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Cell Therapy: The New Approach to Dermatology and Dermatologic Surgery

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1. Abstract

Cell therapy is a viable alternative for the treatment of disease and tissue degeneration that is very effective, reliable and incurs minimal adverse effects on the body. Its successful application extends to all fields of medicine, including dermatology. There are numerous types of stem cells and their extracts based on the procurement, catered to the holistic treatment of an individual. The properties of stems cells like pluripotency and self-renewing make them the ideal remedy for all types of ailments. The application for cell therapy in dermatology is diverse as it can be efficiently utilized from chronic wound therapy to autoimmune blistering diseases to malignancy. This field is evolving rapidly with newer therapies and treatment avenues for more diseases compared to the many pharmacological therapies available.

2. Introduction

The field of cell therapy has been enjoying a period of considerable activity and progress in the last decade [1]. Stem cell therapy has emerged as a viable alternative for the treatment of complex pathologies and tissue regeneration [5]. The challenge has moved on to harness the potential of stem cells for therapeutic uses and more research [1].

Stem cells are undifferentiated cells capable of generating, sustaining, and replacing terminally differentiated cells and tissues [6]. The functionality of stem cells is their wonder, the ability to produce differentiated cells continuously while maintaining their identity and pluripotency through the support of cellular processes [1]. There are two broad categories of natural stem cells: stem cells capable of differentiating into all specialized tissues and stem cells that are important in tissue regeneration [8].

Pluripotent stem cells (PSCs) are undifferentiated cells that have the potential for proliferation, self-renewal, and differentiation into ectodermal, mesodermal, and endodermal cells of all three embryonic germ layers in vitro and vivo83. Embryonic stem cells (ESCs) are natural pluripotent self-renewing cells derived from the preimplantation blastocyst's inner cell mass that differentiate into specialized cell types in response to developmental cues [83]. Mesenchymal Stem Cells (MSCs) are non-hematopoietic progenitor cells of mesodermal and neuroectodermal derivation that can be directed to differentiate in vitro into multiple lineages to aid tissue repair and regeneration [84].

Another form of stem cell is the artificially generated Induced Pluripotent Stem Cells (iPSCs). IPSCs are in vitro manipulated somatic cells that are genetically reprogrammed to revert to a state of pluripotency (immature, undifferentiated cells) [6]. This reprogramming process reverts differentiated adult cells to the undifferentiated stage of (ESCs) [23]. IPSCs potentially combine the advantages of MSCs and ESCs, ushering in a new era of regenerative medicine [23]. IPSCs have provided a unique opportunity to

develop customized, patient-specific cells with a broad spectrum of cellular phenotypes for potential therapeutic applications [1, 6]. The direct reprogramming of somatic cells into iPSCs by the transcription factors (Oct4, Sox2, Klf4, c-Myc) has excellent potential for tissue-specific regenerative therapies, eliminating the ethical issues surrounding the use of ESCs and the rejection problems of using non-autologous cells [10]. The development of iPSC technology can circumvent cell availability limitations [58]. It enables to obtain of cells from limited sources, such as brain cells and uses a limited number of somatic cells to generate a large number of cells with unlimited growth potential [58].

The skin is an organ that can benefit immensely from cell therapy. The application of cell therapy in dermatology has been going on for the last 30 years. One of the main emphasis of cell therapy in dermatology is new and emerging strategies that can exploit and harness human cells' regenerative potential to restore skin tissue [2]. Cell therapy to repair or restore a defective epithelium and possibly deeper skin layers represents an attractive translational research area that could have significant health benefits for many people [2]. This can address a wide spectrum of skin diseases from wound healing, epidermolysis bullosa, and the reversal of skin aging. Another area where cell therapy is making a breakthrough is in addressing chronic inflammatory skin conditions [2]. Cell therapy to regulate cytokines and chemokines may benefit skin conditions like psoriasis and atopic dermatitis [2]. The cascade of inflammation wreaks havoc on the skin, resulting in a significant impact on life quality [2].

Cellular regeneration is a physiological process that has always been going on in the skin. The renewal and timely repair of the human epidermis are sustained by epidermal stem cells (EpSCs), which generate colonies known as holoclones [36]. Holoclones produce meroclone and paraclone-forming cells, which behave as transient amplifying (TA) progenitors, biological intermediates that orchestrate stem cell activity [36, 52]. Both stem cells and TA progenitors are instrumental for proper tissue regeneration in human skin [36].

3. Features of Stem Cells

Stem cells possess characteristics like pluripotency and self-renewing properties [83]. These cells can secrete immunomodulatory cytokines to promote and coordinate angiogenesis, cell proliferation, and tissue regeneration [5]. Generally, stem cells resemble other mature cells but have no unique morphologic feature [5]. They display a high nuclear to cytoplasmic ratio and usually grow large cell- colonies when cultured, reflecting their long-term self-renewal and regenerative potential [6]. Most stem cells show a characteristic behavior in cultures, where they form tightly compact cell colonies that can be repeatedly passaged and transplanted [6]. Stem cells are delineated by the use of specific markers [6]. Their expression profile varies depending on the cell type, their state of activity, and anatomical location [6]. The behavior of stem cells varies according to their task, be in response to activating, amplifying, or inhibiting signals arising from local, environmental, and systemic factors [6].

There are numerous types of stem cells based on their origin. Amongst all the various stem cells, the mesenchymal stem cells (MSCs) have been the most popular simply because they are free of teratoma formation risk and are negligible ethical concerns. MSCs harvested from various sites (bone marrow, adipose tissue, amniotic fluid, and dermis) are considered to be a source for therapeutic approaches owing to their multilineage differentiation, high frequency, the facility of isolation and characterization, and homing ability of MSCs to migrate to injury sites in the body [54]. MSCs are known to secrete a spectrum of cytokines, chemokines, hormones, growth factors, microvesicles, and exosomes that participate in tissue repair and regeneration, primarily via paracrine actions that mediate cell-to-cell signaling [47]. MSCs are stromal cells, and the current definition of MSCs based on the International Society of Cellular Therapy (ICT) includes plastic adherence in cell culture, specific surface antigen expression of the surface markers CD73, CD90, and CD105, by their lack of expression of hematopoietic markers CD14, CD34, CD45, CD11b/CD79, and CD19/HLA-DR, and by their ability to differentiate along osteoblast, adipocyte, and chondrocyte pathways [21, 23]. MSCs secrete immunomodulatory, anti-apoptotic, anti-inflammatory, proangiogenic, promitogenic, and antibacterial factors such as transforming growth factor β-1(TGFβ-1), hepatocyte growth factor (HGF), haemoxygenase-1, prostaglandin E2 (PGE2), interleukin-1(IL-1), and human leukocyte antigen-G5 (HLA-G5) [37]. MSCs also decrease T-cell proliferation, inhibit cytotoxic T-cell production, and suppress the T-cell response to their cognate peptides. Besides, MSCs may activate or inhibit immunoglobulin G secretion by B-cells [37].

Epidermal stem cells (EpSCs) are easily accessible and relatively simple to isolate from skin tissues. The majority of EpSCs resided in the epidermis' basal layer, where they can derive into transient amplifying cells and terminal-differentiated epidermal cells [53]. Immune rejection following autologous transplantation is rare in epidermal stem cells. These cells' tumorigenicity is considered low due to their lesser degree of potency and absence of epigenetic manipulations. They have a high proliferation rate with the ability to double their number within 3-4 days of culture [6]. Adipose stem cells (ADSCs) are plentiful and relatively easily accessed and do not pose any ethical issues [54]. ADSCs are useful for soft tissue repair. Besides the adipose-derived stem cells, adipose tissue also contains endothelial progenitor cells and pericytes [21]. ASCs can promote wound healing and trigger neovascularization through their ability to differentiate endothelial cells and release vascular endothelial growth factor (VEGF) [54].

Endothelial stem cells can be sourced from several tissues, including bone marrow, umbilical cord blood, and adipose tissue. They are effective in stimulating angiogenesis, tissue revascularization, and the remodeling of soft tissue structures [21].

Many diseases, as well as side effects of drugs, manifest themselves through skin symptoms. Skin is a complex tissue that hosts various specialized cell types and performs many roles, including a physical barrier, immune and sensory functions [58]. The skin's continuous exposure to mechanical and chemical stress in the environment makes it require self-renewal of the epidermis, dermis, and adnexa to maintain its diverse functions. This self-renew ability is contributed by the skin's stem cells, which are slow-cycling multipotent cells located in the epidermis, dermis, and hair follicles [6]. However, only specific skin cells called holoclone-forming cells possess full self-renewing capabilities and long-term regenerative potential, harboring the features of EpSCs. Stem cells in the epidermis ensure epidermal homeostasis and hair regeneration maintenance and contribute to repairing the epidermis after injury [8]. Skin cells as a stem cell source are abundant, easy to access, have a high self-renewal ability, and can be used for autologous transplantation for practical therapeutic treatment [9]. Guenou et al. differentiated ESCs into fully functional keratinocytes, subsequently used for reconstitution of the epidermis [23]. Cultured human keratinocytes are the most widely used cell product in the world [53].

4. Stem Cells in Chronic Wounds

The skin is the largest organ in the body, accounting for about 15% of the human body by mass. It is the body's first line of immune and physical defense. A wound is described as an injury and any disorder in the skin's standard structure, which can cause loss of conjunction in the body tissue [54]. Cutaneous wounds can be broadly classed into two categories; acute, which heal uneventfully, and chronic, which do not heal for between 4 weeks to 3 months [10]. An aging population and increasing rates of obesity and diabetes are the main factors associated with poor wound healing [22]. Wound healing is a complex therapeutic process whereby the skin attempts to repair itself after injury [5, 6]. Wound healing is traditionally divided into 4 phases (refer to TABLE 1). The ultimate aim of wound healing is to accelerate skin repair by creating a favorable environment for cell proliferation and differentiation that mimicks the physicochemical and mechanical properties of the skin [26].

Table 1: The 4 phases of wound healing [4]

PHASE	PERIOD	MAJOR CELLS INVOLVED	MAIN ACTIVITY
HEMOSTASIS	Immediate	Platelets	Blood Clotting
			Release of cytokines & Growth factors
INFLAMMATION	Day 1–4	Neutrophils	Phagocytosis
		Macrophages	Release of cytokines & Growth factors
PROLIFERATION	Day 4–21	Macrophages	Repair and restore skin function
		Lymphocytes	
		Fibroblasts	
		Keratinocytes	
REMODELLING	Day-21 onwards	Fibroblasts	Tensile strength

The main clinical focus of stem cell application in wound care is to target improved quality of wound healing [54]. Stem cells improve healing in chronic wounds primarily through the alteration of the microenvironment [10]. Stem cells reduce inflammation through a myriad of different effects. They may directly secrete interleukin-10 (IL-10) to modulate an inflammatory response [10]. They also inhibit local cell signaling to decrease inflammatory cytokine secretion from leukocytes. Stem cell treatment doesn't merely decrease inflammatory signaling; pathogen clearance is enhanced, reducing mortality in a sepsis model. Stem cells have been shown to produce proangiogenic and anti-inflammatory cytokines, especially IL-10, TGFβ, and vascular endothelial growth factor (VEGF). They also reduce the concentration of pro-inflammatory cytokines, mainly IL-1β, tumor necrosis factor α (TNF-α), and interleukin-6 (IL-6) [10]. Resolution of inflammation allows the proliferation of fibroblasts and blood vessels, eventually resulting in wound healing [10].

Every phase in wound healing is mediated by stem cell proliferation and signaling. Impaired stem cell functioning, therefore, leads to chronic wounds. As stem cells directly interact with the wound environment in a complex and multifactorial manner, clinical approaches that utilize them could theoretically be very beneficial. At the inflammatory phase, which is characterized by the migration of neutrophils and macrophages, studies have shown that bone marrow-derived stem cells (BMSCs) play an essential role by homing to injured tissues before proliferating and differentiating into the required lineages [10]. Mast cells, the critical director of the inflammatory phase, are triggered by precursor stem cells present

in the skin. In the proliferative phase, the division and differentiation of tissue-specific adipocyte stem cells (ADSCs) regenerate damaged or lost tissue [10]. Inter follicular and hair follicle bulge epithelial stem cells proliferate and differentiate into cell lineages of keratinocytes for re-epithelialization. BMSCs may also contribute to increasing fibroblast populations in wounds [10]. Revascularization occurs via angiogenesis, the proliferation of endothelial cells in pre-existing blood vessels, and vasculogenesis, the de novo creation of blood vessels by differentiation of endothelial progenitor cells (EPCs). EPCs are critical for wound healing. They exert their effects primarily through the secretion of growth factors rather than cellular proliferation. Hematopoietic stem cells (HSCs) derived from bone marrow produce new endothelial cells [10]. Cell-based treatments are a clear and rational next step in chronic wound care [10].

Bioengineered tissues impregnated with stem cells are the basis of the new approach to wound healing [5]. Rheinwald and Green revolutionized wound healing when they discovered that keratinocytes could be cultured in vitro with the aid of the enzyme dispase to create sheets of cells suitable for grafting, hence a radical new option for clinical translation, including the treatment of burns patients [2]. Newer devices, including cell spray formulations that contain keratinocytes, melanocytes, Langerhans cells, and fibroblasts, have also been developed [2]. Stem cells have shown to work very well when combined with artificial skin in diabetic wound recovery [5]. MSCs can home to damaged tissue and release various immunomodulatory factors that influence the behavior of dendritic cells (DCs), T cells, and natural killer cells (NKCs). MSCs can reduce B cell proliferation, monocyte maturation, secretion of interferon-γ (IFN-γ) and TNF-α while promoting the induction of T-regulatory cells and secretion of anti-inflammatory IL-10 from macrophages in direct contact with MSCs [2]. Although the precise micro environmental contributions to tissue repair are not fully known, a more detailed understanding of the trophic mechanisms associated with MSCs in tissue regeneration is likely to explore clinical utility further [2].

Ongoing inflammation is a trait of non-healing wounds. Although controlled inflammation is an important and necessary phase of wound healing, chronic inflammation damages the wound bed and inhibits the proliferative phase and tissue remodeling [10]. Investigations by Beyth et al. showed that MSCs secrete the anti-inflammatory cytokine IL-10, which decreases T cell reactivity to antigen-presenting cells (APCs) [14]. MSCs cultured with DCs reduced the secretion of the pro-inflammatory cytokine TNF-α and increased the secretion of IL-1014. Later, Aggarwal et al. continued the studies to prove that T-cells cultured with MSCs produced fewer IFN-γ and IL-415. Injection of CD-34β umbilical cord blood cells into a wound decreased expression of the pro-inflammatory factors IL-1b, TNF-α, and IL-6, and it increased IL-10 expression and products [15]. Badiavas et al. from Boston demonstrated the complete closure of wounds in all three patients using BM-MSCs, despite the wounds previously persisting for more than one year with standard therapy [16].

MSCs had shown the ability to differentiate into keratinocytes and epithelial cells [10]. Mesenchymal stem cells stimulate the synthesis of granulation tissue at wound margins by enhancing the proliferation of epidermal cells and angiogenesis. It secretes angiopoietin-1 and growth factors to accelerate endothelial cell recruitment [6, 7]. They reduce the inflammatory response of cytokines and contain connective tissue syntheses such as collagen and fibrosis6. Humpert PM et al. achieved similar success when using topical treatment with autologous BM-MSCs. There was reduced wound size and improved vascularity in a recalcitrant ulcer in a patient with type-2 diabetes mellitus (T2DM) [17].

Wu et al. demonstrated that in pre-clinical trials, the intra-lesional injection of MSCs into wounds significantly shortens the healing time while stimulating angiogenesis, re-epithelialization, and granulation [6, 7]. Wound healing of diabetic ulcers has also been shown to hasten in pre-clinical and early human trials when MSCs were used [6]. Falanga et al. used topically delivered MSCs via a fibrin spray in a clinical study on surgical wounds and chronic wounds. There was an accelerated healing and a significant reduction in the wound's size, if not healed already after 20 weeks of treatment [6]. A study by Lu D et al. on Chronic Limb Ischemia and foot ulcers in 41 diabetic patients demonstrated 100% healing in patients treated with intramuscular BM-MSCs. There were significantly improved quantitative and qualitative measures, including painless walking time, ankle-brachial index results, and MRA analysis when compared to controls. The ability of MSCs to differentiate into keratinocytes and melanocytes and be bioengineered into hair follicles is essential for their use in organ replacement therapy in dermatology. In particular, concerning the differentiation potential of MSCs into keratinocytes, the cells responsible for wound re-epithelialization after wound formation are needed to use MSCs in skin regeneration [37].

BM stem cells have a fundamental role in generating erythrocytes, leukocytes, and platelets but also show plasticity in being able to show lineage differentiation into tissues of mesodermal, endodermal, and ectodermal origin, including the skin. Some subpopulations of BM cells can differentiate into keratinocytes [16]. Further studies have shown that the BM is also a source of fibroblast-like cells in the dermis and that the number of these cells increases after skin wounding [2]. ESCs were initially investigated for skin healing for their ability to self-renew and divide into keratinocytes. They can be induced to undergo epithelial differentiation by the actions of Retinoic Acid (RA) to promote ectodermal fate and Bone Morphogenetic Protein 4 (BMP4) to block neural fate [34]. Adipose-Derived Stem Cells (ADSCs) produce keratinocyte growth factors and vascular endothelial growth factors, both of which accelerate the proliferation phase of wound healing in addition to

aiding keratinocyte migration [24]. A clinical trial by Di et al from Korea proved the use of ADSCs on critical limb ischemia patients resulted in ulcer improvement and an increase in transcutaneous oxygen content [24]. Kim et al investigated that ADSCs had effects on Human Dermal Fibroblasts (HDFs) by increasing collagen synthesis and promoting the proliferation of HDFs, suggesting that ADSCs could be used for the treatment of wound healing [54]. ADSCs have proven to be successful in rapidly recovering wounds using the patient's regenerated tissue, hence no skin graft required [25]. Bura A et al confirmed that ADSCs benefit treatment in a clinical trial involving patients with critical limb ischemia. In a 2-year follow-up, a significant improvement in leg pain, ulcer size, and pain-free walking distance were also described [23]. Although Hematopoietic Stem Cells (HSC)s produce mainly blood cells, they also produce epithelial cells and hepatocytes. HSCs are a major source of fibroblasts during tissue repair. HSCs express a specialized form of CD44 which binds very strongly to E-selectin, resulting in powerful homing to sites of inflammation. It may be possible to deliver HSCs to the site of injury simply by injection into the bloodstream, simplifying clinical treatment [10]. Shroff et al assessed the effect of human ESCs (hESCs) therapy on six patients with non-healing wounds. There was a reduction in the size of wounds and granulation was observed among all the patients, ultimately leading to wound healing [55]. Tanaka R et al., demonstrated that treatment with transplanted autologous G-CSF mobilized peripheral blood CD34+ cells on diabetics with non-healing chronic wounds for more than 3 in all patients [56]. Endothelial Progenitor Cells (EPCs) mainly reside in the bone marrow and are mobilized into the peripheral blood with tissue ischemia or systematic administration of Granulocyte Colony-Stimulating Factor (G-CSF), Vascular Endothelial Growth Factor (VEGF), or estrogen. Mobilized EPCs will be home to ischemic sites for vascular repair [56].

Takahashi and Yamanaka generated iPSC by reprogramming adult fibroblasts into an immature, pluripotent state [23]. Human-induced pluripotent stem cells (iPSCs) are created from differentiated adult somatic cells. Multiple terminally differentiated cells are capable of being reverse-engineered to pluripotency [10]. Keratinocytes have proven to be effective in the synthesis of iPSCs: keratinocytes transduced with OCT4, SOX2, KLF4, and c-MYC produced iPSCs more quickly and efficiently than fibroblasts [10]. The ability to induce pluripotency is only half the challenge. To be therapeutically effective, researchers must be able to direct stem cell differentiation into specific cell types and lineages [10]. Studies conducted by Hewitt KJ et al and Bilousova et al have managed to induce the differentiation of iPSCs into fibroblasts and keratinocytes [11, 12]. The presence of Keratin 14 (K14), a marker for keratinocytes, in the final product suggests that iPSCs can be differentiated into epithelial lineages, including keratinocytes with the aid of RA and BMP434. Significant progress has been made in the differentiation of iPSCs into skin cells by Yang et al, including folliculogenesis human epithelial stem cells, fibroblasts, and keratinocytes—to engineer skin substitutes [23, 54]. The reprogramming of a patient's somatic cells into iPSCs is a promising new approach to establish human models for studying disease mechanisms, testing drugs, and developing cell therapies [34].

Pressure wounds are often persistent and difficult to treat with current practices, in addition to frequent recurrence. In a study by Wettstein et al on 3 patients with non-healing sacral pressure ulcers, treatment with BMSCs decreased wound volume by 60% from baseline in 3 weeks18. In another study by Garcia-Olmo et al, topical application of BMSCs fully healed pressure ulcers in 19 of 22 treated patients within 3 weeks, despite prior persistence for more than four months. Remarkably, none of the ulcers recurred for at least one year following treatment [19].

5. Stem Cells in Blistering Diseases

Epidermolysis Bullosa (EB) refers to a group of inherited skin disorders in where skin fragility results in painful blisters and mucosal erosions after relatively minor trauma to the skin [26, 27]. The four main clinical variants of EB results from mutations of at least 21 genes, mainly encoding structural components of the Dermal-Epidermal Junction (DEJ) or keratinocytes within the lower epidermis, resulting in a spectrum of phenotypic variability of blisters and wounds upon trauma [26, 28]. When superimposed on the individual patient's genetic background and exposure to external trauma, these mutations explain the tremendous variability in the phenotype of this group of disorders [26]. Knowledge of the precise underlying congenital disability is the prerequisite for any molecular therapy approach [43]. Researchers in EB are exploring the applicability of new strategies in regenerative medicine (e.g., induced pluripotent stem cells [iPSCs]) and genome editing (e.g., CRISPR/Cas9) [43]. The delivery of COL7A1 cDNA into the skin does not integrate into the recipient's genome. Therefore, a lifelong continuous application is required to achieve sustained benefits from the treatment [43]. The main principle of care is to manage blisters and erosions, control infection, and prevent complications. Symptom relief is significant as both pain and itch have severely deleterious impacts on quality of life [44]. Collectively, the EB population has a desperate need for innovative therapies that reduce disease burden, improve quality of life, and make advances toward a cure [2].

Dystrophic Epidermolysis Bullosa (DEB) is an inherited blistering skin disorder caused by mutations in COL7A1 that encodes type VII collagen (C7), the main constituent of the anchoring fibrils that links type I collagen to proteins such as laminin-332 at the dermo-epidermal junction [28, 50]. C7 acts as an adhesive that binds the epidermis to the dermis in the skin and mucosa [50]. C7 is synthesized primarily by the keratinocytes and to a lesser extent by dermal fibroblast [50]. Patients with DEB show absent or reduced

or abnormal anchoring fibrils [30]. In the Recessive Dystrophic EB (RDEB), especially the Hallopeau–Siemens variant of epidermolysis bullosa, there may be very extensive fragility of the skin and mucous membranes resulting in extensive mutilating scars and development of aggressive squamous cell carcinomas, with a significantly reduced life span of the affected individuals [27]. Patients with RDEB lack functional C7 and have severely impaired dermal-epidermal stability. This results in profound skin fragility associated with extensive blistering, open wounds, delayed wound healing, and persistent erosions with long-term complications of scarring and increased incidence of malignancy [2]. There is currently no effective or specific treatment available or approved for RDEB [26]. The major challenge is to restore an intact epithelium and provide some protection against mechanical trauma [2].

Cell therapy for RDEB is currently in the early phase of human clinical trials. There have been numerous clinical trials reported using allogeneic fibroblasts (intradermal injections), Mesenchymal Stromal/Stem Cells (MSCs; intradermal or intravenous), and Bone Marrow (BM) transplantation [47].

Keratinocytes and dermal fibroblasts express adhesive proteins that ensure the epidermis remains attached to the skin basement membrane and the papillary dermis [40]. Fibroblasts are much easier to isolate and maintain in culture than keratinocytes. Fibroblasts present an attractive target for cell-based therapies for RDEB. Normal and COL7A1gene-corrected human RDEB fibroblasts overexpressing C7 have been injected intra-dermally into immune deficient mouse skin or transplanted human RDEB skin equivalents [2]. Both these interventions led to sustained human C7 deposition and new anchoring fibril formation at the DEJ [2].

Allogeneic cultured dermal substitutes (CDS) consisting of keratinocytes and fibroblasts supported on a scaffold are used to treat EB skin ulcers with benefit [44]. Atanasova et al. discovered that Amlexanox, a US FDA-approved drug for aphthous ulcer, induced the entire length of the COL7A1 expression after 48 hours of incubation in skin fibroblast and keratinocytes [31]. On further testing, it was discovered that Amlexanox produced similar results in in vitro studies using RDEB patient's skin cells harboring homozygous COL7A1PTC mutation. This pre-clinical study demonstrates the potential of amelxanox for the treatment of RDEB in the form of skin grafts [31, 43]. Eichstadt et al. studied the effects of Epidermal sheets grafts prepared from cells of autologous keratinocytes taken from RDEB patients and transduced with a retrovirus carrying the full-length human COL7A1 gene on the wound site of 7 patients with RDEB. No participants experienced any related severe adverse events. Wound healing of 50% or greater by Investigator Global Assessment was present in 95% of treated wounds [45]. At year 2, 71% of treated wounds had 50% or greater healing. C7 expression persisted up to 2 years after treatment. Treated wounds with 50% or greater healing demonstrated improvement in patient-reported pain, itch, and wound durability [45]. This study provides additional data to support the clinically meaningful benefit of treating chronic RDEB wounds with ex vivo, C7 gene-corrected autologous cell therapy [45]. Long-term renewal function has been shown in transplanted cultured epidermal cell sheets by functional testing for the presence of clonogenic stem cells.

Conversely, clinical failure of transplants is associated with Epidermal stem cell depletion within the transplant [49]. Studies reveal that gene-corrected keratinocytes in the graft are capable of expressing type VII collagen, and there is evidence of assembly of anchoring fibrils at the DEJ [43]. Amniotic membrane and placental material have also been clinically proven to heal ulcers, reduce pain, and improve mobilization of limbs [44]. This approach's durability is questionable as the expression of the COL7A1 fades with time, and the number of stem cells isolated is too small to sustain the graft [43].

Wong et al. reported a study where five RDEB subjects showed that a single intradermal injection of allogeneic dermal fibroblasts increased COL7A1 gene expression for at least three months in most individuals [26, 44]. The study also showed that cell therapy in the form of allogeneic dermal fibroblasts has low immunogenicity and lacks host response at an immunological and histological level [2, 44]. A subsequent study by Nagy et al. was able to show that a single injection of allogeneic dermal fibroblasts with heparin binding-EGF-like growth factor (HB-EGF) could increase the COL7A1 gene expression for up to 6 months while maintaining C7 proteins up to 12 months [2, 44]. Studies by Petrof et al. demonstrated that a single injection of allogeneic dermal fibroblasts into the margins of chronic erosions of an individual with RDEB could speed up wound healing by 28 days compared to vehicle [39, 44]. Transcriptomic analysis of serial skin biopsies following injection of allogeneic dermal fibroblasts in a subject with RDEB revealed that the expression of heparin-binding epidermal growth factor-like growth factor (HB-EGF) was up-regulated. Those expression levels mirrored those seen for the COL7A1 gene [2, 26]. Allogeneic dermal fibroblasts have been used off-license to treat several patients with RDEB. The clinical impression and experience indicate that for a subgroup of individuals with RDEB, notably those with mild to moderate disease severity and some baseline expression of C7 at the DEJ, allogeneic dermal fibroblast therapy may be helpful, in contrast to those individuals with more severe disease that lack C7 expression [2, 44]. Lwim et al. demonstrated that intradermal injections of COL7A1-modified autologous dermal fibroblast to RDEB subjects were able to show an excellent efficacy and safety profile during evaluation after 12 months of treatment [32].

The signaling lymphocyte activating molecule (SLAM)-positive subpopulation of BM cells resulted in new C7 and donor cells' presence at the DEJ. Following infusion of SLAM-positive BM

cells, there was improved healing of blisters on the mouth and paws and histological evidence of rudimentary anchoring fibril formation [2].

Stem cells in the bone marrow were recently found to have pluripotency, which could differentiate into various cell lineages other than hematocytes [33]. Mesenchymal cells are found in several tissues, including the bone marrow, and can migrate to injured tissue and stimulate tissue regeneration, making this therapy potentially relevant to RDEB wounds [44]. BM-derived MSCs can stimulate secretion of C7 with partial restoration of the damaged basement membrane zone and less blister formation. BM stem cells have been shown to correct basement membrane protein expression and lead to phenotypic rescue [2]. The use of allogenic bone marrow stem cell transplantation therapy was first reported in six patients with RDEB by Wagner et al. The MSCs used in the RDEB study were derived from unfiltered marrow stem cells from an HLA identical sibling [38]. RDEB patients are almost or entirely devoid of C7 expression at the DEJ on immunofluorescence microscopy and clinically have severe blistering and wounds that healed slowly with scarring [38]. After the intervention, a Skin biopsy showed that the bone marrow infused stem cells expressed C7 at the DEJ.

Moreover, there was re-epithelialization of chronically ulcerated skin. Most patients in the study showed clinical benefits that lasted more than 6 months [38]. This pilot study showed that cell therapy in the form of infusion administrated allogeneic bone marrow stem cells can lead to de novo C7 expression at the DEJ and prevent blistering and improving wound healing in patients with RDEB [2]. The chemokine CTACK/CCL27 secreted from the injured skin tissue accelerates bone marrow stem cells' differentiation into epidermal keratinocytes [33]. Another chemokine, SLC/CCL21, was found to enhance wound healing via differentiating MSCs into various skin component cells, including keratinocytes. These differentiated keratinocytes function and provide BMZ component proteins [33]. Petrof et al. and Rashidghamat et al. conducted clinical trials using intravenous infusion of allogeneic BM-derived MSCs to subjects with RDEB. The clinical evaluation reported a reduction in blisters, less skin inflammation, better wound healing, and improved quality of life, which lasted for 3-9 months following infusion of MSCs [2, 47]. El-Darouti et al recorded similar results on a study using bone marrow non-hematopoietic stem cells infusions on patients with RDEB, where the number of new blisters was reported to be decreased significantly after treatment, and the rate of healing of blisters was significantly faster. Electron microscopic examination showed increased numbers of anchoring fibrils after treatment [27]. Exogenous induction of mesenchymal stem cells by TGF-β and TNF-α for 48 hours, results in an 8 – fold increase in col 7a1 expression, thus an increase in collagen 7 productions. There was also an increase in the TSG-6 expression, resulting in wound healing acceleration and immunosuppression [42]. MSCs express tumor necrosis factor-alpha (TNFα)-stimulated protein 6 (TSG-6), which is associated with an improvement in wound healing and the downregulation of B-cell proliferation, monocyte maturation, secretion of IFN-γ and TNF-α at wounded tissue sites, while also promoting increased secretion of anti-inflammatory IL-10 from macrophages. In addition to TSG-6, MSCs also mediate immunosuppression through the secretion of nitric oxide, Transforming Growth Factor-Beta (TGF-β) and indoleamine 2, 3-dioxygenase [44]. Clinical studies show the impact and superiority of high-density MSCs compared to fibroblasts, suggesting that further human clinical trials are needed if the maximal benefits of MSC cell therapy in RDEB are to be realized [44].

Ikeda's clinical trials using adipose-derived stem cells (ADSCs) on patients with RDEB showed that 45% of ADSCs differentiated into keratinocyte-like cells and expressed higher levels of Col7 [35]. These findings support ADSCs' therapeutic potential, not only for wound healing but also for the correction of Col7 deficiencies [35].

Cord blood (CB) and other parts of the umbilical cord, such as the Wharton's jelly or tissues associated with the placenta, are rich sources of stem cells. As hematopoietic stem cells, CB is an essential source of other progenitor cells and MSCs, embryonic stem cells, and somatic stem cells, which may have individual or collective value in regenerative medicine [2]. Human embryonic stem cells (hESCs) derived keratinocytes are suitable alternatives for traditionally used immortalized cell lines (i.e., HaCaT cell line) for stem cell-mediated treatment of skin wound [42]. In a previous study regarding mechanical stress effects on hESCs, it has been clarified that mechanical stress increases the expression of some genes, including matrix- metallopeptidase 9, keratinocyte growth-factor-receptor, connexin [43], catenin β1, endothelin1, integrin α6, desmoglein1, interleukin α1, E-cadherin, keratin1, 6, and 10 and laminin α542. Comparing umbilical cord cells versus BM stem cells in individuals with RDEB has shown better skin engraftment with a BM-derived population. Therefore, cord cells' clinical utility in EB or other skin disorders remains to be determined in future clinical trials [2].

A novel therapeutic strategy to treat DEB envisages combining iPSCs derived from patient's somatic cells with gene-editing and tissue engineering technologies58. iPSCs can grow indefinitely while maintaining pluripotency [48]. Tolar J et al. experimented with personalized, gene-corrected, patient-specific cell induced pluripotent stem cells (iPSCs) obtained from both skin fibroblast and keratinocytes of 3 subjects with RDEB. Autologous RDEB iPSCs can form skin-like structures with no Col7 deposition [28].

Sebastiano et al. and Umegaki-Arao et al. successfully used human keratinocyte-derived iPSCs to reconstitute skin in vitro to treat RDEB [23]. There was also the potential for aberrant skin repair in individuals with RDEB, which could, in theory, lead to

rapid growth, self-renewal, and pluripotency states [28]. Gene-corrected RDEB iPS cells could be used to generate an autologous hematopoietic graft and generate non-hematopoietic skin cells [28]. Nakayama et al. developed a technique to convert iPSCs from non-human epidermal keratinocytes (NHEKs) and EB keratinocytes (EBKs) to MSCs [48]. The NHEK-iPSC-MSCs possessed the ability to accelerate wound healing and produce type VII collagen in subcutaneous and intravenous xenotransplantation models [48]. The technique of induced pluripotent stem cells (iPSCs) from a somatic cell, for which the Nobel Prize in Medicine in 2012 was awarded to John B. Gurdon and Shinya Yamanaka, can contribute to broadening the application of revertant mosaicism in the therapy of all genetic diseases. The concept known as “natural gene therapy” or revertant mosaicism is a naturally spontaneous occurring process characterized by genetic repair that can lead to a partial or complete reversal of an affected to a wild-type phenotype. The phenomenon of revert ant mosaicism refers to the sudden rise of clinically normal patches of skin, which is attributed to the re-expression of C7 resulting from the occurrence of secondary mutations that reverse the primary mutation in COL7A1 [50]. The genetic correction appears to be limited to keratinocytes rather than fibroblasts or other cell populations, perhaps reflecting higher proliferation rates in keratinocytes [2]. Revert ant cells can be harvested from a biopsy of reverted skin and used to produce cultured autologous skin grafts. The idea, however, is not to produce large sheets of skin, but rather enough skin to treat wounds that do not heal and have a propensity to progress to carcinoma, as occurs in severe, generalized RDEB [50]. Gostynski et al demonstrated the feasibility of this technique in a patient with junctional EB, by simply transplanting punch biopsy specimens from revert ant skin patches onto persistent wounds. Not only did the transplanted skin last, but there was also a clinical improvement, with no additional blisters developing in the treated areas [50]. Matsumura et al conducted a clinical trial of Cultured Epidermal Autografts (CEA) from clinically revert ant skin for three patients with RDEB. the epithelization rate for each patient at the primary endpoint was 87.7%, 100%, and 57.0%, respectively [51]. The clinical effects were found to persist for at least 76 weeks after CEA transplantation. One of the three patients had apparent revert ant mosaicism in the donor skin and the post-transplanted area. CEAs from clinically normal skin are a potentially well-tolerated treatment for recessive dystrophic epidermolysis bullosa [51].

Junctional EB (JEB) is another primary form of EB, and severe generalized JEB is one of the most severe EB types that leads to the early death of patients, usually within the first few months of life. Severe generalized JEB is caused by mutations in the laminin-332-encoding genes LAMA3, LAMB3, and LAMC2 [37]. Studies by Maviolio et al. demonstrated that autologous epidermal stem cells were reported to help gene therapy for JEB treatment. Epidermal stem cells from a patient affected by LAMB3-deficient JEB were transfected with a retroviral vector expressing LAMB3 cDNA (encoding LAM5-β3). They were successfully used to prepare genetically corrected cultured epidermal grafts transplanted to the patient's leg [37, 49]. One year later, synthesis of normal levels of functional laminin-5 was still observed, together with a normal adherent epidermis in all transplanted areas [49]. Analysis of the regenerated epidermis revealed maintenance by long-lasting, self-renewing transgenic epidermal stem cells [49]. Grafts for patients with JEB were made of a holoclone stem cell population, thus ensuring the cells' longevity after transplantation. A study by Hirsch et al. on a patient with JEB reported that the skin graft functionality was retained several years after the transplantation [43]. Kaipe et al. studied the effects of the intravenous infusions of allogeneic Mesenchymal Stromal Cells (MSCs) in the form of Decidual Stromal Cells (DSCs) obtained from a term placenta to an 11-year-old patient with e Junctional Epidermolysis Bullosa (JEB) over three months.

Improvements in the skin were seen both after the initial two treatments, and the advance continued where the patient's skin lesions healed well [41]. However, there was only a transient clinical improvement with subsequent injections of allogeneic mesenchymal stromal cells. Mesenchymal stromal cell therapy is relevant in regenerative medicine, owing to these cells' ability to secrete factors involved in angiogenesis and tissue repair [41]. The patient survived for 23.5 months compared with the average of 6 months [41]. The JEB patient had high levels of circulating anti-HLA class-I antibodies directed against more than 30 HLA antigens [41]. Tolar J et al. experimented with gene-corrected, patient-specific proliferative cell transfer generated from Induced Pluripotent Stem Cells (iPSCs) cells transduced from the skin of two subjects with JEB. The JEB-iPSCs may be used to establishing a reliable stem cell source for gene therapy interventions in JEB-H [40].

The most evident approach to correcting C7 and other protein deficiencies would be administering the absent or reduced protein [50]. Recombinant type VII collagen, injected locally or intravenously, homes to the DEJZ and promotes wound healing. The advantages of systemically administered recombinant type VII collagen include the possibility that the protein, in addition to homing into the skin, will reach extra cutaneous tissues affected by RDEB, such as the gastrointestinal tract and the cornea of the eye, with subsequent repair [43]. Bone marrow stem cells can be delivered to damaged tissue and facilitate tissue repair and probably by suppressing the inflammation of injured skin [43]. Protein therapies are safer than other novel therapies so that patients can attempt the treatment with a lower dose of protein and that no gene correction is needed [33]. This approach necessitates multiple injections as the corrected resident fibroblasts are not present nor remain active indefinitely [43].

Chemokines and cytokines analysis revealed several EB skin-as-

sociated chemotactic gradients, which contribute to EB pathology, are the way forward to create a favorable milieu to halt the progression of the skin blisters and to heal wounds [46]. Alexeev et al. discovered that blister fluid derived from DEB patients exhibited chemokines, including CXCL1 + 2 and CXCL [5]. Their study also revealed that several chemotactic axes involving CXCR1/2 and CCR4 ligands could be further exploited to effectively recruit and homing of the therapeutic stem cells to EB-affected skin, hence improve cells-based therapies [46].

Tissue engineering and gene therapy together are novel suggested treatment options for EB patients. Still, gene therapy has some obstacles to obtain a particular treatment over stem cells in EB.

6. Stem Cells in Inflammatory Skin Diseases

Inflammatory skin diseases such as psoriasis and Atopic Dermatitis (AD) are considered major health issues with increasing prevalence due to the rapid industrialization of modern society [63]. These diseases' symptoms can deteriorate patients' quality of life due to an impaired skin barrier, itch, insomnia, and the social stigma associated with these skin conditions. However, treatment options for these disorders are limited for patients with moderate-to-severe disease severity who are unresponsive to topical steroids or systemic immunosuppressant's [63].

Psoriasis is a complex chronic immune-mediated inflammatory skin disease that speeds the turnover of epidermal keratinocytes, forming scales and red skin lesions that are itchy and sometimes painful. It is a complex disease of autoimmune origin and has a genetic predisposition with more than ten different loci associated [57]. Some treatments have shown disease remission. However, psoriasis remains incurable. The pathogenesis of psoriasis begins with the activation of T-cell, which leads to the increased release of associated cytokines, expression of Inducible Nitric Oxide Synthase (iNOS), and Vascular Endothelial Growth Factor (VEGF), and increased total antioxidant capacity (total oxyradical scavenging capacity) [68]. The pathogenesis of psoriasis results in the dysregulation in the proliferation and differentiation of keratinocytes (KCs) [59].

Cell therapy for psoriasis has mainly focused on MSCs as the functions of MSCs include, but are not limited to, promoting wound healing, inhibiting T-cell proliferation, affecting angiogenesis, and inducing KCs proliferation. Chen et al. discovered that the therapeutic effects of MSC start by suppressing neutrophil function and then downregulating the production of type I interferon (IFN-I) by plasma cystoid dendritic cells (pDCs) [69]. These biological effects are mainly mediated by direct cell-to-cell contact and, in particular, paracrine effects [59]. MSCs obtained from patients with psoriasis have been shown to have impaired anti-inflammatory function against Th cell subsets, suggesting that allogeneic MSC therapy is expected to be beneficial in treating psoriasis [63]. Chen et al proved that UC-MSC infusions to psoriasis patients were able to keep the patients in remission for more than a year [64]. Niu et al recently discovered that DMSCs as an epigenetically regulated pro-migration factor that is associated with a disorder of immune in psoriasis. Their study on the epigenetics of psoriatic lesions suggests the potential use of CMKLR, COL8A, NRK, SYTL2, and SFRP2 as therapeutic target psoriasis in the future [60]. Kim et al proved that the use of human embryonic stem cell-derived MSCs (hE-MSCs) could suppress the number of psoriatic skin lesions [61]. In the histological analysis, the epidermal thickness was significantly decreased. At the same time, Th1 cytokines (TNF-a, IFN-a, IFN-c, and IL-27) and Th17 cytokines (IL-17A and IL-23) in the serum showed marked inhibition by hEMSCs. These results suggested that hE-MSCs have a potency of immune modulation in psoriasis [61]. De Jesus et al. utilized autologous MSCs from two patients with Psoriasis Vulgaris (PV) who failed to respond to standard treatment modalities [62]. They discovered that both patients showed a significant improvement clinically after a few infusions with the autologous MSCs. The clinical improvements sustained for nine months, and there was a marginal reduction in serum tumor necrosis factor-a (TNF-a) while reactive oxygen species (ROS) activity decreased significantly [62].

Kaffenberger et al. reported that 19 patients experienced psoriasis resolution after allogeneic or autologous hematopoietic stem-cell transplantation (HSCT) [65]. The majority of patients that received allogenic HSCT remained in remission with a mean follow-up of 49 months. The patients in the autologous HSCT developed a recurrence of their psoriasis within two years [65]. Zurita et al. demonstrated that patients with severe psoriasis who underwent autologous HSCT followed by an infusion of hematopoietic stem cells had a very clinically significant improvement maintained for up to 6 months [66]. Comella et al. reported a patient with almost complete clearance of psoriasis within one month of ADSC infusion. The patient continued to be clear of psoriasis for a year, a single ADSC infusion [67].

7. Stem Cells and Cancer

Melanoma is a form of cancer that initiates from the malignant transformation of melanocytes [70, 78]. Amongst the subtypes of melanoma, cutaneous melanoma is the most common and accounts for more than 90% of all melanoma cases [78]. Unfortunately, the incidence of cutaneous melanoma worldwide has been rising annually [5–7], at a rate faster than that of any other malignancy [79]. This is of particular concern given the unusual age demographics of the disease. There is a female preponderance in younger age groups (4:10 in 20– 24-year-olds) [79].

Melanoma has multiple phenotypically distinct subpopulations of cells. Some of them have embryonic-like plasticity, which is involved in self-renewal, tumor initiation, metastasis, and progression and provide a reservoir of therapeutically resistant cells [70]. This subpopulation of self-renewing and expanding cells

known as cancer stem cells (CSCs) [73]. Melanoma is a common type of skin cancer that is frequently associated with poor clinical outcomes [78]. Malignant melanoma is a highly aggressive and drug-resistant cancer [70]. Melanoma treatment becomes difficult, and survival is significantly reduced when the patient develops metastasis [70].

Standard conventional oncology treatments such as chemotherapy, radiotherapy, and surgical resection are only responsible for shrinking the bulk of the tumor mass and the tumor tends to relapse [70]. Targeted therapies against activating mutations occurring in BRAF have significantly prolonged patient survivals, although about 50–60% of melanoma patients lack such mutations and thus are not applicable for BRAF tyrosine kinase inhibitor-based treatment [78]. Thus, targeting CSCs and their microenvironment niche addresses the alternative of traditional cancer therapy [70].

CSCs have similar physiological properties as normal stem cells, like self-renewal, differentiation, and indefinite proliferation ability which might be the main cause of tumor progression [70]. Conventional anticancer treatments eradicate the bulk of tumor mass but it is ineffective for CSCs and hence could be the reason for tumor reoccurrence and progression [70]. CSCs have been identified in hematopoietic cancer and solid tumors like brain, breast, prostate, colon, pancreatic, and lung [70]. Luo et al. have provided significant evidence and shown the existence of CSCs in melanoma by using aldehyde dehydrogenase (ALDH), an intercellular stem cell marker in melanoma [71]. These findings implicate that ALDH isozymes are not only biomarkers of CSCs but also attractive therapeutic targets for human melanoma [71].

To control melanoma growth, it is necessary to target melanoma stem cells because it governs tumor recurrence and metastasis after many years and may act as a reservoir of therapeutically resistant cells [70].

Melanoma-specific CSCs carry specific markers (CD133, CD20, ABCB5, CD271, and ALDH1) or antigens, so targeting these cells using monoclonal antibodies could help combat melanoma growth [70]. Rappa et al. have demonstrated that downregulation of CD133 (Prominin-1) resulted in slower cell growth, reduced cell motility, and decreased capacity to form spheroids under stem cell-like growth conditions in human metastatic melanoma cells (FEMX-1) [70, 72]. Monoclonal antibodies directed against two different epitopes of the Prominin-1 protein induced a specific, dose-dependent cytotoxic effect in FEMX-I cells [72].

Since melanoma-specific CSCs express CD20, rituximab therapy is used in clinical trials to treat metastatic melanoma patients by targeting CD20+ cells [70]. The CD20 antibody therapy depletes CD20 positive melanoma cells and eliminates peripheral B cells that elevate in malignant melanoma patients [70].

Calvani et al. discovered that the combination of Etoposide with Bevacizumab significantly induces apoptosis and abolishes the sphere-forming ability of CD133+ CSCs in melanoma [70, 73]. The combined exposure to Etoposide and Bevacizumab targets melanoma cells endowed with stem like properties [73]. The Etoposide and Bevacizumab treatment reduces cancer cells' ability to induce new colonies, hence a longer latency period [73].

Schatton et al. identified a subpopulation of human malignant melanoma-initiating cells (MMIC) defined by their expression of the chemoresistance mediator ABCB5 and showed specific targeting of this tumorigenic population inhibits tumor growth [74]. ABCB5+ cells in melanoma have been shown to suppress T cell activation and have a particular role in immune evasion [70]. Systemic administration of a monoclonal antibody directed at ABCB5, shown to be capable of inducing antibody-dependent cell-mediated cytotoxicity in ABCB5+ MMIC, exerted tumor-inhibitory effects [74]. Therefore, targeting the immune system in a melanoma patient with IL-2 and IFN-α could be an important therapeutic approach [70]. Identification of tumor-initiating cells with enhanced abundance in more advanced disease but susceptibility to specific targeting through a defining chemo-resistance determinant has important implications for cancer therapy [74].

Andrographolide (Andro), derived from Andrographis paniculata, attenuates tumor growth through abrogation of Notch1-mediated CD133- dependent p38 MAPK activation pathway in CD133+ melanoma cells70,75. Mechanistically, Notch1 upregulates mitogen-activated protein kinase activation through CD133, which ultimately controls vascular endothelial growth factor and matrix metalloproteinase expression in CD133þ stem cells leading to melanoma growth, angiogenesis, and lung metastasis [75]. Blockade or genetic ablation of Notch1 and mitogen-activated protein kinase pathways abolishes melanoma cell migration and angiogenesis [75]. Besides, Andro also impairs the EMT, angiogenesis, and metastasis properties of these CD133+ cells [70, 75]. These data indicated that Andro may act as a potential anti-cancer agent to eradicate CSCs-dependent melanoma progression [70].

Recent advancements in targeting Bcl2 family members are an alternative option to combat melanoma and overcome relapse or resistance of melanoma [70]. To prevent this relapse, it is necessary to develop effective therapies that eradicate all tumor cells' subpopulations, including resistant CSCs subpopulation in melanoma [70]. The BCL2 is essential in regulating the intrinsic apoptotic pathway. Thus BCL-2 protein contributes to melanoma's resistance to apoptosis. Mukherjee et al. proved that the combination of ABT-737, small-molecule BCL-2/BCL-XL/BCL-W inhibitors, with fenretinide (4-HPR), is effective in killing both the bulk of melanoma cells and melanoma initiating cells (MICs) [76]. The combination increased the NOXA, a pro-apoptotic member of the Bcl-2 protein family expression and caspase-dependent MCL-1 degradation [76]. The combination synergistically decreased cell viability and caused cell death in multiple melanoma cell lines but

not in normal melanocytes [76]. Knocking-down NOXA protected cells from combination-induced apoptosis. The combination treatment disrupted MICs and decreased the percentage of ALDH in melanoma cell lines [76]. Moreover, the combination inhibited the self-renewal capacity of MICs [76].

CD44 is present on many types of CSCs, and it binds specially to hyaluronic acid (HA) [77]. Shen et al. have demonstrated that coating solid lipid nanoparticles with hyaluronan (HA-SLNs) allowed targeted delivery of Paclitaxel (PTX) to CD44+ B16F10 melanoma cells [70]. PTX loaded HA-SLNs significantly abrogate tumor growth and lung metastasis [70]. Additionally, combined treatment with engineered VNP20009, carrying shABCB5 and Cyclophosphamide (CTX), drastically reduced ABCB5+ CSCs that leads to attenuation of melanoma tumor growth and enhanced survival time [70, 77]. Administering PTX-loaded HA-SLNs led to efficient intracellular delivery of PTX and induced substantial apoptosis in CD44+ [77]. This treatment exhibited significant antitumor effects with a relatively low dose of PTX, which provided considerable survival benefits without evidence of adverse events [77]. These findings suggest that the HA-SLNs targeting system shows promise for enhancing cancer therapy [77].

8. In Vivo Reprogramming of Injured Skin Tissue

Partial reprogramming of adults specialized within injured or degenerated tissue by creating a temporally and spatially limited pluripotent state is a new strategy to limit components of defect healing with scarring or senescence and to promote regeneration of the affected tissue from within itself [80]. The basic principle of in vivo cell reprogramming is to promote the proliferative capacity of the reprogrammed cells by transforming/dedifferentiating them, thereby generating progenitor cells in the damaged tissue that differentiate into tissue-type target cells [80].

The systematic, time-limited use of a combination of transcription factors that can trigger pluripotency in a variety of tissues - the Oct3/4, Sox2, Klf4, and c-Myc (OSKM) cocktail could, within vivo reprogramming, open up new therapeutic possibilities for the treatment of a variety of diseases in which the loss of certain cell types cannot be treated with established therapies [81].

A study by Ocampo et al (2016) was the first to describe the potential of OSKM to improve the resilience of aged tissue to injury. In this work, it was shown that transient reprogramming removed various molecular aspects of aging and improved the regenerative capacity - including striated muscle and pancreatic tissue in older mice [79].

The results of Doeser et al. showed that OSKM transcription factors delayed wound closure by reducing wound contraction, fibroblast migration, and trans differentiation from fibroblasts to my fibroblasts [79]. Gene expression analyses showed a suppression of the profibrotic gene induction [79]. Importantly, improved wound healing with significantly reduced scar tissue formation was observed. These data suggest that the effect of reprogramming factors on wound healing in the skin is mainly anti-fibrotic and may reduce scar tissue formation [79].

As an alternative explanation for the reduction in scarring, Doeser et al. had suggested that the reduction in OSKM-mediated fibrosis could involve the reactivation of fetal gene expression in adult wound tissue [82]. Mammalian scarless fetal wound healing is characterized in particular by the upregulation of TGF-β3, collagen III, and Il10 and a reduced fibrotic reaction, thus leading to tissue regeneration without the formation of scar tissue [82].

9. Conclusion

Clinical trials on the use of stem cells are underway for a wide variety of conditions, and its therapeutic benefit for patients is increasing [21]. Cell therapy is the transplantation of autologous or allogeneic cells through local delivery or systemic administration to restore deficient tissues' viability or function. Stem cells are the best choice for cell therapy because of their high potential for self-renewal, differentiation, and plasticity [9]. Skin stem cells have the potential to function as an easily accessible autologous source of future stem cell transplantation. It is hoped that cell therapy lessons learned from studies on skin diseases will be relevant to improving future healthcare of patients with more common disorders associated with defective skin.

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