Chapter 8

# The Potential of Cd34+ Hematopoietic Stem Cells to Increase Fibroblast and Collagen Skin in Ultraviolet B-Exposed Skin

Mochamad Syaifudin, Wimpie Pangkahila, Ida Sri Iswari, Basuki Supartono, Mochamad Wildan

## A. Introduction

Aging is a process characterized by the decline and death of cells, which occurs with advancing age. The skin, which accounts for approximately 16% of the human body weight, is frequently exposed to sunlight, toxic substances, air pollution, and heavy metals daily (Parrado et al., 2019). The aging process affects all organs, including the human skin. Skin aging can be attributed to extrinsic factors, such as exposure to ultraviolet (UV) light, cigarette smoke, and air pollution, as well as intrinsic factors, such as genetics, race, and hormones (Krutmann et al., 2021).

Several studies demonstrate that the aging process causes a decline and depletion in the number of Langerhans cells in the

M. Syaifudin, W. Pangkahila, I. S. Iswari, I. G. K. N. Arijana, B. Supartono, M. Wildan

Clinic Regenerative Medicine MMC Lamongan, e-mail: msyaifudin1608@gmail.com



Syaifudin, M., Pangkahila, W., Iswari, I. S., Arijanam I. G. K. N, Supartono, B., & Wildan, M. (2025). The potential of Cd34+ hematopoietic stem cells to increase fibroblast and collagen skin in ultraviolet B-exposed skin. In B. Supartono & A. Noviantari (Eds.), *Discovering the miracle of stem cells* (183–221). BRIN Publishing. DOI: 10.55981/brin.1128.c1302, E-ISBN: 978-602-6303-50-9

epidermis. These cells are known as immunogen-effector cells in the skin and their reduction results in decreased resistance after exposure to the environment (Said et al., 2015). In the epidermal layer, there is a narrowing of the dermo-epidermal junction. Additionally, there is atrophy of the dermal layer, a reduction in fibroblasts, mast cells, and blood vessels, as well as abnormal nerve endings. Other changes include hair loss of pigment, abnormal nail beds, a reduced number of glands in cell size, reduced melanocyte cells, and a decrease in Langerhans cells (Niculet et al., 2020).

Ultraviolet (UV) light is considered one of the most important factors in premature skin aging, often referred to as photoaging (Amaro-Ortiz et al., 2014). Photoaging occurs when the skin is chronically and repeatedly exposed to UV rays over some times. UV light is one of the spectra from sunlight that reaches the earth, in addition to visible light and infrared light (Guan et al., 2021) evidence has also shown their efficacy in the prevention of photoaging, dyspigmentation, DNA damage, and photocarcinogenesis. In the USA, most broad-spectrum sunscreens provide protection against ultraviolet B (UVB.) Based on wavelength, UV rays can be further divided into ultraviolet A (UVA), ultraviolet B (UVB), and ultraviolet C (UVC) rays. Chronic exposure to UVA and UVB rays plays a significant role in photoaging and photocarcinogenesis (Bosch et al., 2015).

Exposure to UVB rays affects skin tissue and produces free radicals, which cause damage at the cellular level and ultimately lead to the death of collagen fiber cells and fibroblast cells (Yin et al., 2019). Collagen is a polypeptide that adopts a triple helix form, with each chain composed of glycine-X-Y linkages. The chains can be damaged by certain enzymes, resulting in the release of glycine groups. Currently, dermal collagen staining is identified based on the amount of glycine. Furthermore, glycine staining can be expressed using the Sirius Red dye (Chen et al., 2019).

A promising method is the utilization of stem cells. Stem cells are cells that can form and structure body tissues. They are early-life cells that can develop into other cells and form various tissues in the

body (multipotent). Furthermore, when stem cells are transplanted into the body, they will form body tissues in that specific location. The characteristics of stem cells are undifferentiated, self-renewal, and the ability to differentiate into more than one cell type (multipotent/pluripotent) (Romito & Cobellis, 2015).

Hematopoietic stem cells are progenitor cells that form blood cells. The sources of these cells are bone marrow and blood. Hematopoietic stem cells can be isolated directly from peripheral blood or through mobilization techniques. These stem cells possess pluripotent and plastic properties, allowing them to differentiate into non-hematopoietic cells (Ogawa et al., 2015). Research on the subcutaneous administration of human peripheral blood CD34+ stem cells to the skin of male Wistar rats exposed to UVB, while observing the number of fibroblast and collagen cells, has never been conducted. This article will discuss the potential of CD34+ hematopoietic stem cells to increase fibroblast and collagen skin in ultraviolet B exposed skin.

## B. Skin

Skin is the largest organ in the human body. The appearance of the skin provides information about the individual, such as their overall health, ethnicity or race, lifestyle, and age. The quality of the appearance of the skin is determined by skin color, texture, and shape. As the largest organ in the human body, the skin has a surface area of 1.5–2 $m^2$ and accounts for about 15% of the total body weight of an adult (Meléndez-Martínez et al., 2019).

Skin is made up of three layers, from outermost to innermost: epidermis, dermis, and hypodermis (subcutaneous tissue). The epidermis, or skin's outermost layer, serves as a waterproof barrier and helps skin tone. The epidermis consists of five layers: stratum corneum, stratum lucidum, stratum spinosum, stratum granulosum, and stratum basale (Yousef et al., 2020). The epidermis is a dynamic structure, of which 95% is composed of differentiated keratinocytes. Other cells in the epidermis include melanocytes, Langerhans cells, and Merkel cells. Melanocytes are melanin-producing cells, which

are the pigment of the skin. Langerhans cells have an immunological function, and Merkel cells play a role in sensory perception (ter Horst et al., 2018).

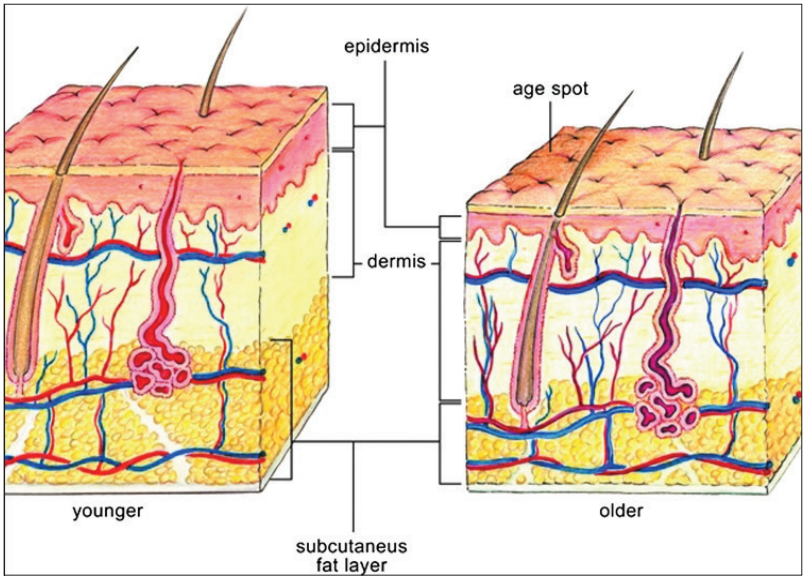
The dermis is divided into two layers: the papillary dermis on the surface and the reticular dermis beneath. The papillary dermis contains collagen, elastin, fibrous, and ground substance (mucopolysaccharides, hyaluronic acid, chondroitin sulfate) and is rich in microcirculation. The reticular dermis contains coarser bundles of collagen with scattered elastin fibers (Brown & Krishnamurthy, 2022).

Aging is an irreversible and natural phenomenon that occurs as a person ages. It is characterized by a gradual decline in the function of various organs and systems in the body. As a result of this reduction in function, many indications and symptoms of aging develop, which are divided into two parts, namely:

1) physical symptoms such as muscle mass, increased fat, wrinkled skin, decreased memory, impaired sexual function, decreased work ability, and bone problems (Amarya et al., 2018); and
2) psychological symptoms include decreased vitality, difficulty sleeping, anxiety, irritability, and feeling worthless (Kang & Kim, 2022).

Anti-aging medicine is defined as a branch of medicine that uses the latest scientific knowledge and medical technology to early detection, prevention, treatment, and reversal of age-related dysfunctions, disorders, and diseases. The goal of anti-aging medicine is to prolong life in a healthy state (Ok, 2022).

The structure of old and younger skin is indeed structurally different (Figure 8.1). In general, the epidermis was thicker in young compared to old skin. Younger epidermis has smoother epidermis. In older skin, the epidermis becomes thinner and flatter, and cell turnover slows down (Mine et al., 2008). The total thickness of the skin in old age decreases due to the loss of collagen and elastin in the dermis; collagen fibers become thicker and irregular than younger skin, reducing the elasticity of the skin. Collagen, the protein molecules made up of amino acids, is the main constituent of the dermis and is




Source: Farage et al. (2013)

**Figure 8.1** Differences between Younger and Older Skin in Skin Structure

made of fibroblast collagen fibers. Young skin has a thick dermis rich in collagen and elastin fibers. As we age, collagen fibers become sparser, more damaged, and less able to support the skin (Karim et al., 2021).

The hypodermis, the deepest skin layer, is the subcutaneous fat layer on the skin. Fat, blood vessels and nerves are the main structural components of the hypodermis. It has many important functions, including storing energy, connecting the dermal layer of the skin to muscles and bones, insulating the body, and protecting against damage. Even though the hypodermis is located in the deepest layer of skin, aging also occurs in this layer, causing loss of fat on the hands and face, as well as increasing fat in the area between the hips and ribs (Liang et al., 2023).

Chronological aging is a natural process of physiological change and is influenced by genetic and hormonal factors. Chronological aging is characterized by xerosis, sagging, wrinkles, sluggishness, and

**Table 8.1** Histological Manifestations of Chronological Skin Aging

| Epidermis | Dermis | Other Tissues |
|---|---|---|
| Flattening of dermo-epidermal junction | Atrophy (decrease in dermis volume) | Hair depigmentation |
| Change in thickness | Changes in skin supporting tissue | Hair loss |
| Varying cell shape and sixe | Decreased fibroblast | Conversion of terminal hair to vellus hair |
| Atypical cell nuclei | Decreased mast cell | Nail plates abnormal |
| Decreased melanocyte | Decreased blood vessel | Abnormal glands |
| Decreased Langerhans cells | Shortening capillary looAbnormal nerve vessels | |

the appearance of seborrheic keratosis and cherry angiomas (Karim et al., 2021). In the epidermal layer (Table 8.1), the most consistent structural changes in aged skin include flattening of the dermo-epidermal junction (Lynch et al., 2022), variation in thickness, changes in the size and shape of cells, occasional atypical nuclei, a decrease in the number of melanocytes, and a reduction in the number of Langerhans cells (Papaccio et al., 2022).

Dermis thickness decreases with age. In the dermis layer occurs atrophy, fibroblasts decrease, mast cells decrease, blood vessels decrease, capillary loops shorten, and nerve endings become abnormal as the result of aging. Other changes include hair loss of pigmentation, hair loss, terminal hair becoming fine hair, abnormal nail bed, and decreased glandular number (Farage et al., 2013).

Relatively few changes occur in the thickness of the epidermis, the shape of keratinocytes, and the cohesion of corneocytes, and there is a significant loss of melanocytes and Langerhans cells. The major skin changes in chronological skin aging are seen at the dermo-epidermal junction, which shows flattening of the rete ridges, which causes a reduction in contact between the epidermis and dermis, leading to a reduction in nutrient and metabolite exchange between these two compartments (Rittié & Fisher, 2015).

Skin aging is a complex biological process that is a consequence of both intrinsic and extrinsic factors. Intrinsic aging, also known as

chronological aging, results in changes in all layers of the skin (Karim et al., 2021). The epidermis undergoes a slowdown in regeneration. In young skin, epidermal turnover takes 28 days, but in old age, it takes 40–60 days. This slowdown results in thinning of the epidermis, making the skin appear translucent. The slowdown in epidermal regeneration also disrupts the skin's defense and repair functions (Kim & Leung, 2012).

Keratinocytes accumulate on the surface of the skin, making it appear rough and scaly. Histology of old skin shows thinning of the dermal-epidermal junction, which increases skin fragility and reduces nutrient transfer to the epidermis and dermis (Karim et al., 2021).

The population of melanocytes in the epidermis decreases and the existing melanocytes experience a decrease in activity. Old skin experiences dyschromic changes such as pigmented spots, freckles, and lentigines. Old skin is also more susceptible to sunburn because the skin is thinner and has fewer melanocytes (Karim et al., 2021). Skin aging also affects Langerhans cells. The number of Langerhans cells decreases by up to 50%, resulting in a decrease in skin immunity and an increased risk of skin cancer (Chambers & Vukmanovic-Stejic, 2020).

The dermis appears hypocellular, with fewer fibroblasts and mast cells and loss of dermal volume. Electron microscopy studies have shown that collagen fibers become loose and there is a moderate increase and thickening of elastin fibers with resorption of most sub-epidermal fibers. In addition, there is a decrease in the number of dermal blood vessels, a shortening of capillary loops, and a decrease in the density of Pacinian corpuscles and Meissner's corpuscles, which are skin-end organs responsible for the perception of pressure and light touch. Loss of sensory and autonomic innervation involves the epidermis or dermis (Russell-Goldman & Murphy, 2020).

## C. UV Rays and Photoaging

Ultraviolet (UV) radiation is a type of nonionizing radiation that can be found at the lower end of the electromagnetic spectrum, between

X-rays and visible light. UV is an invisible electromagnetic radiation with a wavelength range of 100–400 nm. UV can be divided into four wave bands: vacuum UV, UVC (200–280 nm), UVB (280–315 nm), and UVA (315–400 nm) (Williamson & Neale, 2022).

UV radiation is classified as a "complete carcinogen" since it is a mutagen as well as a non-specific damaging agent, as well as a tumor starter and promoter. UV is the most important modifiable risk factor for skin cancer and many other environmental-influenced skin illnesses when it is abundant. UV, on the other hand, enhances human health by mediating natural vitamin D and endorphin synthesis in the skin; thus, UV has complicated and mixed impacts on human health (Amaro-Ortiz et al., 2014).

The three categories of UV radiation markers are as follows (de Jager et al., 2017).

1) UVA (320–400 nm)

   The most common UV radiation encountered since it passes through air ozone with little alteration. When UVA is overexposed, it causes pigment darkening (tanning) followed by sunburn. Although UVA is required for Vitamin D generation in humans, excessive exposure can cause epidermal hardening, immune system suppression, and cataract formation. UVA is often utilized in cosmetics, the production of sunbeds, or tanning booths.

2) UVB (290–320 nm)

   UVB is a crucial contributor to photochemical DNA damage. UVB is also required for the formation of Vitamin D in humans. However, excessive exposure may be damaging to the human body. These negative effects include sunburn, cataracts, and the start of the carcinogenic process in the skin.

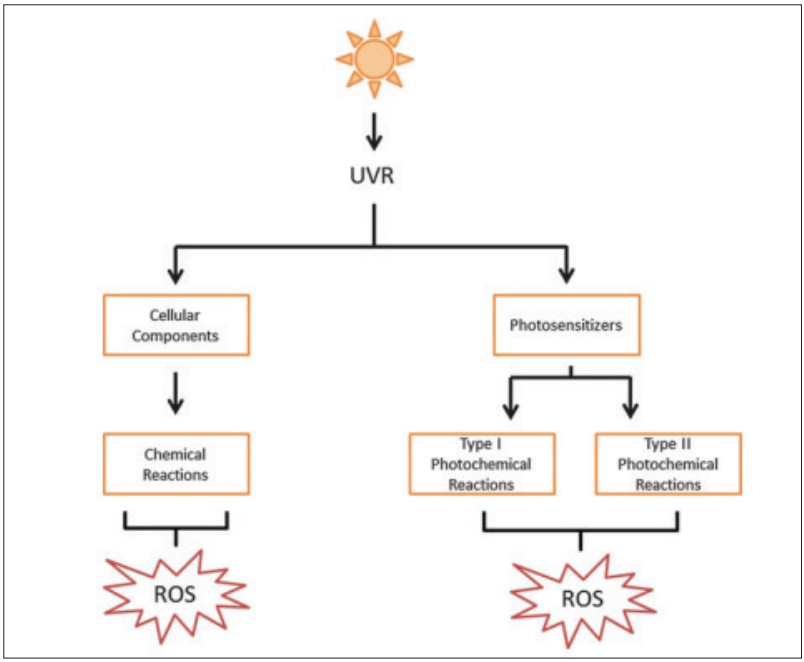
3) UVC (220–290 nm)

   UVC is almost entirely absorbed by ozone in the atmosphere and has little effect on human health. UVC is emitted by germicidal lamps to kill germs. Exposure to UVC by humans may result in

ocular burns and snow blindness. Because UVC is absorbed by the dead outer layer of the dermis, exposure can produce acute pain that subsides in a few days.

UV radiation triggers the formation of reactive oxygen species (ROS), which can damage DNA and inhibit the activity of tyrosine phosphatase. UV can also reduce retinoic acid receptor (RA) and trigger an increase in nuclear factor-kappaB (NF-kappaB), with a final effect of reducing collagen production, and collagen breakdown, due to the activity of matrix metalloproteinases (MMPs) (Gromkowska-Kępka et al., 2021).

UV radiation can mediate damage to cellular components in two ways (Figure 8. 2). The first mechanism involves the direct absorption of incident rays by the cell and its components. This results in the production of an excited state of the components and subsequent




Source: Jager et al. (2017)

**Figure 8.2** Mechanism of UV radiation mediates cellular damage.

chemical reactions. The second mechanism is photosensitization. Incident rays are absorbed by endogenous or exogenous photosensitizers such as bilirubin. As a result, the sensitizers are excited to their triple states. The excited photosensitizers work in two ways. Type I photochemical reactions involve electron transport and the process of hydrogen abstraction to create free radicals. Type II photochemical reactions require the transfer of energy with $O_2$ to produce reactive state singlet oxygen (1 $O_2$) (de Jager et al., 2017).

Photoaging refers to the skin changes that are caused by chronic sun exposure on top of the layers of chronological skin aging. Photoaging is produced from the cumulative damage of UV radiation that causes severe skin disorders (Tanveer et al., 2023). The UVC part of the spectrum is not present in sunlight on earth, except at high latitudes, because the UVC part is absorbed by the ozone layer of the atmosphere through the absorption of UVA and UVB rays by cellular chromophores such as urocanic acid, riboflavin and melanin precursors, which work as photosensitizers that play a major role in the production of reactive oxygen species (ROS) and free radicals (WHO, 2017).

Long-term exposure to UVA radiation can induce the same changes as those induced by UVB, including dermal hyperplasia, thickening of the stratum corneum, thinning of Langerhans cells, dermal inflammation, and accumulation of lysozymes on dermal fibers. Clinically, photoaged skin shows characteristics of roughness, fine and coarse wrinkles, uneven hyperpigmentation that can be in the form of lentigines or spots (freckles), weakness, swelling, and telangiectasia (Maeda, 2018).

## D. The Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) are the definitive architects of hematopoiesis, which functions as a continuous producer of blood cells throughout the life of an organism (Pinho & Frenette, 2019). To keep the immune system and hemostasis functioning normally throughout the life cycle, HSCs continuously produce blood, a process

known as hematopoiesis. Anatomically, hemostasis mostly takes place in the bone marrow of the skull, pelvis, sternum, and vertebral column (Boes & Durham, 2017).

Each HSC is programmed to produce various blood cell components efficiently, thereby enabling red blood cells to transport oxygen, megakaryocytes, and platelet derivatives to interact with injured vascular and immune system cells to protect against microbial attack (Ng & Alexander, 2017). The first appearance of HSCs in the embryonic stage of hematopoiesis was identified in the aorta-gonad-mesonephros area, which then shifted to the fetal liver and continued to the bone marrow over time (Julien et al., 2016).

The hematopoiesis, which serves as a constant maker of blood cells throughout an organism's life, is unquestionably designed by the HSC. Red blood cells that carry oxygen, megakaryocytes, and platelet derivatives can interact because each HSC is engineered to produce different blood cell components effectively (Chapman & Zhang, 2018).

Each HSC is engineered to effectively create different blood cell components, allowing oxygen-carrying red blood cells, megakaryocytes, and platelet derivatives to collaborate with damaged vascular and immune system cells to fend against microbial onslaught (Ng & Alexander, 2017). HSC first appeared in the aortogonado-mesonephros region during the embryonic stage of hematopoiesis. Over time, they moved to the fetal liver and then on to the bone marrow. Since HSCs were originally discovered, there has been a great deal of research into stem cell-specific markers (Chapman & Zhang, 2018). This is predicated on the fundamental tenet that every cell possesses distinctive markers, including blood cells, referred to as clusters of differentiation (CD). CD45-positive hematopoietic cell markers predominate (Yadav et al., 2020). HSC research has focused on examining the differentiation of HSC into progenitor cells in vitro colony assays and HSC/progenitor transplantation experiments in myeloblastic experimental mice (Yadav et al., 2020).

Because mature blood cells have a short lifespan, they must constantly be replenished HSCs, a limited subset of cells with the

capacity for self-renewal and differentiation, carry out this function. HSCs cannot form non-hematopoietic cell groupings like MSCs, which makes it possible for them to differentiate under MSCs (Chen & Ju, 2019).

HSCs are adult stem cells from the bone marrow that show the markers CD34+, CD133+, and Thy1+, but not CD38- or CD33-. They come from the hematopoietic system. Aside from self-renewal, HSCs are capable of actively differentiating by developing all blood components (multipotent). HSCs can also go into the cell cycle phase known as G0, which is known as dormancy and is devoid of cell division activity. However, G0 phase cells are still required, especially in cases of tissue injury (Rix et al., 2022).

## 1. Origins of HSC

Since HSCs mostly comes from the bone marrow, it is typically challenging to locate HSCs in peripheral blood. Therefore, activation with specific cytokines is required to cause HSC migration to peripheral blood arteries. Granulocyte colony-stimulating factor (G-CSF) or cytotoxic drugs (myelosuppressive bone marrow suppressants) are specifically used to stimulate and produce HSCs. Cytotoxic substances can disrupt the communication between hematopoietic cells and bone marrow stromal cells, causing a significant number of progenitor cells and HSCs to be released into the bloodstream (Xie et al., 2021).

## 2. Plasticity of Hematopoietic Stem Cell (HSC)

There is a growing body of evidence that HSCs are plastic and that, at least under some circumstances, they can participate in the generation of tissues other than those of the blood system. A few studies have shown that HSCs can give rise to liver cells. Those findings have scientists speculating about the biological response of HSCs to disease or tissue damage and about the early differentiation of the embryonic tissues into discrete layers. It was unexpected that a component of blood could crossover a developmental separation to form a tissue

type that ordinarily has a completely different embryonic origin (Ogawa et al., 2015).

The findings noted above and other reports of cardiac and muscle tissue formation after bone marrow transplantation in mice and of the development of neuron-like cells from bone marrow have raised expectations that HSCs will eventually be shown to be able to give rise to multiple cell types from all three germ layers. One study has demonstrated that a single HSC transplanted into an irradiated mouse generated not only blood components (from the mesoderm later of the embryo) but also epithelial cells in the lungs, gut, (endoderm layer), and skin (ectoderm layer). If HSCs are truly multipotent, their potential for life-saving regenerative therapies may be considerably expanded in the future (Ogawa et al., 2015).

## 3. The Possible Risk of HSCs

Allogeneic HSCT is used to treat hemoglobinopathies; after conditioned to overcome the immunological barrier, allogeneic stem cells are used as vectors to correct the basic genetic defect by replanting genes that are essential for normal hematopoiesis. Although the benefits of HSCs are widely used in cell therapy, transplanted HSCs can have fatal side effects in bone marrow transplantation in thalassemia patients for indications in high-risk category patients. Allogeneic stem cell transplant from a matched sibling donor is an option to treat certain types of thalassemia and has shown 15-year survival rates reaching near 80%. However, recent retrospective data showed similar overall survival compared to conventional treatments with multiple blood transfusions (Khaddour et al., 2023).

When the allogeneic graft starts to proliferate in the recipient, an immunological graft versus-host reaction graft-versus-host disease (GVHD) may occur. GVHD can affect one or more organs to varying degrees, with the most frequently affected being the skin, gastrointestinal tract, and liver. GVHD is a serious complication of bone marrow transplantation and can be fatal. Therefore, the prophylactic administration of cyclosporine (an immunosuppressive

drug) is an important part of the pretransplant and post-transplant treatment (Lucarelli et al., 2012)

## 4. HSC Marker

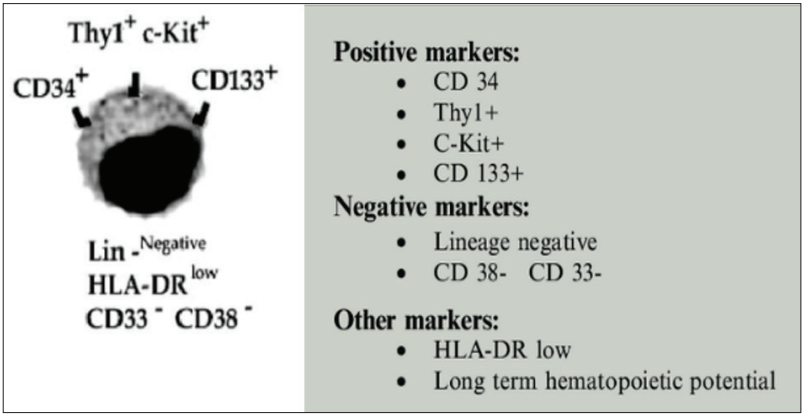
Numerous markers found in HSC serve as intrinsic and signal integration molecules in the activation of transduction pathways. However, CD34+/CD38- can be used as a substitute for the standard HSC markers CD34+/CD133+/Thy1+/CD38-/CD33- (Figure 8.3). Because it is more feasible, using markers is more common (Sudo et al., 2013).

Systematically, HSC markers are divided into two.

1) HSC conventional or traditional markers

   The three categories of traditional HSC markers are as follows (Rix et al., 2022).

   a) CD34+ HSC express the sialomucin transmembrane protein CD34+, which serves as an adhesion molecule. Lymph node endothelial cells that T cell selectin ligands bind to when they enter the lymph nodes also express CD34+ protein.




Source: Rix et al. (2022)

**Figure 8.3** Markers of HSCs

b) Localizing cells is the function of the glycoprotein CD133+, sometimes referred to as prominin 1, which is expressed by HSC, progenitor, and endothelial cells.

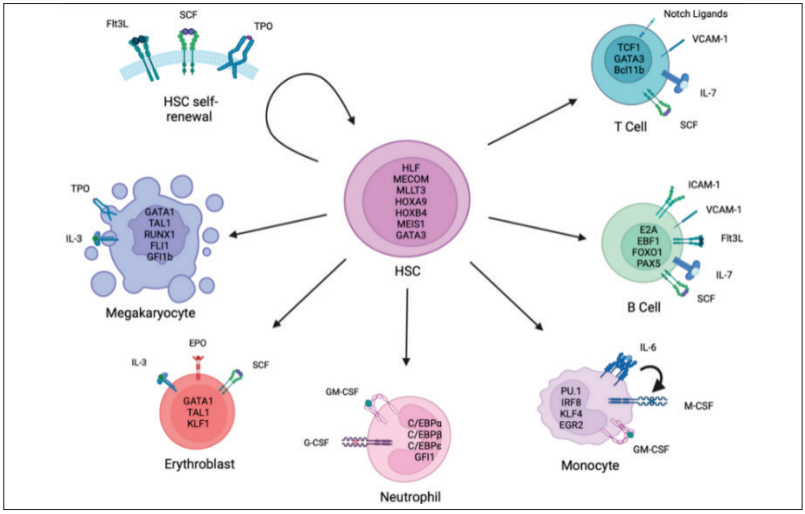
c) Thy1+ Thy1+, also referred to as CD90+, is a glycoprotein that is expressed by HSC, thymocytes (T cell precursors), MSC, NK, neurons, and endothelium and serves as a cell-to-cell communication molecule and matrix contact. The illustration below explains the HSC conventional marker.

The HSCs do not express CD38 or CD33 markers but do express CD34, CD133, and Thy1 markers. Except for self-renewal, HSCs can actively differentiate to generate all blood components.

2) HSC complex markers

Systematically, HSC complex markers are divided into four types (Figure 8.4), namely (Bozhilov et al., 2023):

a) CD34+/CD38-/c-Mpl+, these markers function as cellular physical markers;




Source: Bozhilov et al. (2023)

**Figure 8.4** HSC Molecular Complexity Markers

b) Thrombopoietin (TPO), this marker functions as a self-renewal activity;

c) stem cell factor (SCF), marker functions as a proliferative and differentiation activity;

d) transforming growth-factor-betas (TGF-β); this marker functions as a dormant cell cycle.

HSCs express the standard marker CD34+/CD38-/c-Mpl+, the self-renewal marker TPO, the proliferation and differentiation marker SCF, and the dormant cell cycle marker TGF-β. SCF and TPO are two HSC molecular markers that are important. These two marker proteins have cytokine regulator roles. SCF specifically affects hematopoietic progenitor cell promotion and differentiation, whereas TPO affects self-renewal (Mann et al., 2022).

On the other hand, because TPO cytokines and their receptors, including c-Mpl, are involved in the early stages of HSC hematopoiesis, cells that express the markers CD34+/CD38-/c-Mpl+ have significantly higher HSC engraftment activity (Li et al., 2022). By encouraging HSC adherence to bone marrow osteoblasts and maintaining long-term repopulating activity, signals from angiopoietin-1 via Tie2 control HSC dormancy (He et al., 2014).

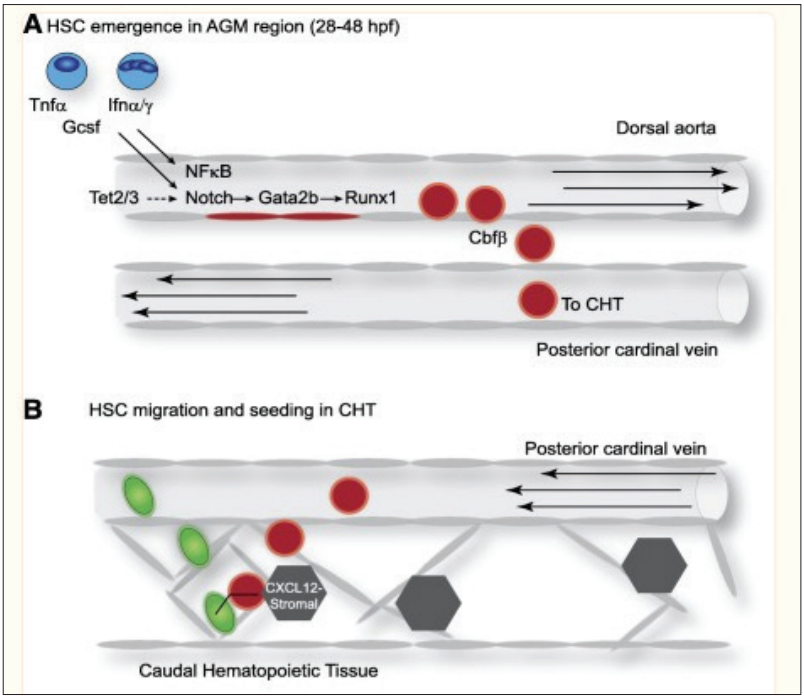
## 5. HSC Development

The integration of several intrinsic factors and signal transduction pathways is necessary for the emergence and specification of HSCs, from early mesodermal precursors to the development of HSCs in the bone marrow. Animal experiments have revealed several regulatory pathways for the emergence of HSCs, including the vascular endothelial growth factor (VEGF) pathway, which can promote cell differentiation and migration, the sonic hedgehog (SHH) pathway, and the bone morphogenetic protein (BMP) pathway, which control arterial wall polarization (HSC specification). Notch lines for HSC shape and specification, as well (Thambyrajah & Bigas, 2022).

Development of HSCs occurs in a region termed the aortagonad-mesonephros (AGM). Within this region, HSCs specifically arise from specialized hemogenic endothelium found in the ventral wall of the dorsal aorta (DA) in a process termed the endothelial-to-hematopoietic transition. The dorsal section of the aorta (DA), which is formed by the vascular cord, which is originally formed by HSC precursors that migrate to the middle region of the embryo from the posterior lateral plate of mesoderm (PLM). HSCs grow from specialized hemogenic endothelial cells after the development of DA; these cells then leave the aorta and enter the bloodstream, where they seed in a niche region for additional development (de la Garza et al., 2017).

The process begins with the release of pro-inflammatory signal molecules Tumor Necrosis Factor Alpha (TNF-α) and Interferon Alpha/Gamma (IFN-α/γ) by myeloid effector cells which can promote the emergence of HSCs via the NF-κB and Notch signaling pathways. The other side of the Tet2/3 protein also regulates Notch signaling. All these conditions trigger the expression of Gata2-b and runx1 in hemogenic endothelial cells. The Cbf-β molecule is needed to encourage extravasation of emerging HSCs from within the dorsal aorta (DA) so that nascent HSCs (newborn HSCs) appear. Nascent HSCs then seed into the caudal hematopoietic tissue (CHT) to induce endothelial remodeling to form a micro-niche consisting of HSCs surrounded by endothelial cells adjacent to stromal cells that express CXCL12 (Figure 8.5) (de la Garza et al., 2017).

Myeloid effector cells emit the pro-inflammatory signaling chemicals TNF- and IFN-α/γ, which can encourage the formation of HSCs via the NF-B and Notch signaling pathways. This is the first step in the process. Contrarily, Tet2/3 protein also controls Notch signaling. The expression of Gata2-b and Runx1 in hemogenic endothelial cells is induced by each of these circumstances. For nascent HSC (newborn HSC) to manifest, the extravasation of HSC emerging from the dorsal aorta (DA) must be stimulated by the Cbf-




Source: de la Garza et al. (2017)

**Figure 8.5** HSC Molecular Development

molecule. To cause endothelial remodeling and create a micro-niche with HSCs surrounded by endothelial cells next to stromal cells that produce CXCL12, nascent HSCs were then sown into CHT sections. The image below explains how HSCs are formed molecularly (de la Garza et al., 2017).

The process of HSC formation begins with the release of TNF-α and IFNα/γ activating the NF-κB and Notch signaling pathways. Together with the Tet 2/3 protein it triggers the expression of Gata2-b and runx1 in hemogenic endothelial cells and together with the Cbf-β molecule promotes the emergence of HSCs as nascent HSCs from within DA. Nascent HSC is then seeded into the CHT section forming a micro-niche consisting of HSC endothelial cells and stromal cells

expressing CXCL12, so that HSCs reside in the bone marrow (de la Garza et al., 2017)

Regulation and coordination of HSC transcription factors namely Gata2, Scl, Runx1, Lmo2, and C-myb. The coordination of HSC transcription factors with epigenetic factors is important in determining the fate of HSCs. Gata2 plays an important role in hematopoiesis, especially downstream of Notch signaling during HSC specification. Gata2-a also plays a role in vascular, conversely, Gata2-b is needed in the formation of HSC (de la Garza et al., 2017).

## 6. HSC Characterization Based On CFU Test

The definition of HSC was based on the capacity of donor HSCs to rebuild (reconstruct) the recipient's bone marrow blood hematopoietic system after radiation ablation had previously caused damage to it. This demonstrates the evolution of the idea that HSCs are bone marrow cells capable of producing the full blood system (Eaves, 2015).

The lymphatic organs of the recipient can be used to directly identify different donor-derived (clonogenic) cell colonies. In particular, the progenitor cells—cells in charge of hematopoietic recovery—allow characterization by examining the recipient lymph colony forming unit (CFU). To preserve stem cell properties, HSC generates progenitor cells that are capable of self-renewal and numerous hematological or multipotent offspring (Kronstein-Wiedemann & Tonn, 2019)

An in vitro test called the CFU test (CFU assay) is used to evaluate hematopoietic progenitor cells, particularly multipotent progenitor cells (MPP) and progenitor cells with a restricted lineage, such as erythroid, granulocytic, and monocyte cells. Although the majority of the CFU found in bone marrow and blood have limited potential in vivo, primitive HSCs and progenitors can colonize under specific culture conditions (Li et al., 2015)

CFU testing is performed by planting low-density single cells in methyl cellulose-based semisolid medium, such as MethoCultTM, together with a specific combination of cytokines. As a result, discrete

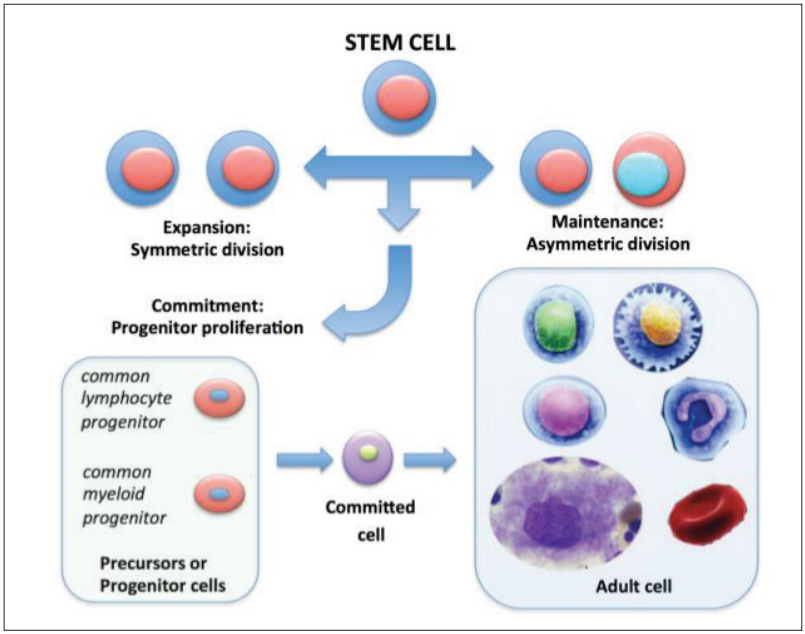
and separate colonies are created as HSCs multiply and develop into certain progenitor cells. Using morphological and phenotypic criteria, colonies originating from several progenitor cell types were categorized and counted according to the quantity and kind of mature cells produced. When long-term transplant studies are both costly and unfeasible, CFU studies are helpful for HSC (Li et al., 2015).

## E. The Physiological Dynamics of Hematopoiesis

Proliferative behavior is a characteristic of in vivo HSC activity. In Basic Molecular Stem Cells, HSCs divide more slowly than progenitor cells. G0 state or quiescence status are two ways to measure the slow rate of HSC cleavage as they progress through the cell cycle. The G0 state is connected to the condition of cells that have stopped dividing (gone dormant) but are still capable of reversing their actions. This state can be distinguished from senescence, which is the condition of cells that are permanently kept in the G1 phase (Cheung & Rando, 2013).

Two modes of cell division are referred to as asymmetric cell division and symmetric cell division (Figure 8.6). The asymmetric division of a stem cell characterized by only produces one differentiated cell and one stem cell, or two distinctly differentiated daughter cells for self-renewal and homeostatic control of the stem cell pool. Therefore, every SC produces copies of itself plus differentiated cells. The second mode, symmetric division of a stem cell characterized only produces two identical stem cells or differentiated daughters (Caocci et al., 2017; Majumda & Tao Liu, 2020).

Intestinal cells and hair follicles are two examples of tissues with high rates of cell turnover that provide evidence for the function of stem cells, including HSCs. Stem cells with a high rate of proliferation are present in this tissue. This shows that the HSCs have an active compartment that can guide hematopoiesis towards a stable state and a dormant compartment that serves as a reserve for preserving the stem cells' capacity for long-term self-renewal (Rompolas & Greco, 2014).




Source: Caocci et al. (2017)

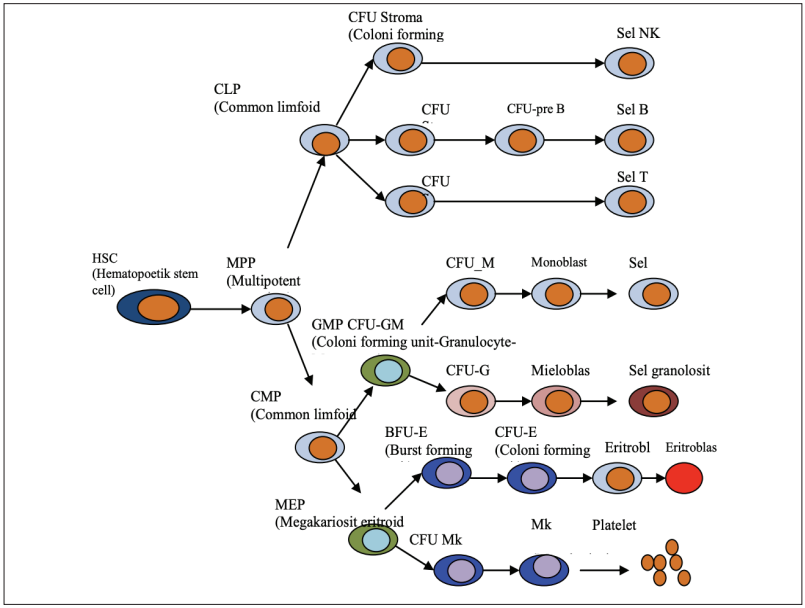
**Figure 8.6** HSC in Bone Marrow

However, HSCs in their latent form can still react to stress. HSC proliferative activity differs from asymmetric cleavage activity, which calls for a correlation between HSC activation and differentiation. When tissue injury occurs, HSC cleavage takes the form of self-renewal activity to replenish HSC that were lost owing to differentiation (Pinho & Frenette, 2019).

Injury signaling molecules are released by damaged tissue, suggesting a significant need for tissue repair and regeneration. To sustain the hematopoiesis system and restore active compartment HSCs that had previously divided asymmetrically to satisfy the needs of injured tissue regeneration, the injury signal causes active dormant compartment senescence HSCs to divide symmetrically (Nugraha & Putra, 2018).

## F. Maturation of HSC Development

HSCs continue to multiply and develop into adult cell lines. HSC derivatives go through various changes during this differentiation process depending on the stages of maturation (Figure 8.7), starting




Notes: LT-HSC : long term-hematopoietic stem cell;
ST-HSC : short-term hematopoietic stem cell;
MPP : multipotential progenitor;
CLP : common lymphoid progenitor;
CMP : common myeloid progenitor;
CFU-GEMM : colony-forming unit-granulocyte/erythrocyte/macrophage/megakaryocyte;
BFU-E : burst-forming unit-erythroid;
CFU-E : colony-forming unit-erythroid;
CFU-Mk : colony-forming unit-megakaryocyte;
CFU-GM : colony-forming unit-granulocyte/Macrophage;
CFU-G : colony-forming unit-granulocyte;
CFU-M : colony-forming unit macrophage.

Source: Wognum and Szilvassy (2015)

**Figure 8.7** HSC Hierarchy

with becoming multi-potential progenitors (MPP), then progenitors that have been committed to certain lineages (lineage-committed progenitors), and so on, until they finally reach maturation into specific cells, such as monocytes or monocytes, lymphocytes, and so forth. An MPP commits in bone marrow to become either common myeloid progenitor (CMP) or common lymphoid progenitor (CLP). The CMP and CLP give rise to mature blood cells in peripheral blood, such as granulocytes, red blood cells (RBC), platelets, monocytes, T cells, B cells, and natural killer (NK) cells (Tober et al., 2018).

## G. HSC Therapy in Skin Aging

Stem cells (SCs) have changed the old paradigm of anti-aging and gained increased attention as a new therapeutic technique in the advancement of biotechnology (Table 8.2). Stem cell (SC) therapies

**Table 8.2** Clinical Trials Applications of Stem Cell for Facial Skin Aging and Photoaging

| SC Preparation | Outcomes | Time | Results | Reference |
|---|---|---|---|---|
| Secretome adipose-derived stem cells (AD-SC) | 1. The epidermal and dermal thickness<br>2. Collagen density | 6 weeks | The epidermal and dermal thickness ↑<br>The expression of TIMP-1 and dermal collagen density ↑ | Putri et al. (2022) |
| Exosomes derived from human umbilical cord blood mesenchymal stem cells | Collagen I dan Elastin synthetis | 4 weeks | Increased expressions of Collagen I and elastin ↑ after 3 days | Kim et al. (2017) |
| Stromal vascular fraction (SVF) | Quality of skin: spots, pores, UV spots, brown spots, and red areas | 6 months | Improved spots, pores, UV spots, brown spots, and red areas | Kuo et al. (2021) |

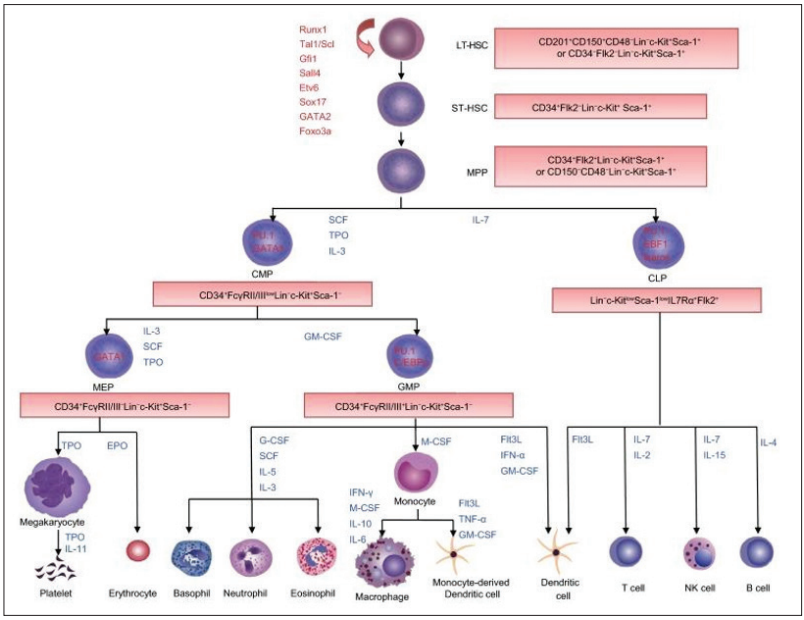
| SC Preparation | Outcomes | Time | Results | Reference |
|---|---|---|---|---|
| Adipose-derived stem cells (AD-SCs) | Collagen synthesis | 4 weeks | Significant wrinkle reduction and hingher collagen density | Jeong et al. (2015) |
| Adipose-derived mesenchymal stem cells (AD-MSCs) | 1. Presences of oxylatan and elaunin 2. Total fibrilin and tropoelastin | 6 weeks | Increased and ordered presence of oxytalan and elaunin fibers in zone 1 Total fibrillin and tropoelastin increased after treatment | Charles-De-Sá et al. (2020) |

have broad application prospects in the field of regenerative medicine due to the inherent biological characteristics of SCs, such as their plasticity, self-renewal, and multidirectional differentiation potential. Thus, SCs could delay or even reverse aging (Chang et al., 2022).

## H. Molecular of HSC Differentiation

Numerous cytokines, chemokines, receptors, and intracellular signaling molecules have an impact on the mechanisms governing HSC self-renewal and differentiation. Growth factors and cytokines, such as CSF and IL, that activate intracellular signaling pathways control the differentiation of HSCs. Traditionally, HSC differentiates into the oligopotent progenitor cell lineages of lymphoid and myeloid-erythroid, which are both confined progenitor cell lineages (Lee & Hong, 2020).

HSC lineage development model of the HSC lineage (Figure 8.8). Common myeloid progenitor cells and lymphoid progenitor cells are produced by the differentiation of HSC. T cells, B cells, NK cells, and dendritic cells are produced by common lymphoid progenitor cells, whereas granulocyte-macrophage progenitor cells and megakaryocyte




Source: Cheng et al. (2020)

**Figure 8.8** HSC Lineage

erythroid progenitor cells are produced by common myeloid progenitor cells. Myeloblasts and monocytes are produced by granulocyte-macrophage progenitor cells, whereas macrophages are produced by monocytes. Erythrocytes and platelets are produced by collections of megakaryocyte erythroid progenitor cells (Cheng et al., 2020)

## I. CD34+

CD34+ is a surface glycophosphoprotein expressed on early hematopoietic stem and progenitor cells, microvascular endothelial fibroblasts, and embryos. Bone marrow (BM) CD34+ cells represent only 1.5% of bone marrow mononuclear cells, but contain precursors of all lymphocyte lineages, as evidenced by the finding that CD34+ cells purified from marrow can restore hematopoietic recovery in primates, humans, or mice undergoing infusion. autologous bone

marrow after keloid treatment. CD34+ hematopoietic cells obtained from marrow or blood are used clinically in transplantation and gene therapy studies, including ongoing efforts to expand hematopoietic progenitor cells ex vivo (Hassanpour et al., 2023).

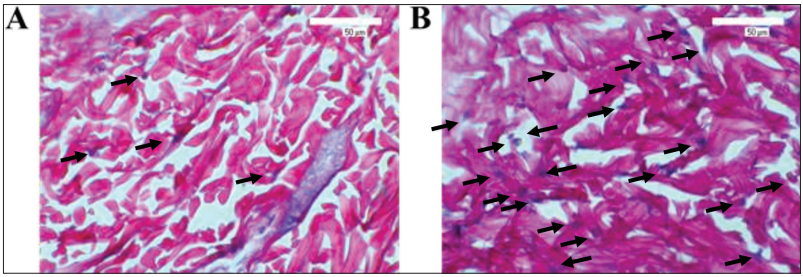
Despite the importance of CD34+ as a marker of early hematopoietic progenitor cells in experimental and clinical hematopoiesis, the function of CD34 remains unclear. Due to its potential role in fundamental processes such as hematopoietic progenitor cell development and inflammation, research into the regulation and function of CD34+ is ongoing in several laboratories (Sidney et al., 2014).

Recent experiments on CD34 function suggest that CD34 expressed on endothelial cells may play a role in leukocyte adhesion and "homing" during inflammation. It is hypothesized that CD34 plays a role in the stable localization of progenitor cells in the BM, CD34 may also play a role, involved in the maintenance of the hematopoietic phenotype of Sted's ancestors (Hughes et al., 2020).

## 1. Effect of CD34+ Hematopoietic Stem Cell to Increase Fibroblast and Collagen Skin in Ultraviolet B-Exposed Skin

CD34+ cells make up roughly 1%–2% of all bone marrow cells. CD34+ cells are typically collected through the process of leukapheresis, whereby blood is processed in a way that concentrates white blood cells and removes many of the red blood cells. Once a concentrated sample containing hematopoietic stem cells is acquired, researchers can apply additional separation methods to extract the desired cell population from the leukoplakia. Even though leukoplakias are specifically designed for further cell separation, harsh isolation methods can result in cell damage that affects viability and cell function. Gentle isolation is especially critical when separating rare or fragile cell populations, such as CD34+ cells, to acquire a sufficient number of cells that retain their normal function (Romito & Cobellis, 2015).

The control group with UVB exposure, there were fewer blue-nucleated fibroblasts overall, but in the CD34+ group with UVB




Notes: A: UVB plus a control group;
B: CD34+ group plus UVB. Increased blue cell nuclei in fibroblast cells were seen. Fibroblasts were marked by white arrows (100× magnification).

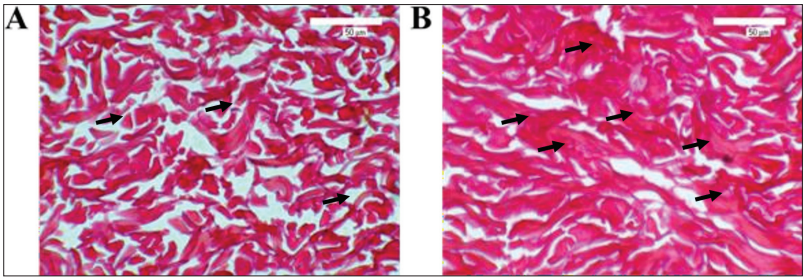
Source: Syaifudin (2015)

**Figure 8.9** Wistar Rat Dermal Tissue Fibroblast Cell Count with HE Staining

exposure, there were more blue cell nuclei in fibroblasts (Figure 8.9 A). Based on the results, the treatment group's fibroblast cell count increased by 68.39%, and its collagen content increased by 18.66% when compared to the control group (Figure 8.9B). It is because stem cells may create and organize bodily tissues. Stem cells are young, multipotent cells that can differentiate into other types of cells and create new bodily tissues. When implanted into the body, stem cells will develop into body tissue at that precise site. Stem cells have the qualities of being undifferentiated, having the capacity for self-renewal, and having the capacity to differentiate into several cell types (Bacakova et al., 2018).

In group with UVB plus as a control group (Figure 8.10A), damage to the structure and content of collagen is evident in the thin-appearing red collagen fibers. Incomplete collagen fibers are shown by white arrows. In group with UVB and CD34+ subset (Figure 8.10B), collagen that has red collagen strands seems to be bigger and broader. Intact collagen fibers are indicated by arrows.

Hematopoietic stem cells, especially CD34+ stem cells, are one such option. Progenitor cells called hematopoietic stem cells produce blood cells. Blood and bone marrow are the origins of these cells. Direct isolation of hematopoietic stem cells from peripheral blood




Notes: A: UVB plus a control group;
B: CD34+ group plus UVB (100× magnification).

Source: Syaifudin (2015)

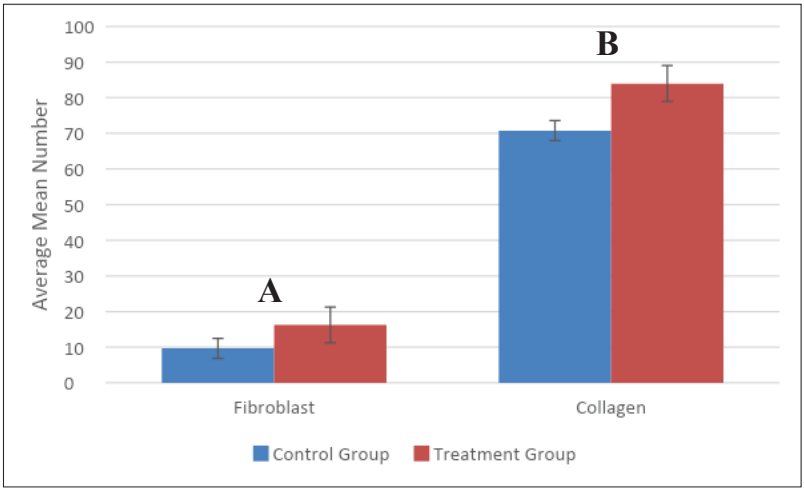
**Figure 8.10** Collagen content in the dermis of male Wistar rats stained with Sirius Red.

is possible, as well as mobilization methods. These stem cells can develop into non-hematopoietic cells because they have pluripotent and plastic features (Supartono et al., 2018).

The differences in the mean number of fibroblasts and collagen between groups after administration of CD34+ stem cell suspension was measured (Figure 8.11). The histogram is presented as mean ± standard deviation. Asterisk symbol shows significant difference in modulators at difference treatment between control group and treatment group ($P < 0.05$).

When donor peripheral blood is isolated directly, no medications are used in the procedure. After being cultivated and identified by cytometric examination, the separated cells are given a medium for growth and application. Peripheral blood can be used to collect hematopoietic stem cells directly, or they can be mobilized. By isolating donor cells without the use of medication, direct isolation is carried out (Supartono et al., 2019).

Hematopoietic stem cells that express the CD34 cell marker are known as CD34+ cells. The most accurate indicator of hematopoietic stem cells is thought to be CD34. Pluripotent blood stem cells, unipotent myeloid cells, vascular endothelium, brain membrane




Notes: A. Fibroblast; B. Collagen (*p<0.05 showed significance compared to the control group).

Source: Syaifudin (2015)

**Figure 8.11** Differences in the Mean Number of Fibroblasts and Collagen between Groups after Administration of CD34+ Stem Cell Suspension

components, and human skin follicular cells all express the CD34 antigen. Studying the plasticity—the capacity of hematopoietic stem cells to differentiate into non-hematopoietic cells—of CD34+ cells is made possible by their features. According to this study, delivering CD34+ hematopoietic stem cells to the skin's dermis may enhance the number of fibroblast and collagen cells there. This has therapeutic importance since it causes the skin to grow firmer and develop a smooth and robust texture (Sidney et al., 2014).

Male Wistar rats exposed to UVB radiation had more fibroblast cells in their skin after receiving subcutaneous injections of human peripheral blood CD34+ stem cells. Additionally, it caused the skin of male Wistar rats exposed to UVB radiation to produce more collagen. By highlighting the potential use of CD34+ hematopoietic stem cells in boosting skin fibroblast cells and collagen, this research aids in the regeneration of face skin (Zorina et al., 2023).

Hyaluronic acid products and laser technology have traditionally been utilized to promote the formation of fibroblasts and collagen. In contrast to typical cosmetic techniques, utilizing hematopoietic stem cells gives the benefit of sustained skin restoration. As only standard injectable syringes were utilized, it is crucial to emphasize that this study had restrictions regarding observation duration, dosage, and depth of subcutaneous injection. Therefore, these elements may have an impact on the fibroblast cells' considerable rise as compared to collagen. It is advised that more studies be conducted to increase the observation period and make use of injection equipment to precisely gauge CD34+ hematopoietic stem cell dose and depth. Clinical studies are required to verify the results of this study in people (Brohem et al., 2013).

## J. Conclusion

Based on the discussion in this chapter, the following four conclusions can be drawn.

1) Anti-aging medicine is a branch of study to the early detection, prevention, treatment, and reversal of age-related dysfunctions, disorders, and diseases.
2) UV radiation can mediate damage to cellular components and photoaging that is produced from the cumulative damage of UV radiation can cause severe skin.
3) CD34 is considered the most reliable marker for hematopoietic stem cells. The characteristics of CD34+ cells provide an opportunity for studying their plasticity, which refers to the ability of hematopoietic stem cells to transform into non-hematopoietic cells.
4) Administration of human peripheral blood CD34+ stem cells subcutaneously increased the number of fibroblasts and collagen in the skin of male Wistar rats exposed to ultraviolet B (UVB) radiation.

## References


Amaro-Ortiz, A., Yan, B., & D'Orazio, J. A. (2014). Ultraviolet radiation, aging and the skin: Prevention of damage by topical cAMP manipulation. *Molecules*, *19*(5), 6202–6219. https://doi.org/10.3390/molecules19056202

Amarya, S., Singh, K., & Sabharwal, M. (2018). Ageing process and physiological changes. In G. D'Onofrio, A. Greco, & D. Sancarlo (Eds.), *Gerontology*. https://doi.org/10.5772/intechopen.76249

Bacakova, L., Zarubova, J., Travnickova, M., Musilkova, J., Pajorova, J., Slepicka, P., Kasalkova, N. S., Svorcik, V., Kolska, Z., Motarjemi, H., & Molitor, M. (2018). Stem cells: their source, potency and use in regenerative therapies with focus on adipose-derived stem cells – A review. *Biotechnology Advances*, *36*(4), 1111–1126. https://doi.org/10.1016/j.biotechadv.2018.03.011

Boes, K. M., & Durham, A. C. (2017). Bone marrow, blood cells, and the lymphoid/lymphatic system. In J. F. Zachary, *Pathologic Basis of Veterinary Disease Expert Consult* (724–804). Elsevier. https://doi.org/10.1016/B978-0-323-35775-3.00013-8

Bosch, R., Philips, N., Suárez-Pérez, J. A., Juarranz, A., Devmurari, A., Chalensouk-Khaosaat, J., & González, S. (2015). Mechanisms of photoaging and cutaneous photocarcinogenesis, and photoprotective strategies with phytochemicals. *Antioxidants (Basel), 4(2),* 248–268. https://doi.org/10.3390/antiox4020248

Bozhilov, Y. K., Hsu, I., Brown, E. J., & Wilkinson, A. C. (2023). In vitro human haematopoietic stem cell expansion and differentiation. *Cells*, *12*(6), Article 896. https://doi.org/10.3390/cells12060896

Brohem, C. A., de Carvalho, C. M., Radoski, C. L., Santi, F. C., Baptista, M. C., Swinka, B. B., Urban, C. de A., de Araujo, L. R. R., Graf, R. M., Feferman, I. H. S., & Lorencini, M. (2013). Comparison between fibroblasts and mesenchymal stem cells derived from dermal and adipose tissue. *International Journal of Cosmetic Science*, *35*(5), 448–457. https://doi.org/10.1111/ics.12064

Brown, T. M., & Krishnamurthy, K. (2022). *Histology, Dermis*. StatPearls Publishing.

Caocci, G., Greco, M., & La Nasa, G. (2017). Bone marrow homing and engraftment defects of human hematopoietic stem and progenitor

cells. *Mediterranean Journal of Hematology and Infectious Diseases*, *9*(1), Article e2017032. https://doi.org/10.4084/MJHID.2017.032

Chambers, E. S., & Vukmanovic-Stejic, M. (2020). Skin barrier immunity and ageing. *Immunology*, *160*(2), 116–125. https://doi.org/10.1111/imm.13152

Chang, L., Fan, W., Pan, X., & Zhu, X. (2022). Stem cells to reverse aging. *Chinese Medical Journal*, *135*(8), 901–910. https://doi.org/10.1097/CM9.0000000000001984

Chapman, J., & Zhang, Y. (2018). *Histology, hematopoiesis*. StatPearls Publishing.

Charles-De-Sá, L., Gontijo-De-Amorim, N. F., Rigotti, G., Sbarbati, A., Bernardi, P., Benati, D., Bizon Vieira Carias, R., Maeda Takiya, C., & Borojevic, R. (2020). Photoaged skin therapy with adipose-derived stem cells. *Plastic and Reconstructive Surgery*, *145*(6), 1037e–1049e. https://doi.org/10.1097/PRS.0000000000006867

Chen, S., Zhu, J., Wang, M., Huang, Y., Qiu, Z., Li, J., Chen, X., Chen, H., Xu, M., Liu, J., She, M., Li, H., Yang, X., Wang, Y., & Cai, X. (2019). Comparison of the therapeutic effects of adipose-derived and bone marrow mesenchymal stem cells on erectile dysfunction in diabetic rats. *International Journal of Molecular Medicine*, *44*(3), 1006–1014. https://doi.org/10.3892/ijmm.2019.4254

Chen, Z., & Ju, Z. (2019). Inflamm-aging of hematopoietic stem cells. *Blood Science*, *1*(2), 141–143. https://doi.org/10.1097/BS9.0000000000000029

Cheng, H., Zheng, Z., & Cheng, T. (2020). New paradigms on hematopoietic stem cell differentiation. In *Protein and cell* (Vol. 11, Issue 1). https://doi.org/10.1007/s13238-019-0633-0

Cheung, T. H., & Rando, T. A. (2013). Molecular regulation of stem cell quiescence. *Nature Reviews Molecular Cell Biology*, *14*(6), 329–340. https://doi.org/10.1038/nrm3591

de Jager, T. L., Cockrell, A. E., & Du Plessis, S. S. (2017). Ultraviolet light induced generation of reactive oxygen species. In S. I. Ahmad, *Ultraviolet light in human health, diseases, and environment* (15–23). Springer. https://doi.org/10.1007/978-3-319-56017-5_2

de la Garza, A., Sinha, A., & Bowman, T. V. (2017). Concise review: Hematopoietic stem cell origins: Lessons from embryogenesis for improving regenerative medicine. *Stem Cells Translational Medicine*, *6*(1), 60–67. https://doi.org/10.5966/sctm.2016-0110

Eaves, C. J. (2015). Hematopoietic stem cells: Concepts, definitions, and the new reality. *Blood*, *125*(17), Article 2605–2613. https://doi.org/10.1182/blood-2014-12-570200

Farage, M. A., Miller, K. W., Elsner, P., & Maibach, H. I. (2013). Characteristics of the aging skin. *Advances in Wound Care*, *2*(1), 5–10. https://doi.org/10.1089/wound.2011.0356

Gromkowska-Kępka, K. J., Puścion-Jakubik, A., Markiewicz-Żukowska, R., & Socha, K. (2021). The impact of ultraviolet radiation on skin photoaging — review of in vitro studies. *Journal of Cosmetic Dermatology*, *20*(11), 3427–3431. https://doi.org/10.1111/jocd.14033

Guan, L. L., Lim, H. W., & Mohammad, T. F. (2021). Sunscreens and Photoaging: A Review of Current Literature. *American Journal of Clinical Dermatology*, *22*, 819–828. https://doi.org/10.1007/s40257-021-00632-5

Hassanpour, M., Salybekov, A. A., Kobayashi, S., & Asahara, T. (2023). CD34 positive cells as endothelial progenitor cells in biology and medicine. *Frontiers in Cell and Developmental Biology*, *11*. https://doi.org/10.3389/fcell.2023.1128134

He, N., Zhang, L., Cui, J., & Li, Z. (2014). Bone marrow vascular niche: Home for hematopoietic stem cells. *Bone Marrow Research*. https://doi.org/10.1155/2014/128436

Hughes, M. R., Canals Hernaez, D., Cait, J., Refaeli, I., Lo, B. C., Roskelley, C. D., & McNagny, K. M. (2020). A sticky wicket: Defining molecular functions for CD34 in hematopoietic cells. *Experimental Hematology*: *Journal for Hematology, Stem Cell Biology, and Transplantation*, *86*, 1–14. https://doi.org/10.1016/j.exphem.2020.05.004

Jeong, J. H., Fan, Y., You, G. Y., Choi, T. H., & Kim, S. (2015). Improvement of photoaged skin wrinkles with cultured human fibroblasts and adipose-derived stem cells: A comparative study. *Journal of Plastic, Reconstructive and Aesthetic Surgery*, *68*(3), 372–381. https://doi.org/10.1016/j.bjps.2014.10.045

Julien, E., El Omar, R., & Tavian, M. (2016). Origin of the hematopoietic system in the human embryo. *FEBS Letters Special Issue*, *590*(22), 3987–4001. https://doi.org/10.1002/1873-3468.12389

Kang, H., & Kim, H. (2022). Ageism and psychological well-being among older adults: A systematic review. *Gerontology and Geriatric Medicine*, *8*. https://doi.org/10.1177/23337214221087023

Khaddour, K., Hana, C. K., & Mewawalla, P. (2023). *Hematopoietic stem cell transplantation*. StatPearls Publishing.

Karim, P. L., Inda Astri Aryani, & Nopriyati. (2021). Anatomy and histologic of intrinsic aging skin. *Bioscientia Medicina: Journal of Biomedicine and Translational Research*, *5*(11), 1065–1077. https://doi.org/10.32539/bsm.v5i11.417

Kim, B. E., & Leung, D. Y. M. (2012). Epidermal barrier in atopic dermatitis. *Allergy, Asthma and Immunology Research*, *4*(1), 12–16. https://doi.org/10.4168/aair.2012.4.1.12

Kim, Y. J., Yoo, S. M., Park, H. H., Lim, H. J., Kim, Y. L., Lee, S., Seo, K. W., & Kang, K. S. (2017). Exosomes derived from human umbilical cord blood mesenchymal stem cells stimulates rejuvenation of human skin. *Biochemical and Biophysical Research Communications*, *493*(2), 1102–1108. https://doi.org/10.1016/j.bbrc.2017.09.056

Kronstein-Wiedemann, R., & Tonn, T. (2019). Colony formation: An assay of hematopoietic progenitor cells. In G. Klein & P. Wutcher (Eds.), *Stem cell mobilization: Methods and protocols* [Springer protocols] (29–40). Humana Press. https://doi.org/10.1007/978-1-4939-9574-5_3

Krutmann, J., Schikowski, T., Morita, A., & Berneburg, M. (2021). Environmentally-induced (extrinsic) skin aging: Exposomal factors and underlying mechanisms. *Journal of Investigative Dermatology*, *141*(4),1096–1103. https://doi.org/10.1016/j.jid.2020.12.011

Kuo, S. C. H., Huang, F., Chi, S. Y., Lin, H. P., Chien, P. C., & Hsieh, C. H. (2021). Investigate the improvement of facial skin texture with the VISIA system after total thyroidectomy. *BMC Surgery*, *21*(1), Article 94. https://doi.org/10.1186/s12893-021-01108-3

Lee, J. Y., & Hong, S. H. (2020). Hematopoieticstem cells and their roles in tissue regeneration. *International Journal of Stem Cells*, *13*(1), 1–12. https://doi.org/10.15283/ijsc19127

Li, C., Lu, L., Zhang, J., Huang, S., Xing, Y., Zhao, M., Zhou, D., Li, D., & Meng, A. (2015). Granulocyte colony-stimulating factor exacerbates hematopoietic stem cell injury after irradiation. *Cell and Bioscience*, *5*(1): Article 65. https://doi.org/10.1186/s13578-015-0057-3

Li, F., Xiong, Y., Yang, M., Chen, P., Zhang, J., Wang, Q., Xu, M., Wang, Y., He, Z., Zhao, X., Huang, J., Gu, X., Zhang, L., Sun, R., Sun, X., Li, J., Ou, J., Xu, T., Huang, X., … Zhang, Q. (2022). c-Mpl-del, a c-Mpl alternative splicing isoform, promotes AMKL progression and

chemoresistance. *Cell Death and Disease*, *13*(10): Article 869. https://doi.org/10.1038/s41419-022-05315-5

Liang, Y., Su, W., & Wang, F. (2023). Skin ageing: A progressive, multi-factorial condition demanding an integrated, multilayer-targeted remedy. *Clinical, Cosmetic and Investigational Dermatology*, *16*, 1215–1229. https://doi.org/10.2147/CCID.S408765

Lucarelli, G., Isgrò, A., Sodani, P., & Gaziev, J. (2012). Hematopoietic stem cell transplantation in thalassemia and sickle cell anemia. *Cold Spring Harbor Perspectives in Medicine*, *2*(5), Article a011825. https://doi.org/10.1101/cshperspect.a011825

Lynch, B., Pageon, H., Le Blay, H., Brizion, S., Bastien, P., Bornschlögl, T., & Domanov, Y. (2022). A mechanistic view on the aging human skin through ex vivo layer-by-layer analysis of mechanics and microstructure of facial and mammary dermis. *Scientific Reports*, *12*, Article 849. https://doi.org/10.1038/s41598-022-04767-1

Maeda, K. (2018). Analysis of ultraviolet radiation wavelengths causing hardening and reduced elasticity of collagen gels in vitro. *Cosmetics*, *5*(1), Article 14. https://doi.org/10.3390/cosmetics5010014

Majumda, S., & Tao Liu, S. (2020). Cell division symmetry control and cancer stem cells. *AIMS Molecular Sciences*, *7*(2), 82–98. https://doi.org/10.3934/molsci.2020006

Mann, Z., Sengar, M., Verma, Y. K., Rajalingam, R., & Raghav, P. K. (2022). Hematopoietic stem cell factors: Their functional role in self-renewal and clinical aspects. *Frontiers in Cell and Developmental Biology*, *10*. https://doi.org/10.3389/fcell.2022.664261

Meléndez-Martínez, A. J., Stinco, C. M., & Mapelli-Brahm, P. (2019). Skin carotenoids in public health and nutricosmetics: The emerging roles and applications of the UV radiation-absorbing colourless carotenoids phytoene and phytofluene. *Nutrients*, *11*(5), Article 1093. https://doi.org/10.3390/nu11051093

Mine, S., Fortunel, N. O., Pageon, H., & Asselineau, D. (2008). Aging alters functionally human dermal papillary fibroblasts but not reticular fibroblasts: A new view of skin morphogenesis and aging. *PLoS ONE*, *3*(12), Article e4066. https://doi.org/10.1371/journal.pone.0004066

Ng, A. P., & Alexander, W. S. (2017). Haematopoietic stem cells: Past, present and future. *Cell Death Discovery*, *3*, Article 17002. https://doi.org/10.1038/cddiscovery.2017.2

Niculet, E., Bobeica, C., & Tatu, A. L. (2020). Glucocorticoid-induced skin atrophy: The old and the new. *Clinical, Cosmetic and Investigational Dermatology, 13*, 1041–1050. https://doi.org/10.2147/CCID.S224211

Nugraha, A., & Putra, A. (2018). Tumor necrosis factor-α-activated mesenchymal stem cells accelerate wound healing through vascular endothelial growth factor regulation in rats. *Universa Medicina*, *37*(2), 135–142. https://doi.org/10.18051/univmed.2018.v37.135-142

Ogawa, M., LaRue, A. C., & Mehrotra, M. (2015). Plasticity of hematopoietic stem cells. *Best Practice & Research Clinical Haematology*, *28*(2–3), 73–80. https://doi.org/10.1016/j.beha.2015.10.003

Ok, S. C. (2022). Insights into the anti-aging prevention and diagnostic medicine and healthcare. *Diagnostics*, *12*(4), Article 819. https://doi.org/10.3390/diagnostics12040819

Papaccio, F., D'arino, A., Caputo, S., & Bellei, B. (2022). Focus on the contribution of oxidative stress in skin aging. *Antioxidants*, *11*(6), Article 1121. https://doi.org/10.3390/antiox11061121

Parrado, C., Mercado-Saenz, S., Perez-Davo, A., Gilaberte, Y., Gonzalez, S., & Juarranz, A. (2019). Environmental Stressors on Skin Aging. Mechanistic Insights. *Frontiers in Pharmacology, 10*. https://doi.org/10.3389/fphar.2019.00759

Pinho, S., & Frenette, P. S. (2019). Haematopoietic stem cell activity and interactions with the niche. *Nature Reviews Molecular Cell Biology*, *20*(5), 303–320. https://doi.org/10.1038/s41580-019-0103-9

Putri, W. E., Endaryanto, A., Tinduh, D., Rantam, F., Notobroto, H. B., & Prakoeswa, C. R. S. (2022). Effect of secretome of adipose stem cell (ASC) in photoaging skin. *Bali Medical Journal*, *11*(3). https://doi.org/10.15562/bmj.v11i3.3543

Rittié, L., & Fisher, G. J. (2015). Natural and sun-induced aging of human skin. *Cold Spring Harbor Perspectives in Medicine*, *5*(1), 1–15. https://doi.org/10.1101/cshperspect.a015370

Rix, B., Maduro, A. H., Bridge, K. S., & Grey, W. (2022). Markers for human haematopoietic stem cells: The disconnect between an identification marker and its function. *Frontiers in Physiology*, *13*. https://doi.org/10.3389/fphys.2022.1009160

Romito, A., & Cobellis, G. (2015). Pluripotent stem cells: Current understanding and future directions. *Stem Cells International*. https://doi.org/10.1155/2016/9451492

Rompolas, P., & Greco, V. (2014). Stem cell dynamics in the hair follicle niche. *Seminars in Cell and Developmental Biology*, *25–26*, 34–42. https://doi.org/10.1016/j.semcdb.2013.12.005

Russell-Goldman, E., & Murphy, G. F. (2020). The pathobiology of skin aging: New insight into an old dilemma. *The American Journal of Pathology*, *190*(7), 1356–1369. https://doi.org/10.1016/j.ajpath.2020.03.007

Said, A., Bock, S., Müller, G., & Weindl, G. (2015). Inflammatory conditions distinctively alter immunological functions of Langerhans-like cells and dendritic cells in vitro. *Immunology*, 144(2), 218–230. https://doi.org/10.1111/imm.12363

Sidney, L. E., Branch, M. J., Dunphy, S. E., Dua, H. S., & Hopkinson, A. (2014). Concise review: Evidence for CD34 as a common marker for diverse progenitors. *Stem Cells, 32*(6), 1380–1389. https://doi.org/10.1002/stem.1661

Sudo, T., Yokota, T., Ishibashi, T., Ichii, M., Doi, Y., Oritani, K., & Kanakur, Y. (2013). Canonical HSC markers and recent achievements. In K. Alimoghaddam, *Stem cell biology in normal life and diseases*. InTech. https://doi.org/10.5772/54474

Supartono, B., Hutagalung, E., Ismail, Boediono, A., Shirakawa, T., Djauzi, S., Yusuf, A. A., Siregar, N. C., Pandelaki, J., Bachtiar, A., & Shigemura, K. (2018). Hyaline cartilage regeneration on osteochondral defects by intraarticular injection of human peripheral blood cd34+ cells, hyaluronic acid and growth factor in a rat model. *Biomedical Journal of Scientific & Technical Research*, *7*(1), 5617–5626. https://doi.org/10.26717/bjstr.2018.07.001436

Supartono, B., Yusriana, B., & Amalia, M. (2019). The effect of granulocyte colony stimulating factors (gcsf) on expansion of cd34+hematopoietic stem cells. *Biomedical Journal of Scientific & Technical Research*, *12*(5), 1–5. https://doi.org/10.26717/bjstr.2019.12.002304

Syaifudin, M. (2015). *Pemberian sel punca CD34+ darah tepi manusia secara subkutan meningkatkan jumlah sel fibroblas dan kolagen pada tikur jantan wistar yang dipajan sinar ultra violet B* [Unpublished]. Universitas Udayana.

Tanveer, M. A., Rashid, H., & Tasduq, S. A. (2023). Molecular basis of skin photoaging and therapeutic interventions by plant-derived natural product ingredients: A comprehensive review. *Heliyon*, *9*(3), Article E13580. https://doi.org/10.1016/j.heliyon.2023.e13580

ter Horst, B., Chouhan, G., Moiemen, N. S., & Grover, L. M. (2018). Advances in keratinocyte delivery in burn wound care. *Advanced Drug Delivery Reviews*, *123*, 18–32. https://doi.org/10.1016/j.addr.2017.06.012

Thambyrajah, R., & Bigas, A. (2022). Notch signaling in HSC emergence: When, why and how. *Cells*, *11*(3), Article 358. https://doi.org/10.3390/cells11030358

Tober, J., Maijenburg, M. M. W., Li, Y., Gao, L., Hadland, B. K., Gao, P., Minoura, K., Bernstein, I. D., Tan, K., & Speck, N. A. (2018). Maturation of hematopoietic stem cells from prehematopoietic stem cells is accompanied by up-regulation of PD-L1. *Journal of Experimental Medicine*, *215*(2), 645–659. https://doi.org/10.1084/jem.20161594

WHO. (2017, October 16). *Radiation: Ultraviolet (UV) radiation and skin cancer*. https://www.who.int/news-room/questions-and-answers/item/radiation-ultraviolet-(uv)-radiation-and-skin-cancer

Williamson, C. E., & Neale, P. J. (2022). Ultraviolet radiation. *Encyclopedia of Inland Waters Second Edition*, *1*, 83–94. https://doi.org/10.1016/B978-0-12-819166-8.00023-2

Wognum, A., & Szilvassy, S. (2015). *Hematopoietic stem and progenitor cells*. Stem Cell Technology. https://cdn.stemcell.com/media/files/minireview/MR29068-Hematopoietic_Stem_and_Progenitor_Cells.pdf

Xie, M., Zhang, S., Dong, F., Zhang, Q., Wang, J., Wang, C., Zhu, C., Zhang, S., Luo, B., Wu, P., & Ema, H. (2021). Granulocyte colony-stimulating factor directly acts on mouse lymphoid-biased but not myeloid-biased hematopoietic stem cells. *Haematologica*, *106*(6), 1647–1658. https://doi.org/10.3324/haematol.2019.239251

Yadav, P., Vats, R., Bano, A., & Bhardwaj, R. (2020). Hematopoietic stem cells culture, expansion and differentiation: an insight into variable and available media. *International Journal of Stem Cells*, *13*(3), 326–334. https://doi.org/10.15283/IJSC19157

Yin, S., Wang, Y., Liu, N., Yang, M., Hu, Y., Li, X., Fu, Y., Luo, M., Sun, J., & Yang, X. (2019). Potential skin protective effects after UVB irradiation afforded by an antioxidant peptide from Odorrana andersonii. *Biomedicine and Pharmacotherapy*, *120*, Article 109535. https://doi.org/10.1016/j.biopha.2019.109535

Yousef, H., Alhajj, M., Fakoya, A. O., & Sharma, S. (2020). *Anatomy, skin (integument), epidermis*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK470464/

Zorina, A., Zorin, V., Isaev, A., Kudlay, D., Vasileva, M., & Kopnin, P. (2023). Dermal fibroblasts as the main target for skin anti-age correction using a combination of regenerative medicine methods. *Current Issues in Molecular Biology*, *45*(5), 3829–3847. https://doi.org/10.3390/cimb45050247