokeratin 5 [40]. This fact suggested that ADSCs could possibly be used as keratinocyte progenitor cells in skin regeneration.

4. Heterogeneity of MSCs

MSCs are a heterogeneous population of cells that have variable expression of markers depending on the tissue source. The minimal criteria for defining human MSCs are: (1) plastic adherence when maintained in standard culture conditions; (2) expression of CD105, CD73, and CD90; lack of expression of CD45, CD34, CD14, or CD11b, CD79α, or CD19, and HLA-DR surface molecules; and (3) differentiation to osteoblasts, adipocytes, and chondroblasts *in vitro* [42].

Cell sorting, which is the method of separating cells according to their properties such as size, morphology, or surface protein expression, is thought to be useful to isolate putative stem cells. Multilineage-differentiating stress enduring (Muse) cells are a new type of pluripotent stem cells derived from MSCs [43]. Muse cells are isolated from human skin fibroblasts, ADSCs, and BM-MSCs, and can be identified by stage-specific embryonic antigen 3 as a marker. Muse cells were demonstrated to differentiate into functional melanocytes [44]. Cell sorting techniques such as fluorescent-activated cell sorting and magnetic cell sorting will be important to increase the efficacy of stem cell therapy.

5. MSCs for the Treatment of Skin Diseases

Numerous studies have already shown the beneficial uses of MSCs in both preclinical and clinical trials, which have highlighted the potential of MSCs for the treatments of several disorders, including hematological disease, multiple sclerosis, diabetes, osteoarthritis, chronic spinal cord injury, and feline chronic kidney disease [45,46]. In the dermatology field, MSCs may be applied in wound repair after a deep wound or burn. In addition, because of their immunomodulatory properties, MSCs have been used to successfully treat steroid-resistant acute graft-versus-host disease [26,47–51]. MSCs may be also of potential use for inflammatory skin diseases, including atopic dermatitis and psoriasis, and autoimmune skin diseases such as lupus erythematosus.

The human skin has two primary layers: the epidermis and dermis. The epidermis is an ectoderm-derived, keratinized, stratified layer with a unique cytokeratin expression pattern, while the dermis is a mesoderm-derived layer composed of connective tissue, nerves, vessels, and appendages. The epidermis acts as a barrier against infection and regulates the amount of water released from the body. In a healthy state, the epidermis is a rapidly regenerating tissue that is maintained by the continuous transformation from transient amplifying cells and epidermal stem cells located in the basal layer of the interfollicular epidermis (interfollicular epidermal stem cells), bulge region of hair follicles (hair follicle stem cells), and sebaceous glands (sebaceous gland stem cells). Epidermal stem cells differentiate into keratinocytes that are directed to the upper layers of the epidermis and are involved in wound re-epithelialization. Hair follicle stem cells, which have the functions of hair follicle remodeling and epidermal regeneration, show potential for the treatment of patients with alopecia [52,53] and for inducing repigmentation in the patients with vitiligo [54,55]. Sebaceous gland stem cells are able to differentiate into both sebocytes and the interfollicular epidermis [11].

Moreover, melanocytes are directly involved in pigmentation, and ultraviolet (UV) B exposure induces the differentiation of melanocyte stem cells into melanoblasts [56]. Melanoblasts migrate from the neural crest to the hair follicles where they undergo proliferation, differentiation, and maturation into melanocytes [57–59].

However, mesenchymal compartments contain a greater number of adult stem cells than the epidermis. MSCs can differentiate into multiple cell lineages, including keratinocytes. Thus, MSCs can be used for the treatment of congenital or acquired skin defects, including burns, chronic ulcers, or deep wounds, owing to not only their wound-healing properties but also their differentiation potential into keratinocytes.

Skin aging is induced by a decrease or functional disorder of stem cells [2]. Therefore, MSCs may induce anti-aging effects by promoting skin regeneration, maintaining skin homeostasis, and affecting the activity of various humoral factors. The anti-aging activity of MSCs should be determined before their clinical application for anti-aging therapy such as acne scars, wrinkles, and photoaging.

6. Vitiligo

Vitiligo vulgaris is characterized by the development of white patches on various parts of the body. Although vitiligo is not a contagious or life-threatening disease, it significantly reduces the quality of life of patients with widespread lesions. Vitiligo is clinically divided into two types: the generalized and segmental type [60,61]. Although the pathogenesis of vitiligo is unclear, autoimmunity against melanocytes and melanins, and impaired peripheral nerve function are suggested to play a role in vitiligo development. Therefore, UV therapy and topical steroid therapy are the first-line treatments for both types of vitiligo, based on their immunosuppression effect against autoimmunity. Intractable and stable vitiligo lesions can be treated by autologous epidermal transplantation, including suction blister grafting and mini-grafting [62–64].

Among MSCs, Muse cells were reported to have the ability to differentiate into melanocytes [44,65]. For the treatment of large vitiligo lesions, autologous MSCs might be useful as an immunosuppressant or in cultured autologous grafting, although no clinical reports of these applications have been reported to date.

7. Alopecia

Scarring alopecia develops as a result of injury or autoimmune skin diseases such as discoid lupus erythematosus of the scalp. Patients with scarring alopecia show destruction of the hair follicles indicating the need for excision and plasty or hair transplantation. Androgenic alopecia (male pattern alopecia) is currently treated using drugs and hair transplantation. However, the drug treatment exerts temporary effects, and the total transplantable hair number of the donor site is limited. To overcome these problems, *in vitro* culturing and implantation of hair follicles using autologous stem cells may be

useful for treating scarring and androgenic alopecia. Yoo et al. [66] showed that dermal papilla-like tissues formed using BM-MSCs and UC-MSCs induced the growth of new hair follicles in mice. Thus, autologous MSCs are expected to be a novel cell source for regenerating hair in the treatment of scarring alopecia and androgenic alopecia.

Conversely, alopecia areata is characterized by round or oval patches of non-scarring hair loss across the entire body. In severe cases, the hair loss may progress to complete scalp hair loss. Although the pathogenesis of alopecia areata is unclear, autoimmunity is thought to be involved in its development [67–69]. Therefore, alopecia areata is treated with topical steroids, steroid injections, UV therapies, and immunosuppressants. Because some cases are intractable, the immunomodulatory and anti-inflammatory effects of autologous MSCs could be considered as one of the options for the treatment of alopecia areata. In animal models, alopecia areata-induced mice were successfully treated with MSCs [70]. However, no successful strategies for treating scarring alopecia, androgenic alopecia, and alopecia areata using MSCs have been reported in human studies.

8. Epidermolysis Bullosa

Epidermolysis bullosa (EB) is a group of genetic skin fragility disorders characterized by blisters and skin erosions in response to minor injury. Recessive dystrophic EB (RDEB) is one of the more severe forms of EB. The pathogenesis of RDEB involves mutations in *COL7A1* that encodes type VII collagen (Col7), the main constituent of the anchoring fibrils that anchor the epidermal basement membrane to the papillary dermis [71], resulting in blistering and repeated wounding of the skin, oral mucosa, and gastrointestinal tract components [72,73]. In these cases, persistent skin erosion frequently results in intractable ulcers. As the ulcers heal, they result in severe scarring, fusion of the fingers and toes, loss of nails, and contractures of the finger joints.

Various biological dressings, including autologous and allogeneic cultured skin substitutes, have been used for intractable ulcers in patients with RDEB [74–80]. However, allogeneic cultured skin substitutes cannot be permanently adopted. Autologous cultured skin can serve as a permanent covering, but their use does not alleviate the effects of *COL7A1* mutations. Thus, both autologous and allogeneic cultured skin substitutes are only used only as temporary biological dressings.

Stem cell therapy involving autologous and allogeneic MSCs may be used for EB, including RDEB. Previous studies have shown that stem cell therapies using adult stem cells, including topical administration of MSCs and bone marrow transplantation, could alleviate RDEB in both animal models and human subjects [81–88]. To determine the utility of MSCs from a healthy donor in an allogeneic transplantation for repairing basement membrane alterations in patients with RDEB, it is important to examine Col7 expression in these cells because patients with RDEB have mutations in Col7. Thus, allogeneic MSCs may enhance wound healing, correct Col7 insufficiency, repair defective/reduced anchoring fibrils, and improve skin integrity in patients with RDEB. However, it is necessary to consider the presence of anti-Col7 antibodies, which are relatively common in patients with RDEB [89].

Junctional EB (JEB) is another major form of EB, and severe generalized JEB is the most severe type of EB that leads to the early death of patients, usually within the first few months of life [72,90,91]. Severe generalized JEB is caused by mutations in the laminin-332-encording genes *LAMA3*, *LAMB3*, and *LAMC2*. Autologous epidermal stem cells were reported to be useful in gene therapy for the treatment of JEB [92]. Epidermal stem cells from a patient affected by *LAMB3*-deficient JEB were transfected with a retroviral vector expressing *LAMB3* cDNA (encoding *LAM5-β3*), and were successfully used to prepare genetically corrected cultured epidermal grafts. Autologous MSCs might be used similarly in gene therapy for the treatment of various types of EB.

In addition to the transplantation of autologous or allogeneic MSCs, mobilization of endogenous MSCs from the bone marrow to the affected site has been attempted for skin regeneration. High-mobility group box 1 (HMGB1) is a non-histone nuclear protein that regulates chromatin structure remodeling [93]. In injured tissues, HMGB1 is secreted by macrophages and dendritic cells and is released from necrotic cells to induce tissue remodeling and regeneration by activating inflammatory reactions and angiogenesis [94–100]. HMGB1 is also a strong chemoattractant for mesoangioblasts and endothelial precursor cells [101,102]. Intravenously administered recombinant HMGB1 was shown to mobilize endogenous platelet-derived growth factor receptor α-positive mesenchymal cells, which are epithelial progenitors, from the bone marrow in mice [103]. HMGB1 could be used to stimulate endogenous mesenchymal cells to the site epithelial injury such as in RDEB patients. This method is more convenient than bone marrow transplantation.

9. Delivery Route

Systemic delivery routes such as intravenous injection or organ-specific local delivery methods should be examined for MSC administration. Systemic administration would be preferable over topical administration when MSCs are used for achieving anti-inflammation and immunosuppression effects. Although intravenous delivery is a simple route of administration, this method can possibly cause pulmonary embolism, which has not been recorded in humans. Since dermal substitutes are an appropriate environment for MSCs [104], local injection and the use of suitable scaffolds, including commercial dermal substitutes, in combination with MSCs may also be used for skin diseases, especially for the purpose of organ replacement. It is also necessary to establish an optimal delivery dose for MSC administration, together with additional preclinical and clinical studies. Because cells can be directly administered to the skin, the skin is suitable for practical application in regenerative medicine as well.

10. Conclusion

This review describes the potential use of MSCs in cell-based therapies for the clinical management of various dermatoses. The work conducted thus far shows the potential of MSCs for

treating skin diseases owing to their immunomodulatory effect and transdifferentiation capacities. MSCs have proven to be useful in skin regeneration. Among the different types of MSCs, the advantages of using ADSCs include their abundance in donors and their convenient isolation by less invasive methods such as liposuction. Although the optimal method for administering MSCs is still debatable, MSCs show the possibility to be widely used for treating skin diseases in the near future.

Conflicts of Interest

There is no conflict of interest regarding this paper.

References

1. Charbord P (2010) Bone marrow mesenchymal stem cells: historical overview and concepts. *Hum Gene Ther* 21: 1045-1056.
2. Sellheyer K, Krahl J (2010) Skin mesenchymal stem cells: prospects for clinical dermatology. *J Am Acad Dermatol* 63: 859-865.
3. Shi C, Zhu Y, Su Y, et al. (2006) Stem cells and their applications in skin-cell therapy. *Trends Biotechnol* 24: 48-52.
4. Sousa BR, Parreira RC, Fonseca EA, et al. (2014) Human adult stem cells from deverse origins: An overview from multiparametric immunophenotyping to clinical applications. *Cytometry A* 85: 43-77.
5. Ullah I, Subbarao RB, Rho GJ (2015) Human mesenchymal stem cells – current trends and future prospective. *Biosci Rep* 35: e00191.
6. Ding DC, Shyu WC, Lin SZ (2011) Mesenchymal stem cells. *Cell Transplant* 20: 5-14.
7. Chamberlain G, Fox J, Ashton B, et al. (2007) Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem cells* 25: 2739-2749.
8. Caplan AI (1991) Mesenchymal stem cells. *J Orthop Res* 9: 641-650.
9. Pittenger MF, Mackay AM, Beck SC, et al. (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284: 143-147.
10. Wegmeyer H, Bröske A-M, Leddin M, et al. (2013) Mesenchymal stromal cell characteristics vary depending on their origin. *Stem Cells Dev* 22: 2606-2618.
11. Zouboulis CC, Adjaye J, Akamatsu H, et al. (2008) Human skin stem cells and the aging process. *Experimental Gerontol* 43: 986-997.
12. Fraser JK, Wulur I, Alfonso Z, et al. (2006) Fat tissue: An underappreciated source of stem cells for biotechnology. *Trends Biotechnol* 24: 150-154.
13. Zuk PA, Zhu M, Mizuno H, et al. (2001) Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng* 7: 211-228.

14. Dominici M, Le Blanc K, Mueller I, et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cryotherapy* 8: 315-317.

15. Barry FP, Murphy JM, English K, et al. (2005) Immunogenicity of adult mesenchymal stem cells: lessons from the fetal allograft. *Stem Cells Dev* 14: 252-265.

16. Caplan AI, Correa D (2011) The MSC: An injury drugstore. *Cell Stem Cell* 9: 11-15.

17. Chen L, Tredget EE, Wu PY, et al. (2008) Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS ONE* 3: e1886.

18. Wang J, Liao L, Tan J (2011) Mesenchymal-stem-cell-based experimental and clinical trials: current status and open questions. *Expert Opin. Biol Ther* 11: 893-909.

19. Hoogduijn MJ (2015) Are mesenchymal stromal cells immune cells? *Arthritis Res Ther* 17: 88.

20. Nauta AJ, Kruisselbrink AB, Lurvink E, et al. (2006) Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells. *J Immunol* 177: 2080-2087.

21. Bartholomew A, Sturgeon C, Siatskas M, et al. (2002) Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol* 30: 42-48.

22. McIntosh K, Zvonic S, Garrett S, et al. (2006) The immunogenicity of human adipose-derived cells: temporal changes in vitro. *Stem Cells* 24: 1246-1253.

23. Lombardo E, DelaRosa O, Mancheño-Corvo P, et al. (2009) Toll-like receptor-mediated signaling in human adipose-derived stem cells: implications for immunogenicity and immunosuppressive potential. *Tissue Eng Part A* 15: 1579-1589.

24. Puissant B, Barreau C, Bourin P, et al. (2005) Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells. *Br J Haematol* 129: 118-129.

25. Gonzalez MA, Gonzalez-Rey E, Rico L, et al. (2009) Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. *Arthritis Rheum* 60: 1006-1019.

26. Fang B, Song Y, Liao L, et al. (2007) Favorable response to human adipose tissue-derived mesenchymal stem cells in steroid-refractory acute graft-versus-host disease. *Transplant Proc* 39: 3358-3362.

27. Cho KS, Roh HJ (2010) Immunomodulatory effects of adipose-derived stem cells in airway allergic diseases. *Curr Stem Cell Res Ther* 5: 111-115.

28. Badillo AT, Redden RA, Zhang L, et al. (2007) Treatment of diabetic wounds with fetal murine mesenchymal stromal cells enhances wound closure. *Cell Tissue Res* 329: 301-311.

29. Li H, Fu X, Ouyang Y, et al. (2006) Adult bone-marrow-derived mesenchymal stem cells contribute to wound healing of skin appendages. *Cell Tissue Res* 326: 725-736.

30. Ma K, Liao S, He L, et al. (2011) Effects of nanofiber/stem cell composite on wound healing in acute full-thickness skin wounds. *Tissue Eng Part A* 17: 1413-1424.

31. Schneider RK, Pullen A, Kramann R, et al. (2010) Long-term survival and characterisation of human umbilical cord-derived mesenchymal stem cells on dermal equivalents. *Differentiation* 79: 182-193.

32. Wu Y, Chen L, Scott PG, et al. (2007) Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells* 25: 2648-2659.

33. Kwon DS, Gao X, Liu YB, et al. (2008) Treatment with bone-marrow derived stromal cells accelerates wound healing in diabetic rats. *Int Wound J* 5: 453-463.

34. Yoshikawa T, Mitsuno H, Nonaka I, et al. (2008) Wound therapy by marrow mesenchymal cell transplantation. *Plast Reconstr Surg* 121: 860-877.

35. Falanga V, Iwamoto S, ChartierM, et al. (2007) Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. *Tissue Eng* 13: 1299-1312.

36. Borue X, Lee S, Grove J, et al. (2004) Bone marrow-derived cells contribute to epithelial engraftment during wound healing. *Am J Pathol* 165: 1767-1772.

37. Sasaki M, Abe R, Fujita Y, et al. (2008) Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. *J Immunol* 180: 2581-2587.

38. Shokrgozar MA, Fattahi M, Bonakdar S, et al. (2012) Healing potential of mesenchymal stem cells cultured on a collagen-based scaffold for skin regeneration. *Iran Biomed J* 16: 68-76.

39. Pằnescu V, Deak E, Herman D, et al. (2007) In vitro differentiation of human mesenchymal stem cells to epithelial lineage. *J Cell Mol Med* 11: 502-508.

40. Hasegawa T, Sakamoto A, Wada A, et al. (2015) Keratinocyte progenitor cells reside in human subcutaneous adipose tissue. *PLoS One* 10: e0118402.

41. Chavez-Munoz C, Nguyen KT, Xu W, et al. (2013) Transdifferentiation of adipose-derived stem cells into keratinocyte-like cells: Engineering a stratified epidermis. *PLoS One* 8: e80587.

42. Dominici M, Le Blanc K, Mueller I, et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8: 315-317.

43. Kuroda Y, Kitada M, Wakao S, et al. (2010) Unique multipotent cells in adult human mesenchymal stem cell populations. *Proc Natl Acad Sci USA* 107: 8639-8643.

44. Tsuchiyama K, Wakao S, Kuroda Y, et al. (2013) Functional melanocytes are readily reprogrammable from multilineage-differentiating stress-enduring (muse) cells, distinct stem cells in human fibroblasts. *J Invest Dermatol* 133: 2425-2435.

45. Squillaro T, Peluso G, Galderisi U (2016) Clinical Trials With Mesenchymal Stem Cells: an update. *Cell Transplant* 25: 829-848.

46. Oraee-Yazdani S, Hafizi M, Atashi A, et al (2016) Co-transplantation of autologous bone marrow mesenchymal stem cells and Schwann cells through cerebral spinal fluid for the treatment of patients with chronic spinal cord injury: safety and possible outcome. *Spinal Cord* 54: 102-109.

47. Le Blanc K, Rasmusson I, Sundberg B, et al. (2004) Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymalstem cells. *Lancet* 363: 1439-1441.

48. Ball LM, Bernardo ME, Roelofs H, et al. (2013) Multiple infusions of mesenchymal stromal cells induce sustained remission in children with steroid-refractory, grade III-IV acute graftversus- host disease. *Br J Haematol* 163: 501-509.

49. Kurtzberg J, Prockop S, Teira P, et al. (2014) Allogeneic human mesenchymal stem cell therapy (remestemcel-L, Prochymal) as a rescue agent for severe refractory acute graft-versus-host disease in pediatric patients. *Biol Blood Marrow Transplant* 20: 229-235.

50. Introna M, Lucchini G, Dander E, et al. (2014) Treatment of graft versus host disease with mesenchymal stromal cells: A phase I study on 40 adult and pediatric patients. *Biol Blood Marrow Transplant* 20: 375-381.

51. Ringdén O, Erkers T, Nava S, et al. (2013) Fetal membrane cells for treatment of steroid-refractory acute graft-versus-host disease. *Stem Cells* 31: 592-601.

52. Cotsarelis G (2006) Epithelial stem cells: a folliculocentric view. *J Invest Dermatol* 126: 1459-1468.

53. Ohyama M, Kobayashi T (2012) Isolation and characterization of stem cell-enriched human and canine hair follicle keratinocytes. *Methods Mol Biol* 879: 389-401.

54. Staricco RG, Miller-Milinska A (1962) Activation of the amelanotic melanocytes in the outer root sheath of the hair follicle following ultra violet rays exposure. *J Invest Dermatol* 39: 163-164.

55. Quevedo WC Jr, Isherwoob JE (1961) Influence of hair growth cycle on melanocyte activation in rabbit skin after a single application of methylcholanthrene. *J Invest Dermatol* 27: 93-101.

56. Yamada T, Hasegawa S, Inoue Y, et al. (2013) Wnt/β-catenin and kit signaling sequentially regulate melanocyte stem cell differentiation in UVB-induced epidermal pigmentation. *J Invest Dermatol* 133: 2753-2762.

57. Cichorek M,Wachulska M, Stasiewicz A, et al. (2013) Skin melanocytes: biology and development. *Postepy Dermatol Allergol* 30: 30-41.

58. Watt FM, Jensen KB (2009) Epidermal stem cell diversity and quiescence. *EMBO Mol Med* 1: 260-267.

59. Sieber-Blum M, Grim M, Hu YF, et al. (2004) Pluripotent neural crest stem cells in the adult hair follicle. *Dev Dyn* 231: 258-269.

60. Koga M, Tango T (1988) Clinical features and course of type A and type B vitiligo. *Br J Dermatol* 118: 223-228.

61. Njoo MD, Westerhof W, Bos JD, et al. (1999) The development of guidelines for the treatment of vitiligo. Clinical Epidemiology Unit of the Istituto Dermopatico dell'Immacolata-Istituto di Recovero e Cura a Carattere Scientifico (IDI-IRCCS) and the Archives of Dermatology. *Arch Dermatol* 135: 1514-1521.

62. Spencer GA (1951) Skin transplantation in extensive vitiligo. *AMA Arch Derm Syphilol* 64: 514-515.

63. Hasegawa T, Suga Y, Ikejima A, et al. (2007) Suction blister grafting with CO2 laser resurfacing of the graft recipient site for vitiligo. *J Dermatol* 34: 490-492.

64. Falabella R (1983) Repigmentation of segmental vitiligo by autologous minigrafting. *J Am Acad Dermatol* 9: 514-521.

65. Yamauchi T, Yamasaki K, Tsuchiyama K, et al. (2017) A quantitative analysis of multilineaage-differentiating stress-enduring (Muse) cells in human adipose tissue and efficacy of melanocytes induction. *J Dermatol Sci* 86: 198-205.

66. Yoo BY, Shin YH, Yoon HH, et al. (2010) Application of mesenchymal stem cells derived from bone marrow and umbilical cord in human hair multiplication. *J Dermatol Sci* 60:74-83.

67. McElwee KJ, Hoffmann R (2002) Alopecia areata-animal models. *Clin Exp Dermatol* 27: 410-417.

68. Randall VA (2001) Is alopecia areata an autoimmune disease? *Lancet* 358: 1922-1924.

69. Gilhar A, Landau M, Assy B, et al. (2001) Melanocyte-associated T cell epitopes can function as autoantigens for transfer of alopecia areata to human scalp explants on Prkdc(scid) mice. *J Invest Dermatol* 117: 1357-1362.

70. Byun JW, Kin HJ, Na K, et al. (2017) Bone marrow-derived mesenchymal stem cells prevent alopecia areata development through the inhibition of NKG2D expression: A pilot study. *Exp Dermatol* 25: 532-535.

71. Hilal L, Rochat A, Duquesnoy P, et al. (1993) A homozygous insertion-deletion in the type VII collagen gene (COL7A1) in Hallopeau-Siemens dystrophic epidermolysis bullosa. *Nat Genet* 5: 287-293.

72. Fine JD, Eady RA, Bauer EA, et al. (2008) The classification of inherited epidermolysis bullosa (EB): Report of the Third International Consensus Meeting on Diagnosis and Classification of EB. *J Am Acad Dermatol* 58: 931-950.

73. Freeman EB, Köglmeier J, Martinez AE, et al. (2008) Gastrointestinal complications of epidermolysis bullosa in children. *Br J Dermatol* 158: 1308-1314.

74. McGrath JA, Schofield OM, Ishida-Yamamoto A, et al. (1993) Cultured keratinocyte allografts and wound healing in severe recessive dystrophic epidermolysis bullosa. *J Am Acad Dermatol* 29: 407-419.

75. Roseeuw D, De Raeve L, Dangoisse C, et al. (1994) Treatment of epidermolysis bullosa with human cultured epidermal allografts. *Dermatology* 189: 68-70.

76. Beele H, Naeyaert JM, Monstrey S, et al. (1995) Ulcers in pretibial epidermolysis bullosa. Grafting with autologous meshed split-thickness skin and allogeneic cultured keratinocytes. *Arch Dermatol* 131: 990-992.

77. Verplancke P, Beele H, Monstrey S, et al. (1997) Treatment of dystrophic epidermolysis bullosa with autologous meshed split-thickness skin grafts and allogeneic cultured keratinocytes. *Dermatology* 194: 380-382.

78. Eisenberg M, Llewelyn D (1998) Surgical management of hands in children with recessive

dystrophic epidermolysis bullosa: use of allogeneic composite cultured skin grafts. *Br J Plast Surg* 51: 608-613.

79. Hasegawa T, Suga Y, Mizoguchi M, et al. (2004) Clinical trial of allogeneic cultured dermal substitute for the treatment of intractable skin ulcers in 3 patients with recessive dystrophic epidermolysis bullosa. *J Am Acad Dermatol* 50: 803-804.

80. Hasegawa T, Mizoguchi M, Haruna K, et al. (2007) Amnia for intractable skin ulcers with recessive dystrophic epidermolysis bullosa: Report of three cases. *J Dermatol* 34: 328-332.

81. Kiuru M, Itoh M, Cario MS, et al. (2010) Bone marrow stem cell therapy for recessive dystrophic epidermolysis bullosa. *Dermatol Clin* 28: 371-382.

82. Wagner JE, Ishida-Yamamoto A, McGrath JA, et al. (2010) Bone marrow transplantation for recessive dystrophic epidermolysis bullosa. *N Eng J Med* 363: 629-639.

83. Chino T, Tamai K, Yamazaki T, et al. (2008) Bone marrow cell transfer into fetal circulation can ameliorate genetic skin diseases by providing fibroblasts to the skin and inducing immune tolerance. *Am J Pathol* 173: 803-814.

84. Conget P, Rodriguez F, Kramer S, et al. (2010) Replenishment of type VII collagen and re-epithelialization of chronically ulcerated skin after intradermal administration of allogeneic mesenchymal stromal cells in two patients with recessive dystrophic epidermolysis bullosa. *Cryotherapy* 12: 429-431.

85. Liao Y, Itoh M, Yang A, et al. (2014) Human cord blood-derived unrestricted somatic stem cells promote wound healing and have therapeutic potential for patients with recessive dystrophic epidermolysis bullosa. *Cell Transplant* 23: 303-317.

86. Liao Y, Ivanova L, Zhu H, et al. (2015) Rescue of the mucocutaneous manifestations by human cord blood derived nonhematopoietic stem cells in a mouse model of recessive dystrophic epidermolysis bullosa. *Stem Cells* 33: 1807-1817.

87. Petrof G, Lwin SM, Martinez-Queipo M, et al. (2015) Potential of systemic allogeneic mesenchymal stromal cell therapy for children with recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 135: 2319-2321.

88. Iinuma S, Aikawa E, Tamaki K, et al. (2015) Transplanted bone marrow-derived circulating PDGFRα+ cells restore type VII collagen in recessive dystrophic epidermolysis bullosa mouse skin graft. *J Immunol* 194: 1996-2003.

89. Woodley DT, Cogan J, Wang X, et al. (2014) De novo anti-type VII collagen antibodies in patients with recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 134: 1138-1140.

90. Bauer J, Schumann H, Sonnichsen K (2002) Molecular diagnostics facilitate distinction between lethal and non-lethal subtypes of junctional epidermolysis bullosa: a case report and review of the literature. *Eur J Pediatr* 161: 672-679.

91. Yuen WY, Duipmans JC, Molenbuur B (2012) Long-term follow-up of patients with Herlitz-type junctional epidermolysis bullosa. *Br J Dermatol* 167: 374-382

92. Mavilio F, Pellegrini G, Ferrari S, et al. (2006) Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermolysis bullosa by transplantaation of genetically modified epidermal stem cells. *Nat Med* 12: 1397-1342.

93. Osmanov T, Ugrinova I, Pasheva E (2013) The chaperone like function of the nonhistone protein HMGB1. *Biochem Biophys Res Commun* 432: 231-235.

94. Tang D, Shi Y, Kang R, et al. (2007) Hydrogen peroxide stimulates macrophages and monocytes to actively release HMGB1. *J Leukoc Biol* 81: 741-747.

95. Dumitriu IE, Baruah P, Valentinis B, et al. (2005) Release of high mobility group box 1 by dendritic cells controls T cell activation via the receptor for advanced glycation end products. *J Immunol* 174: 7506-7515.

96. Scaffidi P, Misteli T, Bianchi ME (2002) Release of chromatin protein HMGB1 by necrotic cell triggers inflammation. *Nature* 418: 191-195.

97. Park JS, Gamboni-Robertson F, He Q, et al. (2006) High mobility group box 1 protein interacts with multiple Toll-like receptors. *Am J Physiol Cell Physiol* 290: C917-924.

98. Yanai H, Ban T, Taniguchi T (2012) High-mobility group box family of proteins: ligand and sensor for innate immunity. *Trends Immunol* 33: 633-640.

99. Takahashi K, Fukushima S, Yamahara K, et al. (2008) Modulated inflammation by injection of high-mobility group box 1 recovers post-infarction chronically failing heart. *Circulation* 118: S106-114.

100. Straino S, Di Cario A, Mangoni A, et al. (2008) High-mobility group box 1 protein in human and murine skin: involvement in wound healing. *J Invest Dermatol* 128: 1545-1553.

101. Palumbo R et al. (2004) Extracellular HMGB1, a signal of tissue damage, induces mesoangioblast migration and proliferation. *J Cell Biol* 164: 441-449.

102. Clavakis E, Hain A, Vinci M, et al. (2007) High-mobility group box 1 activates integrin-dependent homing of endothelial progenitor cells. *Circ Res* 100: 204-212.

103. Tamai K, Yamazaki T, Chino T, et al. (2011) PDGFRα-positive cells in bone marrow are mobilized by high mobility group box 1 (HMGB1) to regenerate injured epithelia. *Proc Natl Acad Sci USA* 108: 6609-6614.

104. Jeremias TS, Machado RG, Visoni SB, et al. (2014) Dermal substitutes support the growth of human skin-derived mesenchymal stromal cells. *PLoS One* 9: e89542.