Review

Current regenerative medicine-based approaches for skin regeneration: A review of literature and a report on clinical applications in Japan

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ABSTRACT

Current trends indicate a growing interest among healthcare specialists and the public in the use of regenerative medicine-based approaches for skin regeneration. The approaches are categorised in either cell-based or cell-free therapies and are reportedly safe and effective. Cell-based therapies include mesenchymal stem cells (MSCs), tissue induced pluripotent stem cells (iPSCs), fibroblast-based products, and blood-derived therapies, such as those employing platelet-rich plasma (PRP) products. Cell-free therapies primarily involve the use of MSC-derived extracellular vesicles/exosomes. MSCs are isolated from various tissues, such as fat, bone marrow, umbilical cord, menstrual blood, and foetal skin, and expanded *ex vivo* before transplantation. In cell-free therapies, MSC exosomes, MSC-derived cultured media, and MSC-derived extracellular vesicles are collected from MSC-conditioned media or supernatant. In this review, a literature search of the Cochrane Library, MEDLINE (PubMed), EMBASE, and Scopus was conducted using several combinations of terms, such as 'stem', 'cell', 'aging', 'wrinkles', 'nasolabial folds', 'therapy', 'mesenchymal stem cells', and 'skin', to identify relevant articles providing a comprehensive update on the different regenerative medicine-based therapies and their application to skin regeneration. In addition, the regulatory perspectives on the clinical application of some of these therapies in Japan are highlighted.

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1. Background

Current trends indicate a growing interest among healthcare specialists and the public in the use of regenerative medicine-based therapies for skin regeneration, especially around the face and neck to address facial ageing, and cutaneous wound healing. Facial ageing can negatively impact the quality of life. Facial ageing is attributed to the interplay of several factors, categorised as either internal or external (environmental) factors. The internal factors are mainly genetic, whereas the primary environmental factor is ultraviolet-B (UVB) radiation exposure [1]. Extrinsic ageing, also known as photoageing, involves fine and coarse wrinkling, roughness, dryness, laxity, and pigmentary lesions resulting from decreased epidermal thickness and keratinocyte atypia. When the stratum corneum of the epidermis is exposed, it is often damaged by UVB radiation, leading to loss of water retention capacity. In addition, exposure of the dermis to UVB radiation denatures collagen, leading to the loss of skin elasticity and the formation of wrinkles.

Conventional aesthetic treatment approaches for the reduction of facial wrinkles include, *inter alia*, dermal fillers [2] and various injectable preparations [3], sometimes a combination of the two approaches with a microneedle fractional laser [4,5], and several cosmeceuticals, such as derivatives of retinol, vitamin C, and topical growth factors [6]. Conventional approaches are popular in many countries and are commonly used to treat facial ageing. However, most of these approaches have been found to be inadequate because of their limited clinical efficacy and safety. For instance, there are two categories of dermal fillers: absorbent and non-absorbent. Absorbent fillers include hyaluronic acid, calcium hydroxyapatite, poly L-lactic acid, polymethylmethacrylate, and collagen. Non-absorbent fillers include gelled silicon, and polyacrylamide hydrogel. Non-absorbent fillers are not recommended for use in Japan. According to the country's cosmetology practice guidelines stipulated by the Japanese Society of Cosmetic Surgery, non-absorbent fillers are associated with a high risk of difficulty in removal and unknown long-term safety issues [7]. Absorbent fillers are approved for use in Japan; however, because the active materials are absorbed by the body over time, their impact on skin regeneration is considered temporary. Moreover, dermal fillers are generally associated with adverse effects, such as infections, allergies, and intravascular embolism. Serious complications, including skin necrosis and blindness, have been reported with the use of hyaluronic acid preparations [7]. The injectable preparations are mostly *Clostridium botulinum* toxin-based products. Compared with all dermal fillers, *Clostridium botulinum* toxin preparations are relatively safe, but their efficacy is challenged by neutralizing antibodies produced in the body against toxins [7].

In this regard, regenerative medicine, which aims at improving the tissue regeneration process through a multi-prong problem solving approach of repairing and correcting physiological deficiencies related to cutaneous wound healing [8], offers several opportunities to enhance and promote wound healing. Such opportunities include the use of stem cells, growth factors, and direct application of biomaterials to induce regeneration or modify the skin wound environment and provoke a more effective healing process. The regenerative medicine-based therapies for different pathologies, including those of the skin, are generally considered safe [9], although there is still a need to overcome some limitations, especially those related to tissue origin and donor factors, discrepancies in isolation and culture procedures, risk of adverse effects, such as tumorigenicity and some ethical regulatory restrictions. Clinical investigations involving stem cells and stem cell-derived products are on-going, and industry and academic researchers across the world continue to explore new accelerated applications in the treatment of several diseases including skin ailments. The International Society for Stem Cell Research (ISSCR) has set global standards for stem cell research and clinical translation, stipulating new guidelines for preclinical research, clinical translation, and practice [10]. These guidelines emphasize the importance of high standards of cell processing and manufacturing and good manufacturing practice (GMP) in the preparation of stem cell-based therapeutics. The production or handling of regenerative medicine products must adhere to the guidelines set by individual nations or regions in accordance with the ISSCR and other international standards, such as the Declaration of Helsinki. In Japan, the regulations are implemented by following two authorities: the Pharmaceuticals and Medical Devices Agency (PMDA) and the Ministry of Health, Labor and Welfare (MHLW) [11]. Moreover, two laws have been enacted, viz., The Act on the Safety of Regenerative Medicine (RM Act) and the Pharmaceuticals and Medical Devices Act (PMD Act) [12]. The PMD Act defines regenerative medicine products as (1) processed human or animal cells intended for either (a) the reconstruction, repair, or formation of the structure or function of the human (or animal) body (i.e., tissue-engineered products), (b) the treatment or prevention of human (or animal) diseases (i.e., cellular therapy products); (2) articles intended for the treatment of disease in humans (or animals) and are genetically manipulated to express in human (or animal) cells (i.e., gene therapy products) (PMD Act Article 2 (9)). Articles 1—2 further specify following three product categories as regenerative medicine products: (1) Processed human cell products, such as adult stem cell products; (2) Processed animal cell products; (3) Gene therapy products, which are products that introduce genes to cells that are already in the human body (*in vivo*) or have been extracted from and then transplanted back into the human body (*ex vivo*) [12]. All regenerative medicine products are reviewed by the PMDA and approved by the MHLW following the Act on the Safety of Regenerative Medicine under one of the three risk categories. The classification depends on the degree of risk ranging from low to high. For instance class I is high risk for treatments using cells from a riskier source, such as embryonic stem cells, gene edited cells, or cells from another person, class II is medium risk for treatments

using cells from a patient, but performing a different function, such as stem cells derived from fat used to treat atherosclerosis or amyotrophic lateral sclerosis, and class III is low risk for treatments using cells from a patient and performing a function similar to the one they originally served, such as immune cells activated to fight cancer.

Regenerative medicine-based products can be classified into following two categories: cell-based and cell-free therapies. Both these categories can be autologous or allogeneic. The most common regenerative medicine-based products in the Japanese market include cell-based methods utilising autologous adult stem cells, such as mesenchymal stem cells (MSCs) [13], fibroblasts [14], emerging blood-derived cellular therapies, such as platelet-rich plasma (PRP) products [15,16], and the clinical application induced pluripotent stem cells (iPSCs) is progressing, with cell therapies for several ailments being under clinical investigations [17], especially those in line with the use of iPSC-derived products with less risk, such as the iPSC-derived MSCs (iPSC-MSCs) [18]. In a recent review by Hantae et al. [19] skin wound healing was described as comprising four superimposing phases including haemostasis, inflammation, proliferation, and maturation. The application of MSCs aids all phases of the wound healing process and reduces scarring through migration to skin injury site, inhibiting inflammation, and elevating the proliferation and differentiation potential of fibroblasts, epidermal cells, and endothelial cells [19]. Cell-free products are mostly based on the secretory components of MSCs, such as MSC-derived exosomes (MSC-exo), MSC-derived conditioned media (MSC-CM), and MSC-derived extracellular vesicles (MSC-EVs) [5,16]. Autologous transplantation is performed by isolating and culturing MSCs from the patient's own tissue sources, such as adipose MSCs (AD-MSCs), bone marrow MSCs (BM-MSCs), umbilical cord MSCs (UC-MSC), foetal dermis MSCs (FD-MSC), endometrial and menstrual blood (eMSC/MenSC). Autologous PRP is obtained by collecting and centrifuging the patient's own blood. The molecular mechanisms underlying the action of regenerative medicine-based therapies continue to be studied. However, the unique properties of MSCs, such as self-renewal, multidirectional differentiation, regulation of inflammation, immunomodulation, angiogenesis, and the haematopoietic abilities of PRP exhibited through the release of cytokines and various growth factors, are considered to play important roles in skin regeneration. This review provides a detailed account of the different regenerative medicine therapies and their application to skin improvement, and the Japanese regulatory guidelines concerning their usage.

2. Methodology

2.1. Literature search strategy

The literature search mainly focused on original articles on regenerative medicine and skin ageing treatment approaches that were written in English. Non-English articles were evaluated for pertinence and content of the relevant information. Databases including, inter alia, Medline, Embase, and Web of Science, were searched using various combinations of the following search terms: 'stem', 'cell', 'aging', 'wrinkles', 'nasolabial folds', 'therapy', 'mesenchymal stem cells', and 'skin'. A web search was conducted between July 2021 and August 2021 to investigate the clinical trial implementation status of cell therapy for skin rejuvenation. The following websites were surveyed: ClinicalTrials.gov (https://clinicaltrials.gov/), a list of submitted regenerative medicine provision plans (Ministry of Health, Labour and Welfare) (https://saiseiiryo.mhlw.go.jp/published_plan/index/1/2), (https://saiseiiryo.mhlw.go.jp/published_plan/index/1/3), and jRCT (https://jrct.niph.go.jp/search).

2.2. Cell-based therapies

2.2.1. Treatment for skin using MSCs

MSCs are adult stem cells known for their multi-lineage differentiation capacity, immunomodulation, regulation of inflammation, as well as homing and protection of other cells from peroxide-mediated damage [9,20]. MSCs can be isolated from various tissues for therapeutic and cosmetic applications. In a review by Ntege et al. [9], the primary MSC tissue sources among others include AD-MSC, BM-MSC, UC-MSC, and eMSC/MenSC. In addition, adult stem cells, such as the iPSC-derived MSCs, may perform equally or even better than the primary MSCs for different tissue treatments including those for skin [21]. However, unlike iPSC-derived MSCs, the primary MSCs are easier to cultivate and may be subjected to lesser stringent regulations [22—24]. Specifically, the immune tolerance and the ability of MSCs to migrate to damaged tissue by chemo-attraction, stimulate angiogenesis, and modulate inflammation renders them suitable for skin regeneration and associated wound healing [19,24].

1) AD-MSCs

AD−MSCs have been widely studied and are attracting attention in skin anti-ageing treatment because of their efficiency in re-epithelialization and secretion of multiple growth factors necessary for skin regeneration. They are also preferred because of their abundance, ease of isolation, and resilience during collection. AD-MSCs are known to improve skin regeneration by increasing angiogenesis and synthesizing collagen and other dermal elastic matrix components, such as oxytalan, elaunin, and elastin fibres [25,26]. In skin photoageing, the elastin network in the deep dermis undergoes progressive degeneration. The elastin fibres become thickened, tangled, tortuous, degraded, and non-functional, leading to loss of skin elasticity. AD-MSCs play a role in the restoration of damaged or lost elastic fibre networks. As a result, the three-dimensional structure of the reticular layer of the dermis is modified, and the microvascular bed is increased. Furthermore, by activating the proteolytic enzymes, cathepsin K and matrix metalloproteinase-12 (MMP-12), and expanding the infiltration of the anti-inflammatory cytokine, M2 macrophages, elastin is formed in the deep dermis [25].

Experimental studies have demonstrated that AD-MSCs can improve the quality of ageing skin. For example, Charles-de-Sá et al. [20] reported a rejuvenating effect in six patients aged 45−65 years from their clinical trial wherein they administered AD-MSCs for a face lift. A combination of hyaluronic acid and AD-MSCs has also demonstrated effectiveness in the improvement of skin wrinkles, as hyaluronic acid is an excellent vehicle for MSCs in tissue repair [27]. In 2020, Brazilian researchers combined AD-MSCs with meglumine antimoniate and controlled lesion development and parasite load in cutaneous leishmaniasis, a neglected disease caused by Leishmania spp. [28]. Using a murine model of cutaneous leishmaniasis caused by Leishmania amazonensis, Ramos et al. [28] observed a reduction in lesion size and parasite load in AD-MSCs combination groups compared with that in the control infected C57BL/6 mice treated with meglumine antimoniate and phosphate-buffered saline. Production of small amounts of IL-10 was detected at the infection site, but the production of either IL-4 or interferon-γ was not detected, indicating resolution of infection without effect on the percentage of regulatory T cells. These results suggest that combination treatment of cutaneous leishmaniasis with AD-MSCs and meglumine antimoniate may be a viable alternative in treating cutaneous leishmaniasis.

According to the regulatory framework for regenerative medicine in Japan, a number of stem cell-based clinical investigations are on-going under the provisional approval by MHLW; these include AD-MSC-based treatment for skin conditions, such as wrinkles and loss of elasticity, which is commonly implemented in

dermatology or aesthetic surgery clinics under class II and III of the regenerative medicine risk classification [11,12]. For instance, in a recent review [9], we reported about our MHLW-approved clinical treatment for depressed skin lesions using cultured AD-MSCs, conducted in compliance with other ethical regulatory bodies, such as the International Conference on Harmonization's Good Clinical Practice standards, and the Declaration of Helsinki (registered under one identifier at UMIN-CTR Clinical Trial (https://www.umin.ac.jp/ctr/) with unique ID number; UMIN000020530). This was the first such phase I/II trial for clinical evaluation of the concept of repairing soft tissue defects with fat grafts combined with culture expanded ASCs in Japan and revealed an important role in the treatment of skin depressed or altered scars and other surgical problems. Other examples in Japan include the several investigation plans, provisionally accepted by MHLW, at Ometesando Helene Clinic in Tokyo; these include autologous facial fat transfer and application of stem cells for external facial use [29].

2) BM-MSCs

Unlike AD-MSCs, BM-MSCs have the disadvantages of limited donor availability and low yield [30]. However, BM-MSCs exhibit high differentiation and proliferation rates. In addition, BM-MSCs have demonstrated regenerative abilities, such as promotion of angiogenesis and scarless healing, increased collagen production, as well as excellent homing and interaction abilities with local cells in the skin [31–33]. BM-MSCs promote wound healing through indirect down-regulation of inflammatory mediators, converting tissue-resident macrophages from pro-inflammatory (M1) to anti-inflammatory macrophages (M2) [34].

Several preclinical studies have demonstrated the effectiveness of BM-MSCs in skin regeneration, including those on mouse and rat models that showed increased angiogenesis and increased type I collagen and integrin $\alpha 2\beta 1$ levels, which accelerated the healing of burns without tumorigenesis [35–37].

The potential of BM-MSCs in treating skin diseases was previously reviewed by Nemeth et al. in 2015 [38]. Thereafter, numerous clinical investigations have either been completed or are still under way for a number of diseases including photoageing (NCT01771679), scleroderma (NCT02213705), epidermolysis bullosa (NCT02582775), atopic dermatitis (NCT04723303), and diabetic skin ulcers [39]. The application of autologous BM-MSCs for burn wound therapy showed a reduction in scar formation and restoration of elastin fibres [40,41], and the generation of subcutaneous tissue from BM-MSCs showed effectiveness in tissue regeneration in 20 patients with intractable skin diseases [42]. Leonardi et al. [30] combined BM-MSCs with artificial dermal substitutes and demonstrated increased vascular density and re-epithelialisation. In Japan, as a cellular and tissue-based product, BM-MSCs have been approved by MHLW; these include TEMCELL HS Inj., a human (autologous) BM-derived MSC marketed by JCR Pharmaceuticals Co. Ltd., for the treatment of acute graft-versus-host disease (aGvHD) in children and adults [43]. aGVHD can principally target the skin with clinical manifestation of mostly a pruritic or painful maculopapular rash, which initially involves the nape of the neck, ears, shoulders, the palms of the hands, and the soles of the feet in the acute phase, and then spreads to involve the whole integument, eventually becoming confluent. In severe GVHD, the maculopapular rash forms bullous lesions with toxic epidermal necrolysis mimicking the Stevens–Johnson syndrome.

3) UC-MSCs

Similar to other MSCs, a clinical study of UC-MSC therapy in patients with psoriasis (NCT03765957) confirmed it as a safe alternative. In addition, a research group from South Korea recently reported successful treatment, without reoccurrence, of a 47-year-old male patient with long-term intractable psoriasis for 25 years, with three rounds of minimally manipulated UC-MSCs for a period of over 2 weeks [44]. UC-MSCs have good homing abilities, reduce inflammatory cells, and increase the levels of interleukin-1 (IL-1), IL-6, IL-10, tumour necrosis factor alpha (TNF-α), and tumour necrosis factor-stimulated gene-6 (TSG-6), which promotes wound healing and skin regeneration [45]. In addition, UC-MSCs promote neo-vascularisation by increasing the release of vascular endothelial growth factor (VEGF) and the production of collagen types I and III. Liu et al. [45] examined the effect of human UC-MSCs on wound healing in a rat model with severe burns and observed accelerated wound healing through UC-MSCs increased migration, modulation of the inflammatory environment, and promotion of the formation of a well-vascularised granulation matrix and collagen scaffold. In Japan, Nagamura et al. from the Institute of Medical Science at the University of Tokyo have for a long time been interested in the exploration of UC-MSCs in treating severe aGVHD. In 2019, they demonstrated that UC-MSCs have diverse immunosuppressive potency through migration towards activated lymphocytes, suppressing activated T cell proliferation, regulatory T cell induction, and transition of the monocyte phenotype. Moreover, UC-MSCs are feasible and abundant sources of immunotherapy [46].

4) Human endometrial and mesenchymal stem-like cells (eMSCs)

eMSCs are recently identified MSCs, which are easily obtained using minimally invasive procedures on the highly regenerative endometrial lining of the uterus [47]. Endometrial stem/progenitor cells in the menstruation blood are defined as menstrual stem cells (MenSCs). Therefore, eMSCs/MenSCs are similar peri-vascular cells identified by comparing the cloning efficiencies of endometrial stromal cells purified either through cell sorting by determining the co-expression of CD140b and CD146 or by magnetic bead sorting using a single marker, sushi domain containing 2 protein identifier, the W5C5 antibody [48]. eMSCs/MenSCs have excellent therapeutic potential for tissue repair and regeneration probably owing to their specific roles in regulating angiogenesis, inflammation, and fibrosis [49]. In a recent review by Bozorgmehr et al. [49], the reparative properties of MenSCs demonstrated by researchers from Chile were reported, providing evidence of consistent wound healing and increased angiogenesis following intradermal injection of MenSCs in a murine excisional wound splinting model. The Chile scientists demonstrated an up-regulation of two pro-angiogenic genes, *Il-8* and *Vegf*, and maturation of vasculature in the MenSC-injected group of murine wounds. In addition, high density and well-organised collagen fibres were observed, suggesting the potential of MenSCs in wound healing and cutaneous regeneration. Moreover, the application of eMSCs promotes wound healing by reducing the secretion of inflammatory cytokines, IL-1β, IL-6, and IL-12β. Moreover, fibroblasts differentiated from eMSCs travel to wound sites and synthesise matrix proteins, such as collagen and fibronectin [50]. In addition, eMSCs increase IL-13 production and anti-inflammatory effects by activating nuclear factor-κB (NF-κB) [51]. Studies in mouse pressure ulcer models have shown increased angiogenesis and wound healing in the eMSC-treated group compared with those in the control group [50]. Additional evidence for tissue reparative properties of eMSCs was reported in 2018; Darzi et al. [47] attempted to develop and evaluate a new type of mesh for potential clinical use in pelvic organ prolapse treatment. They showed the efficacy of delivering human eMSCs in a small animal model of wound repair through the mesh. The human eMSCs exerted a paracrine effect promoting wound healing, angiogenesis, and new tissue formation in this xenogeneic model.

5) iPSCs

The application of iPSCs in skin regeneration has recently been described [52]. In disease conditions with complications, such as chronic non-healing of cutaneous wounds, application of primary adult stem cells is often challenged by the small number of cells and invasive methods of obtaining them. However, these challenges can be minimised by using iPSCs as a promising alternative source of MSCs. Currently, there are advanced technologies and innovative methods of cell controlled differentiation which can be utilised to produce iPSC-derived cells from all three germ layers that accelerate each phase of cutaneous regeneration and wound healing. Such iPSC-derived products include iPSC-MSCs, iPSC-derived keratinocytes, endothelial cells (ECs), as well as growth factors. iPSC-derived keratinocytes and ECs were reported to be widely used in preclinical models of acute and chronic wounds including diabetic wounds, burn wounds, and skin diseases, like epidermolysis bullosa [52]. Furthermore, Choudhury et al. [52] reported on the evidence of increased healing through transplantation of iPSC-derived ECs into full-thickness skin wounds in non-diabetic-severe combined immunodeficiency mice by other researchers. In Japan, investigations on clinical application of iPSCs are on-going for several diseases [17] and iPSC-derived MSCs were demonstrated by Nakayama et al. [53] to increase type VII collagen in mouse wound models that promoted epithelialisation and release the anti-inflammatory cytokine, TSG-6 [54], in the healing of skin injuries.

2.2.2. Treatment for skin using fibroblasts

Fibroblasts are major connective tissue cells that synthesise collagen and other extracellular matrix (ECM) proteins. Furthermore, they secrete soluble cytokines and growth factors, such as TGF-β, keratinocyte growth factor (KGF), VEGF, and insulin-like growth factor (IGF), to maintain skin structure [55]. The ECM is mainly composed of type I collagen produced by fibroblasts and determines the firmness and strength of the skin. Since the function of fibroblasts and their ability to synthesise the ECM decreases with age, it is predicted that, among other methods, transplantation of fibroblasts could promote the synthesis of ECM and improve skin wrinkles [56]. Epicel, the earliest successful cell-based therapy for structural repair, uses cultured epidermal keratinocytes. Epicel was developed and commercialised by Biosurface Technology [57]. Epicel is a skin graft grown from healthy skin that provides skin replacement for patients with dermal burns that cover more than 75% of the total body surface area and is approved by the Food and Drug Administration (FDA) as a 'humanitarian use device'. Epicel is currently manufactured and marketed in the United States by Vericel Corporation without prior clinical trials [58]. In 1990, the effects of autologous fibroblast transplantation on wrinkles and acne scars were reported [59]. In 1995, Isolagen (currently Fibrocell Science) from the United States transplanted autologous dermal fibroblasts into the skin and put into practical use a treatment method to improve the properties of the skin [60]. Apligraf, a bioengineered allogenic skin substitute, was developed by Organogenesis (Canton, MA), for the treatment of venous leg ulcers and diabetic foot ulcers. Apligraf is constructed by culturing human foreskin-derived neonatal fibroblasts in a bovine type I collagen matrix over which human foreskin-derived neonatal epidermal keratinocytes are subsequently cultured. Apligraf received FDA approval in 1998 for the treatment of venous leg ulcer and in 2000 for the treatment of diabetic foot ulcer [58].

In Japan, treatment of skin ageing using fibroblasts has been approved as a class II regenerative medicine risk classification [11]. First, the skin behind the auricle of a patient is harvested, and the fibroblasts are isolated and cultured before being transplanted back to the same patient. In the United States, Isolagen's autologous fibroblast product (trade name: LAVIV®) was approved by the US FDA in 2001, and the product is manufactured with the aim of improving the nasolabial fold in adults. Moreover, LAVIV® was demonstrated to be effective in improving acne scars [61]. In Japan, human (autologous) epidermis derived cell sheet (JACE) was approved by the MHLW for the treatment of severe burns (in 2007), giant congenital melanocytic nevi (in 2016), and epidermolysis bullosa (in 2018) [43].

2.2.3. Treatment for skin using melanocytes

The epidermis, beginning from the external surface, consists of the stratum granulosum, stratum spinosum, and stratum basale. The stratum basale, or the basal layer, contains melanocytes that produce melanin. Vitiligo is a disease in which melanocytes disappear or decrease, resulting in loss of pigment and whitening of the skin [62]. The exact prevalence is unknown but the incidence has been reported to be 1 in 20,000 [63]. Treatment of vitiligo includes topical steroids and immunosuppressants combined with phototherapy. However, this treatment approach is inadequate for some patients. Such patients can benefit from autologous epidermal transplantation [62]. However, epidermal transplantation without culture necessitates the collection of epidermis from normal skin tissue around the same location affected by vitiligo, thereby, restricting the amount of skin that could be transplanted. In contrast, culture expansion in autologous epidermis transplantation favours transplantation to large vitiligo-affected areas, even if the skin collection area is small. In Japan, autologous epidermal transplantation is classified as a class II regenerative medicine risk classification [11]. After collecting and culturing the epidermis from the patient, a sheet-shaped cultured epidermis is transplanted into the vitiligo-affected areas. Upon transplantation, melanocytes settle in the area and produce melanin [62]. This aids in restoring skin colour in the affected areas. A melanocyte-containing cultured epidermis sheet product (ACE02) is currently developed and marketed by Japan Tissue Engineering Co., Ltd. (J-TEC) in Miyakitadori, Gamagori, Aichi, Japan.

2.2.4. Treatment for skin using blood-derived cellular therapy

PRP is plasma in which platelets are concentrated, and it is collected from the patient's own blood through centrifugation. By causing an agglutination reaction in platelets and activating them, cytokines and growth factors that are effective in wound healing are released. PRP growth factors include fibroblast growth factor (FGF-2), platelet-derived growth factor, VEGF, epidermal growth factor, TGF-β, and IGF-1. These growth factors are involved in the activity of specific biomolecules and are known as wound healing factors. In vitro studies in dermatology have shown that PRP can stimulate the proliferation of human skin fibroblasts and increase the synthesis of type I collagen [64]. Furthermore, subcutaneous injection of PRP deep into the human dermis has been shown to induce fibroblast activation, new collagen deposition, and formation of new blood vessels and adipose tissue [65]. In Japan, PRP therapy for skin ageing symptoms, such as wrinkles and loss of skin elasticity, is approved as a class III regenerative medicine risk classification [12,43].

2.3. Cell-free therapies

MSC derived extracellular vesicles/exosomes demonstrate tissue repair effects similar to those of MSCs, through the modification of gene expression and protein production in recipient cells. They also activate pathways, such as the Wnt/β-catenin, AKT, ERK, and STAT3 pathways associated with regeneration [66—68]. The regenerative impact of exosomes derived from MSCs on lung, heart, kidney, liver, and brain tissues was recently investigated and they were found to

be promising as therapeutic agents for cell-free regeneration medicine-based therapy. Moreover, emerging evidence indicates MSC-derived exosomes increase the proliferation and migration of skin cells and inhibit apoptosis during wound healing.

2.3.1. Treatment of skin using cell-derived extracellular materials

Treatments using the secretory components of MSCs, such as MSC-exos, MSC-CM, and MSC-EVs, have been reported [16].

2.3.1.1. Adipose-derived MSC-derived extracellular materials.
AD-MSC-exo promotes the proliferation and migration of fibroblasts and improves wound healing by optimising collagen deposition [69]. It also promotes epidermal barrier repair by inducing de novo synthesis of ceramide [70]. Non-clinical studies include mouse models of atopic dermatitis [71], acne scars [72], photoageing [73], and skin injury [74]. Acne is a stressful chronic skin disease. As previously described in section 2.2.1, AD-MSCs increase wound healing. Zhou et al. [75] demonstrated that conditioned media of MSCs derived from adipose tissue was effective in treating 13 patients with atrophic acne scars via increased hydration and skin elasticity, higher concentrations of dermal collagen, and a higher elastin density. Because of its ability to release different growth factors, including fibroblast growth factor-basic (bFGF), KGF, TGF-β1, HGF, and VEGF, AD-MSC-exo can eliminate wrinkles and improve facial defects through an increased production of dermal collagen and migration of fibroblasts into the dermis.

AD-MSC-CM has antioxidant activity and protects fibroblasts from oxidative damage by reducing apoptosis [14]. It also increases the expression of type I and type III collagen, elastin, and endogenous protease inhibitors, such as tissue inhibitor of metalloproteinase-1, and decreases the expression of MMP-1 and MMP-9 [76]. Non-clinical studies have also been conducted in mouse models of oxidative damage [14].

AD-MSC-EV increases the activity of fibroblasts and protects them from ageing caused by UVB waves. It also suppresses the cell cycle arrest of fibroblasts by suppressing the differentiation of M0 into M1 macrophages, reducing the production of active enzymes in cells, and promoting the expression of antioxidant enzymes [16]. Non-clinical studies have been conducted in a mouse photoageing model [16].

2.3.1.2. Bone marrow MSC-derived extracellular materials.
BM-MSC-exo induces the differentiation of M2 macrophages, an anti-inflammatory cytokine [77]. In addition, preclinical studies in mouse and dog injury models have shown that BM-MSC-exo-derived microRNA accelerates wound healing by promoting collagen synthesis and angiogenesis at the wound site [77,78].

2.3.1.3. Umbilical cord mesenchymal stem cell-derived extracellular materials.
MicroRNAs derived from UC-MSC-exo reduce the expression of inflammatory factors, such as toll-like receptor-4 and the activation of NF-κB p65, and control or suppress inflammation [16]. In addition, preclinical studies in rat burn models and rat wound models showed that UC-MSC-exo suppressed the expression of excessive smooth muscle actin and reduced the deposition of type I collagen by inhibiting TGF-β2 and SMAD2 [79,80].

2.3.1.4. Amnion MSC-derived extracellular materials.
AM-MSC-CM suppresses TGF-β-induced expression of α-smooth muscle actin [81]. In clinical trials, AM-MSC-CM was administered three times at 2-week intervals to prevent light-induced ageing [81].

2.3.1.5. Foetal dermal MSC-derived extracellular materials.
Preclinical studies in mouse injury models showed that FD-MSC-exo promotes the wound-healing ability of fibroblasts by activating the Notch signalling pathway, which plays an important role in the MSC self-renewal process [75].

2.3.1.6. Induced pluripotent stem cell-derived MSC-derived extracellular materials.
IPSC-MSC-exo increased the production of collagen and fibronectin in keratinocytes and fibroblasts, respectively [82]. In addition, preclinical studies in rat wound models showed that iPSC-MSC-exo promoted the proliferation of skin cells by activating the extracellular signal-regulated kinase-1/2 pathway [83].

3. Conclusions

Epidermal cell replacement processes decrease with age, resulting in the loss of elasticity and wrinkling of the skin. Skin ageing is mainly caused by decreased production of collagen and fibroblasts. In addition, disruption of the relationship between fibroblasts and other cells, such as dermal mast cells, epidermal keratinocytes, and adipocytes, exacerbates skin ageing. The suitability of regenerative medicine-based therapies for skin regeneration is based on properties, such as multi-directional differentiation, self-replication, immuno-modulation, regulation of inflammation, angiogenesis, and haemostasis, which are exhibited through the release of various cytokines and growth factors. This review updates the therapeutic effects of MSCs and their extracellular components on skin regeneration. It also highlights regulatory perspectives on the clinical application of some of these therapies in Japan. Although the clinical application remains challenging, regenerative medicine-based products are expected to offer relatively safe and more effective skin regeneration treatment options in the future.

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