Stem Cells and Cloning: Advances and Applications

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REVIEW

A Comprehensive Review of Stem Cell Conditioned Media Role for Anti-Aging on Skin

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Abstract: Various studies have been widely conducted on conditioned medium for the development of anti-aging preparations, including the utilization of stem cells, which present a promising alternative solution. This narrative review aims to understand the latest developments in various conditioned medium stem cell applications for anti-aging on the skin. A search of the Scopus database yielded publications of interest. The research focused on articles published without restrictions on the year. After finding 68 articles in the search results, they moved on to the checking phase. Upon comprehensive literature review, 23 articles met the inclusion criteria, while 45 articles were deemed ineligible for participation in this research. The results of the review indicate that conditioned medium from various stem cells has demonstrated success in reducing risk factors for skin aging, as proven in various tests. The successful reduction of the risk of skin aging has been established in vitro, in vivo, and in clinical trials. Given the numerous studies on the progress of exploring and utilizing conditioned medium, it is expected to provide a solution to the problem of skin aging.

Keywords: conditioned medium, stem cell, anti-aging, skin aging

Introduction

Anti-aging treatment is an area of study with many unknowns, particularly when it comes to the use of Conditioned Medium (CM). Prior research has shown that MSCs derived from CM possess anti-aging properties. Superior secretory capabilities can be derived from fetal MSCs sourced from human umbilical cord blood (CB).[1] Stem cells secrete a diverse array of growth factors and compounds that induce tissue renewal, all of which are found within CM.[2] Cellular medicines (CMs) are anticipated to encounter less stringent regulatory constraints when contrasted with stem cell therapy products in their cellular preparation format because they are rich in cytokines and growth factors that meet regulatory requirements.[3] CM contains a variety of cytokines and growth factors, such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and transforming growth factor-beta (TGF-β), which are important in cell growth and maintenance of skin tissue.[4,5] Additionally, CM is rich in vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), macrophage stimulating protein (MSP), keratinocyte growth factor (KGF), and insulin-like growth factor 1 (IGF-1).[6] As long as the donor of umbilical cord tissue is properly screened, tested, and approved, CM is devoid of cells, thereby eliminating the potential for Graft Versus Host Disease (GvHD) and mitigating adverse consequences.[3] Currently, there are no specific regulations governing the use of stem cells in cosmetics, so testing requires approval from a regulatory body before initiating clinical trials.[7]

Skin aging is a multifaceted and progressive biological process associated with both photoaging, induced by exposure to sunlight, and chronological aging, occurring naturally over time.[8] Photoaging and chronological aging may share similar mechanisms, as evidenced by reduced responses to growth factors associated with chronological aging, The deterioration of the extracellular matrix, reduced production of collagen molecules, and decreased procollagen functionality are evident in photodamaged skin.[9] Numerous internal and external elements play a role in the phenomenon of skin

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aging by weakening the skin's structural integrity and impairing regular physiological processes. UV light, commonly known as a contributor to photoaging, is the most significant external factor among these components.[10] Signs of aging skin include wrinkles, dryness, thinning, sagging, uneven pigmentation, and loss of elasticity.[11,12] Additionally, aging can manifest as drooping skin, age spots or blemishes, changes in pigmentation, loss of skin tone, and dehydration.[13] The manifestation of skin aging through clinical symptoms like wrinkles, sagging, and irregular texture predominantly stems from alterations in the extracellular matrix (ECM), particularly affecting collagen and elastin components.[14–18] This results in the buildup of impaired large molecules like lipids, proteins, and DNA. Reactive molecules like free radicals, produced during regular cellular metabolism, chemically alter these macromolecules. As people age, the generation of these molecules increases significantly.[19]

Ultraviolet (UV) radiation plays a pivotal role in the process of skin aging. The effects of both ultraviolet B (UVB) and ultraviolet A (UVA) rays on the skin cells manifest through the induction of considerable oxidative stress. This stress arises from the interaction between UV rays and specific substances within the cells known as chromophores and photosensitizers. This interaction leads to detrimental effects such as genetic damage, cellular aging, and damage to connective tissues.[20] The extent of harm induced by ultraviolet (UV) radiation differs based on the specific wavelength of the light.[21,22] UVB radiation, specifically, has the potential to induce genetic alterations within cellular DNA, resulting in manifestations such as skin erythema and the heightened risk of developing skin malignancies, resulting in approximately 800–1000 times more skin damage than an equivalent dose of UVA radiation.[23] Extended contact with UV radiation leads to solar elastosis, a condition marked by the degradation of the extracellular matrix (ECM) within the human skin, consequently precipitating the formation of wrinkles. Additionally, UV light stimulates the production of reactive oxygen species (ROS) in the skin.[24] It is believed that UVB-irradiated fibroblasts contain ROS that activate cytoplasmic signaling pathways associated with both cell growth and the aging process.[25,26] As tissues age, there is typically a rise in the generation of reactive oxygen species (ROS), accompanied by heightened concentrations of 8-Hydroxyguanine (8-oxo-dG) within mitochondrial DNA (mtDNA). These observations imply that the progressive buildup of oxidative damage to DNA plays a substantial role in the aging phenomenon.[27] Fibroblasts exposed to UVB radiation exhibit a reduction in the synthesis of type I collagen along with the upregulation of MMP-1 expression.[28–33]

Anti-aging medicine, a rapidly evolving field, focuses on utilizing cutting-edge scientific and medical advancements to prevent, detect, treat, and cure age-related dysfunctions. Its primary objective, however, goes beyond merely extending lifespan. Rather, anti-aging medicine aims to sustain a healthy existence for an extended duration. This involves safeguarding against age-related ailments, including atherosclerosis, neurodegenerative disorders, cancer, diabetes, and molecular-level skin wrinkles, by employing antioxidants.[34–36] Several compounds have been identified as having geroprotective properties. These include anesthetics like procaine, plant polyphenols such as antioxidants and resveratrol, rapamycin, and vitamins E, C, and A.[37] Other compounds with geroprotective effects include coenzyme Q, lipoic acid, carotenoids, selenium, hormones like growth hormone, thyroid hormones, and adrenaline, as well as sex hormones. Additionally, melatonin, bioregulatory peptides like thymalin and epithalamin, biguanides such as phenformin and metformin, and adaptogens like ginseng have been studied for their anti-aging properties. These geroprotective compounds, which are being researched globally, disrupt the oxidative balance and include both natural components like resveratrol, rapamycin, and procaine, as well as synthetic molecules.[35] Substances possessing innate anti-aging attributes, including polysaccharides, hydroxy acids, polyphenols, vitamins, and various other compounds, significantly contribute to skincare.[38] Hyaluronic acid, collagen, and elastin play vital roles in maintaining the integrity and appearance of the skin. These three elements act as the building blocks, imparting strength, flexibility, and structure to the skin's composition. With collagen's robustness, elastin's elasticity, and hyaluronic acid's moisture-retaining properties, the skin achieves a harmonious combination of resilience, suppleness, and smoothness. These structural components work in unison to create a solid foundation for healthy and youthful-looking skin.[39]

In the development of anti-aging science, many therapies are being explored to achieve the best anti-aging results. Among these, cell therapy stands out, encompassing both unicellular and multicellular therapies, including stem cell-based and non-stem cell approaches. These therapies differ mainly in their characteristics, sources of isolation, and areas of application.[40] Stem cell research is a rapidly advancing field that holds promise in the realm of regenerative medicine. It offers hope for treating numerous severe illnesses by addressing unmet medical needs.[41] Stem cells possess an

inexhaustible potential for proliferation and possess an extraordinary capacity to undergo differentiation into diverse cellular lineages, rendering them a captivating focus of research within the realm of anti-aging investigations. These remarkable cells possess the capacity to mitigate oxidative stress, prevent programmed cell death, and counteract aging effects. They also have the ability to promote the generation of the extracellular matrix (ECM) to rejuvenate the skin, regulate inflammatory reactions, and offer advantages such as reducing wrinkles and enhancing brightness.[42,43] Mesenchymal stem cell (MSC)-derived treatments represent a promising avenue in addressing skin disorders, as they provide replenishing cells that aid in regulating epidermal equilibrium and rejuvenating compromised tissues.[44–46] Human mesenchymal stem cells (hMSCs) possess significant advantages owing to their remarkable capacity for differentiation into diverse cellular lineages. These cells can be readily obtained, cultured, and efficiently expanded within laboratory environments. Furthermore, they possess the versatility to be utilized in both allogeneic therapies and exhibit robust paracrine functions. Additionally, researchers have utilized the capabilities of conditioned medium (CM) obtained from these stem cell cultures for diverse pragmatic purposes.

Material and Methods

The literature search was conducted using the Scopus database to capture all high-quality research articles. The search for articles was not restricted by publication date to ensure the comprehensiveness of the review. Relevant research articles were identified using the following keywords: ((“conditioned medium“ OR “secretome“) AND ‘stem cell’ AND (”anti-aging” OR ”skin rejuvenation” OR ‘skin aging’ OR ‘cellular senescence’)). Following identification, the articles underwent manual screening in accordance with predefined inclusion and exclusion criteria (outlined in Table 1). The initial search yielded 68 articles, which were subsequently subjected to screening. Following a thorough evaluation, 23 articles were deemed to meet the inclusion criteria, whereas 45 articles failed to meet the criteria established for this research endeavor.

Results

Human Skin Aging Process and Factors

Skin aging, characterized primarily by a decrease in elasticity and the development of wrinkles, has puzzled humanity for centuries, driving us to tirelessly combat its effects, yet it remains an enigma. The skin experiences extrinsic aging, influenced by external factors, in addition to intrinsic aging, resulting in alterations in both the structure and function of the skin tissue.[47,48] Other research indicates that aging causes changes in inflammatory responses as well as scar remodeling, maturation, proliferation, and cell migration, all of which play a crucial role in effective skin healing.[49] The aging process negatively impacts the mechanisms that facilitate wound healing. This is mainly due to a reduced rate of cell migration and proliferation, as well as a deficiency in the production of the components comprising the extracellular matrix. Furthermore, there is a shortage of growth factors that promote cell division. In older animals, wounds exhibit diminished cell proliferation and noticeable alterations in matrix deposition across all biological components, including keratinocytes, fibroblasts, and endothelial cells.[50] An overview of the various characteristics of aging is provided in Figure 1.

Both the processes of intrinsic and extrinsic aging are subject to the influence of diverse mechanisms and conditions. Emerging studies suggest that oxidative harm plays a pivotal role in the initiation of numerous age-related ailments, including but not limited to arthritis, Alzheimer’s disease, and allergic conditions.[51] As individuals grow older, the incidence of these conditions tends to rise steeply. Increased concentrations of reactive oxygen species (ROS) within cellular structures are strongly linked to the aging of cells, with hydrogen peroxide (H2O2) potentially playing a pivotal

Table 1 Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
Studies were restricted to articles published in English Studies that used the secretome as the active substance	Articles that did not include in vitro, in vivo, or clinical trials Articles with results that were not relevant to anti-aging outcomes were excluded

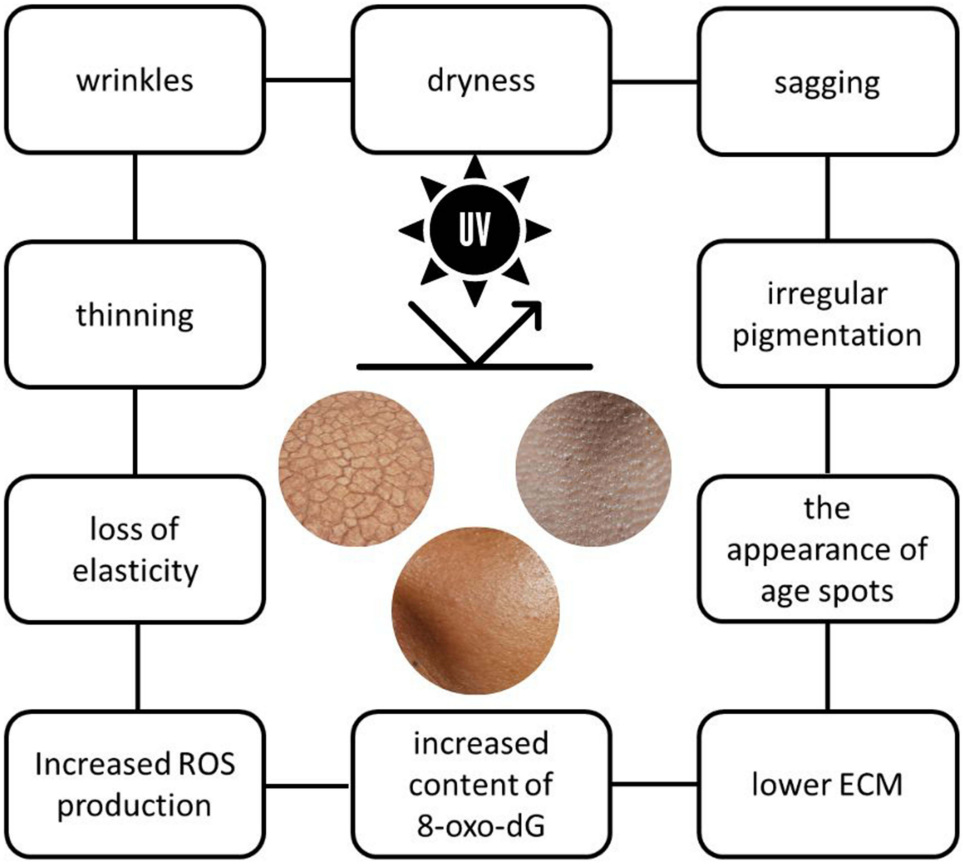

Figure 1 Aging Characteristics. The hallmark indicators of aging include the deterioration of the extracellular matrix (ECM), heightened generation of reactive oxygen species (ROS) within the skin, and raised concentrations of 8-hydroxyguanine (8-oxo-dG) in mitochondrial DNA (mtDNA). These changes manifest in various ways, including wrinkles, dryness, loss of elasticity, thinning, sagging, uneven pigmentation, the appearance of age spots or blemishes, sagging skin, changes in pigmentation, dehydration, and loss of skin tone. Additionally, there is an accumulation of damaged lipids, proteins, and DNA. Reactive molecules like free radicals chemically modify these macromolecules, contributing to the aging process.

role in initiating this aging phenomenon. Cells undergoing aging can be identified by enhanced Senescence-Associated β-Galactosidase (SA-β-gal) activity. Senescence can be induced by markers of cellular aging, including p53/p21Waf1/Cip1, INk4a, and Id-1 (inhibitor of differentiation or DNA binding-1). Senescence can inhibit DNA replication and result in cell cycle arrest.[52,53]

Anti-Aging Treatment and Care Using Conditioned Medium

CM-MSCs have various developmental aspects that act as chemoattractants, recruiting endothelial and macrophage cells for wound healing through cytokines.[54] Chondrocyte-derived factors (CM) have been observed to stimulate the proliferation, migration, and synthesis of Extracellular Matrix (ECM) in fibroblasts exposed to exogenous agents. This implies the potential utility of CM in facilitating skin regeneration and wound healing processes.[55] Therapies combining CM with appropriate cell populations could serve as alternatives or potentially enhance existing procedures. Immunocompatibility is one benefit that could make CMs and their component medicines preferable to cell-based therapies. The absence of the need for donor and recipient selection in therapy due to cell exclusion is another notable benefit.[15,56]

Within the cell culture microenvironment, factors such as cell contact inhibition, physical and chemical characteristics, capacity for cell growth and differentiation, and cell aggregation ability all affect its structure.[57] Like any method, there are uncertainties regarding the application of CM in regenerative medicine, similar to any new technique. A significant concern arises from the absence of universally accepted protocols and criteria governing the biological

processing and quality assurance of treatments derived from cell media (CM). This absence results in a diverse array of compositions and deviations, influenced by factors such as the methodology of cultivation and duration.[58]

Furthermore, cells prepared for therapy may undergo cell death in culture or during transport to the injury site, a challenge that CM avoids due to its lack of living material, thereby avoiding such inconveniences.[59] CM is easy to produce, package, freeze, and transport.[15] The production phase of cell-based therapies requires stringent laboratory sterility measures, while CM application can take place under non-sterile conditions.[15,56]

Using secretome MSCs in biomedical contexts is perceived to present fewer risks compared to the utilization of medicinal formulations containing viable cells.[15,60] Aged mouse MSCs display decreased antioxidant capacity.[61,62] It is worth noting that there may be a risk of hypersensitivity reactions associated with the base medium used for cell culture to produce conditioned medium. Due to limited clinical trials in this field, the long-term effects of CM remain poorly understood.[59]

Therefore, regenerative therapies requiring only outpatient conditions are well-suited to CM. Therapies involving CM can also save time and money because media can be obtained from cells introduced once without waiting for them to mature.[63] As several researchers indicate, the secretome can sustain its curative capabilities over time.[15,64,65] Through the examination of individual patient reactions to the administration of contrast media via in vitro studies, it becomes possible to gauge to some extent the semi-retention duration of medium constituents within the body. Incorporating patient-specific variables into the medium's structure during the manufacturing process can also significantly increase the effectiveness of CM-mediated implementation, enabling the advancement of secure and efficient tailored treatments for diverse ailments.[56]

Conditioned Medium as Stem Cell Carrier

Numerous studies have showcased the effectiveness of stem cells conditioned in a medium for combating aging, with a predominant emphasis on non-dermal stem cells, notably those sourced from adipose tissue or umbilical cord blood.[66–68] Umbilical Cord-Conditioned Medium (UC-CM) has been shown in numerous clinical and preclinical studies to support tissue homeostasis by promoting skin regeneration and protecting against harmful skin conditions.[69–72] It is well-known that conditioned media (CM), with an assortment of cytokines and growth factors stimulate the restoration of damaged tissues, facilitating their regeneration. Therefore, stem cell conditioned media represent a cutting-edge technology that can be applied to treat dark spots around the eyes, hair, and skin care.[15,41] Below are examples of various conditioned media formulations, each containing different types of stem cells for anti-aging purposes (Table 2).

Various Anti-Aging Tests

In anti-aging testing, various methods are employed to analyze the effectiveness of conditioned medium containing stem cells. UVB rays can damage DNA, age cells, and potentially trigger skin cell cancer.[91] UVB-induced apoptosis is thought to represent a protective mechanism aimed at maintaining skin integrity while eradicating aberrant precancerous cells.[92] The G1 phase of the cell cycle is arrested, and the cell's capacity to divide is reduced due to cellular degeneration.[93] Intense light exposure can lead to increased cell apoptosis and inflammation. Cell migration was assessed via a wound healing assay. HaCaT cells were cultured on six-well plates. When they reached full coverage, a deliberate wound was created in the center of each well. The migration rate of HaCaT cells was photographed and quantified using inverted microscopy.[88]

A summary of in vitro tests involving conditioned medium for anti-aging purposes is provided in Table 3. Clinical trials have also been conducted by Sohn et al in 2018,[14] where twenty-five female participants with mild to moderate signs of aging, aged 29 to 69, were enrolled. Over the course of four weeks, participants applied a cosmetic solution infused with 5% EPC-CM to their cheeks twice daily. Initial assessments were conducted to evaluate Fitzpatrick skin attributes and dimensions of crow's feet wrinkles,[94] doctor's global rating scale,[95] Before the application, measurements of the wrinkle index using the ANTERA 3D system from Miravex in Dublin, Ireland, were conducted. This index encompasses various aspects such as skin texture, skin surface, and the depth of wrinkles (specifically, crow's feet). The evaluation of these components was performed by two dermatologists. The wrinkle index is calculated by determining the spot with the least facial muscle activity. The deepest point in a given area is used to determine wrinkle depth, while

Table 2 Conditioned Medium Carrier of Stem Cell as Anti-Aging

Stem Cell	Conditioned Medium	Application	Reference
Umbilical Cord (UC)	UC-CM	Anti-aging, detoxification, anti-inflammatory, cell apoptosis	[73]
	hUC-MSC-CM	Anti-photoaging	[74]
	hUC-MSC-CM	Skin Rejuvenation	[75]
	Flask Medium (FM)	Anti-apoptosis, Anti-aging, Anti melanogenic	[76]
	Bioreactor Medium (BM)	Anti-apoptosis, Anti-aging, Anti melanogenic	[76]
	USC-CM	Skin Rejuvenation	[77]
Probiotic	Probiotik-CM	Anti-photoaging, Anti pigmentation	[78]
Adipose Tissue Derived (ATd)	CM-hATMSCs	Anti-aging	[79]
	CM-hATMSCs	Anti-aging	[80]
Adipose Stem Cell (ASC)	ASC-CM	Anti-photoaging	[81]
Trophoblast derived (TBd)	TB-CM	Regenerative, Anti-aging	[82]
Adipose Mesenchymal	MSC-CM	Anti-aging	[83]
Adipose Derived Stem Cell (ADSC)	ADSC-CM	Anti-photoaging	[84]
	ADSC-CM	Anti-aging	[46]
	ADSC-CM	Anti-aging	[85]
Natural Killer Cell (NKc)	NK-CdM	Anti-skin aging	[86]
Dermal Fibroblast	DFCM	Anti-aging	[87]
MRC-5 Cell	AMC-CM	Anti-aging	[88]
Epidermal Progenitor Cell (EPC)	EPCCM	Anti-aging	[14]
Fetal Dermal Mesenchymal	Fetal Dermal MSC-CM	Anti-aging	[89]
Cord Blood Mesenchymal (CBMSC)	CBMSC-CM	Anti-aging	[90]

Table 3 Various Conditioned Medium Tests as Anti-Aging in vitro

Conditioned Medium	Stem Cell	Testing	Result	Reference
UC-CM	Umbilical cord	- UV irradiation - Cytotoxicity assay - Skin irritation test - Assessment the EpiKutis inflammatory model's shape and inflammatory variables - Quantitative real-time polymerase chain reaction (RT-qPCR) for reverse transcription - Enzyme-linked immunosorbent assay (ELISA) - Western blotting - Cellular senescence detection - Intracellular ROS generation - UVA/UVB contact, Masson stains, and ex vivo epidermal cultivation	- No irritation to normal skin and has a low level of cytotoxicity - Enhances the skin barrier - Eliminates UV-induced error of the skin's microenvironment by improving detoxification power - Protects and helps to preserve skin homeostasis and shields HSF from oxidative stress.	[73]
hUC-MSC-CM		- Assay for expansion - Apoptosis detection - Cell cycle detection - Detection of reactive oxygen species - Cell migration assay - qRT-PCR - Western blot analysis	- UVB irradiation impacts the cell cycle and proliferation, among other biological processes induces aging-related secretory phenotypes - Minimizes cell apoptosis, prevents the cell cycle's G1 phase arrest, reduces the production of oxidative oxygen species and enhances cellular motility. - The genes MYC, IL-8, FGF-1, and EREG were pinpointed as vital elements in the anti-photoaging effects of hUC-MSC-CMs, according to qRT-PCR outcome. - According to findings of Western blotting, the main proteins responsible for the anti-photoaging action of hUC-MSC-CM were cyclin A2, TGFβ, FGF-1, p53, C-JUN, and C-FOS.	[74]

(*Continued*)

Table 3 (Continued).

Conditioned Medium	Stem Cell	Testing	Result	Reference
Flask Medium (FM) dan Bioreactor Medium (BM)		- Quantification of pro-collagen, pro-melanin, and hyaluronic acid (HA) content - The assay for hyaluronic acid (HA) - The pro-collagen tests - Assay for tyrosinase activity - Growth aspect, HA, and pro-collagen secretion seemed substantially greater on BM treatment compared to FM care, according to reverse transcription (RT)-PCR. - BM prevented UVB-induced oxidative stress from killing CCD-986SK cells. - After being exposed to UVB radiation, BM downregulated the collagen collapse accelerating gene MMP-1 and elevated the promoter activity of the antioxidant genes GP, CAT, and SOD1. - In SK-MEL-31 cells encouraged by melanocyte-stimulating hormone (A-MSH), BM decreased the creation of melanin and downregulated the levels of TRP-2, tyrosinase, TRP-1, and MITF. - Because of its anti-melanogenic, anti-aging, and anti-apoptotic functions, BM can be used as a skin protective agent (72).	- BM treatment resulted in significantly rise in the release of growth factor, hyaluronic acid (HA), and pro-collagen. BM offered a shield to CCD-986SK cells against the damage induced by UVB radiation-induced oxidative stress. - After UVB radiation exposure, BM inhibited the MMP-1 gene responsible for hastened collagen breakdown while promoting the activation of antioxidant genes such as GP, CAT, and SOD1. - In SK-MEL-31 cells triggered by melanocyte-stimulating hormone (A-MSH), BM decreased the production of melanin and decreased the levels of TRP-2, TRP-1, tyrosinase, and MITF. - Because of its anti-melanogenic, anti-aging, and anti-apoptotic qualities, BM can be made as a skin shielding agent.	[76]
USC-CM		- People growth element antibody array-Scratch Assay-Collagen synthesis of HDFs stimulated by exosomes - USC-CM Exos penetration of a person's skin - Exosomes stimulate people skin's synthesis of collagen.	- There are significant amounts of growth variables in USC-CM and USC-CM Exos. - HDFs' integrating of USC-CM Exos - In vitro, USC-CM Exos stimulates HDF migration and collagen synthesis. - USC-CM Exos encourage the synthesis of collagen in a person's skin - USC-CM is a major factor that promotes the growth and secretion of ECM proteins in HDF, which is crucial for dermatological rejuvenation. It can be used as an exosome make for skin regeneration components.	[77]
Probiotic-CM	Probiotic	- Water-soluble tetrazolium salt assay - UVB irradiation and reagent treatment - Analysis of intracellular ROS levels - High-performance liquid chromatography analysis - Immunoblot assay - Reverse-transcription quantitative polymerase chain reaction (RT-qPCR) - Determination of melanin content - Measurement of mushroom tyrosinase activity	- Exerting protective effects of CM from Bi and La species on UV-irradiated NHDFs - Exerting inhibitory effects of CM from Bi and La species on melanogenesis - Exerts anti-pigmentation effect of CM from B. lactis via down regulation of PKA and ERK pathways - The anti-pigmentation effect of CM pHO is mediated by UV-induced inhibition of MITF and tyrosine expression through dephosphorylation/inactivation of upstream regulators, PKA, ERK, and CREB in melanocytes	[78]
CM-hATMSCs	Adipose Tissue Derived	- Viability Assay Fibroblast Cell Culture and Cells - Human Skin - Measurement of ROS Level - Measurement of Collagen Content	- Effect of CM-hATMSCs on Proliferation of Aging Cell Model - Effect of CM-hATMSCs on ROS Level of Aging Cell Model - Effect of CM-hATMSCs on Collagen Level of Aging Cell Model	[79]
CM-hATMSCs		- Type of growth factors (EGF, FGF, TGF-b1, TGF-b2, VEGF2, VCAM1) determined from CM-hATMSCs	- FGF was the most abundant substance in CM-hATMSCs, both in cells treated with FFP and in cells not. - FGF as the composition of growth elements in CM-hATMSCs	[80]

(*Continued*)

Table 3 (Continued).

Conditioned Medium	Stem Cell	Testing	Result	Reference
TB-CM	Trophoblast derived	- Nanoparticle Tracking Analysis - Western Blot Analysis - CCK8 Assay - Wound Scratch Assay - SA-β-Gal Staining - Real-time tangible RT-PCR and RNA extraction - Ultraviolet B Irradiation on HNDFs - RNA-seq	- TB-Exos and TB-CM Encourage the Spread of HNDF - TB-Exos and TB-CM Have Impacts on HNDFs That Promote Moving - TB-CM Restores HNDF Aging - The presence of dermal skin extracellular matrix constituents was markedly elevated in HNDFs treated with TB-CM and TB-Exos.	[82]
ADSC-CM	Adipose Derived Stem Cell	- Cell Culture and UVB Irradiation - Intracellular ROS Generation - Hoechst 33258 Staining - MMP-1 and IL-6 Inhibition Assay - RNA Preparation and Reverse Transcription (RT)-PCR - Western Blot Analysis	- The effect of ADSC-CM was evaluated on both the UVB-exposed human keratinocyte cell line HaCaT and normal human dermal fibroblasts (NHDFs). - The potential for ADSC-CM's presence to change the expression of signaling molecules engaged in early UVB-responsive signaling pathways is evident. - ADSC-CM can enhance the activity of the antioxidant response element (ARE) and stimulate the expression of the gene responsible for promoting collagen synthesis, namely, transforming growth factor-β (TGF-β). - Treatment with ADSC-CM led to the suppression of matrix metalloproteinase-1 (MMP-1) expression and the inhibition of type I procollagen production.	[84]
ADSC-CM		- Concentration and efficiency detection of PDGF-AA in ADSC-CM - ADSC-CM treatment and UVB radiation of HDFs - Cell proliferative activity detection - Cellular senescence detection - Quantitative real-time polymerase chain reaction (qRT-PCR) - Western Blot analysis - PIK3/Akt pathway inhibition - PDGF-AA pathway inhibition	- The produced ADSC-CM's PDGF-AA focus dropped with time while maintaining good bioactivity at low temperatures. - In irradiated HDF, the ADSC-CM pretreatment has the potential to slightly or dramatically enhance cellular proliferation and reduce cellular senescence. - Before exposure, pretreatment with ADSC-CM led to increased expression of collagen I, collagen III, elastin, and TIMP-1, while reducing the expression of MMP-1 and MMP-9 in both irradiated and non-irradiated HDFs. - The implementation of LY294002 considerably reduced the expression of ECM protein, while ADSC-CM pretreatment considerably raised the presentation of pAkt.	[46]
ADSC-CM		- Culture, UVB radiation, and ADSC-CM treatment of HDFs - Cell proliferative activity detection - Cellular senescence detection - Cellular apoptosis detection - Quantitively real time polymerase chain reaction (qRT-PCR)	- In both exposed and non-irradiated HDF, CM dramatically increased cellular proliferative activity and restored functionality. - When it came to cellular senescence brought on by intrinsic senescence factors, ADSC-CM had no apparent impact, but it did reduce cellular apoptosis and senescence brought on by UVB. In all three HDF generations, the outcomes were comparable, albeit to various types.	[85]
NK-CdM	Natural Killer Cell	- NK cell enrichment and expansion - Neonatal human dermal fibroblast culture - CCK-8 assay - Quantitative determination of type I collagen secretion - MMP-1 inhibition assay - Quantification of elastin - Antibody array - Reverse transcription quantitative polymerase chain reaction (RT qPCR) analysis and isolating - Western blot analysis - Total antioxidant capacity assay - Immunocytochemistry - A three-dimensional reconstructed human full skin model (Keraskin FT™) subjected to UVB irradiation. - Picrosirius red staining	- Procollagen presentation rose, UV B-treated NHDF proliferated, and matrix metalloproteinase (MMP)-1 expression dropped in response to NK-cdM. - Total the antioxidant potential demonstrated the strong antioxidant action of NK-cdM. - By deactivating MAPK signaling, NK-cdM prevented UV-B-induced collagen damage. - Inhibition of UV-B-induced JNK activation and MMP-1 presentation by NK-cdM	[86]

(*Continued*)

Table 3 (Continued).

Conditioned Medium	Stem Cell	Testing	Result	Reference
DFCM	Dermal Fibroblast	- Protein quantification - Sodium dodecyl sulfate polyacrylamide gel electrophoresis (sds-page) - Growth rate and migration rate of hdfs - Scratch assay - Gene expression analysis via rt2 profiler pcr assay	- By incorporating bFGF, EGF, and insulin into serum-free fibroblast (FM) media, a conditioned medium (CM) named DFCM-GM was generated, which contained a higher protein concentration. - DFCM-GM consistently exhibited effects on wound healing characteristics, particularly in terms of cell proliferation and migration rates during and after the healing process. - HDFs were compared using DFCM-KM and DFCM-FM. - DFCM-GM supplementation showed anti-aging gene expression by HDFs	[87]
AMC-CM	MRC-5 Cell	- Assessing paeonol's effect on the viability of MRC-5 cells - Analyzing senescent MRC-5 cells through the SA-β-gal assay - Determining the impact of paeonol on the concentration of reactive oxygen species (ROS) in MRC-5 cells - Evaluating paeonol's influence on the cloning efficiency of HaCaT cells - Measuring IL-6 and IL-8 levels in HaCaT cells - Investigating the impact of paeonol on the migration and invasion capabilities of HaCaT cells - Immunofluorescence assay - Western blot analysis	- Paeonol diminishes the aging progression in MRC cells induced by H2O2. The cell count is reduced by five. - Paeonol decreases the manifestation of aging markers in senescent MRC-5 cells. - Paeonol lowers the levels of reactive oxygen species (ROS) induced by hydrogen peroxide (H2O2) and enhances the translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) into the cell nucleus in MRC-5 cells. - Both AMC-CM and paeonol have the potential to significantly decrease the expression levels of IL-6 and IL-8, which could impede the epithelial-mesenchymal transition (EMT) process in HaCaT cells. - In the model group, the protein expression of p-ERK, TGF-B1, p-Smad2, and p-Smad3 was higher compared to the control group. - Paeonol effectively inhibits EMT in highly metastatic pre-malignant HaCaT cells by modulating the activity of the ERK and TGF-B1/Smad pathways.	[88]

Abbreviations: CM, Conditioned medium; CB, Cord Blood; GvHD, Graft Versus Host Disease; UV, Ultraviolet; ECM, Extracellular Matrix; ROS, Reactive Oxygen Species; 8-oxo-dG, 8-Hydroxyguanine; mtDNA, Mitochondrial DNA; MSC, Mesenchymal Stem Cell; H_20_2, Hydrogen Peroxide; SA-β-Gal, Senescence-Associated β-Galactosidase; UC, Umbilical Cord; HDFs, Human Dermal Fibroblast; UCB, Umbilical Cord Blood; FM, Flask Medium; BM, Bioreactor Medium; Atd, Adipose Tissue Derived; ASC, Adipose Stem Cell; TB, Trophoblast; ADSC, Adipose Derived Stem Cell; NK, Natural Killer; EPC, Epidermal Progenitor Cell.

the skin's surface represents the volume of the entire affected area in a given region. The Ra value of the ANTERA 3D program index itself reflects skin texture, indicating the level of surface roughness. Various tests of conditioned medium as anti-aging agents in vivo and clinically are summarized in Figures 2 and 3. Figure 3 represents the rapid advancement of clinical testing for conditioned media as an anti-aging agent. This progress can provide more safety data on the use of conditioned media, which would be useful for regulatory bodies in each region. However, there are still numerous obstacles in testing that have prevented several countries from issuing specific regulations for the use of conditioned media (CM), particularly in clinical trials. Currently, there are no regulations governing the use of stem cells in cosmetics, so testing requires approval from a regulatory body before starting clinical trials.[7] The use of CM for regenerative medicine remains a new field with many uncertainties. There is a lack of recommendations and general standards for bioprocesses and quality control of CM-based therapies, leading to significant variations in content that depend on the method and duration of culture.[96] Furthermore, hypersensitivity reactions may occur due to the components in the basic media used to produce CM. Given the limited number of clinical trials in this field, the long-term effects of its use are not yet fully understood.[97] The use of stem cells has certain limitations, including maintaining biological activity, quantifying biologically active substances, and managing logistics and distribution.[98] Storage temperatures must always be low, and storage times must be relatively short.[46] In some studies, small sample sizes and a lack of pathological assessments are the main limitations.[75] A challenge that cannot be ignored is the difficulty of maintaining conditioned medium during storage until its application.

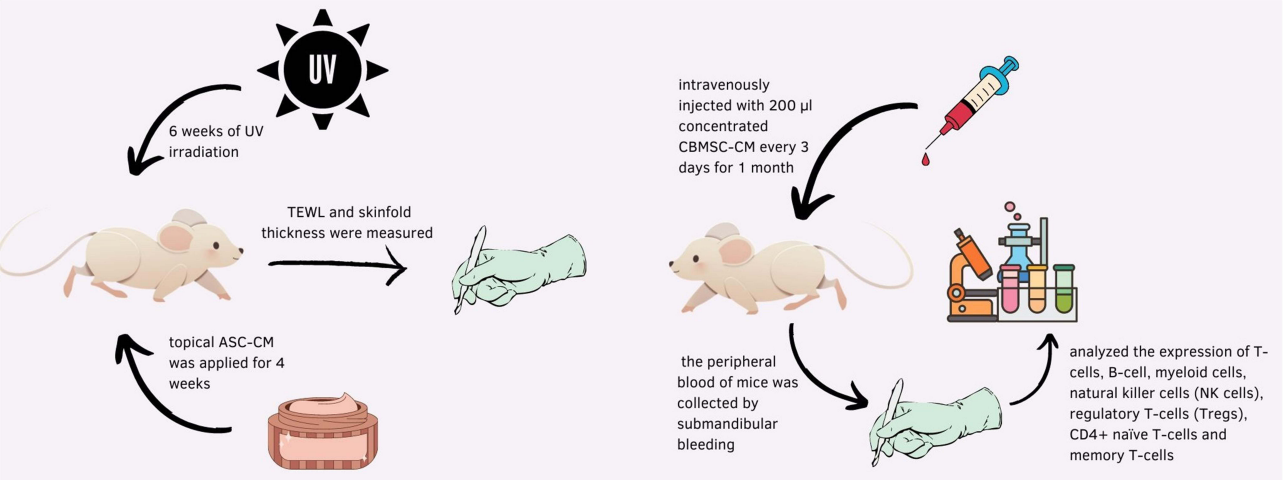

Figure 2 Assessment of Conditioned Medium for Anti-Aging Effects In Vivo. In an experiment comprising 28 male Wistar rats aged between 10 and 12 weeks, the rodents underwent a one-week acclimatization period before being randomly assigned to four distinct groups. For four weeks, ASC-CM was administered twice daily, resulting in a total radiation dose of 4.2 J/cm2 over six weeks. Data analysis was conducted using SPSS. According to the assessment of Transepidermal Water Loss (TEWL) and skin thickness, ASC-CM shows potential as a viable remedy for skin photoaging. Nevertheless, additional investigation is warranted to ascertain the enduring impacts of ASC-CM. In aged mice, the immune response exhibited greater efficacy when conditioned with human cord blood mesenchymal stem cells (CBMSC-CM). This led to a decreased cell count associated with aging in kidney tissue. CBMSC-CM, with its significantly reduced hydrogen peroxide levels, stimulates T-cell responses and reduces oxidative stress, thereby inhibiting cellular senescence.

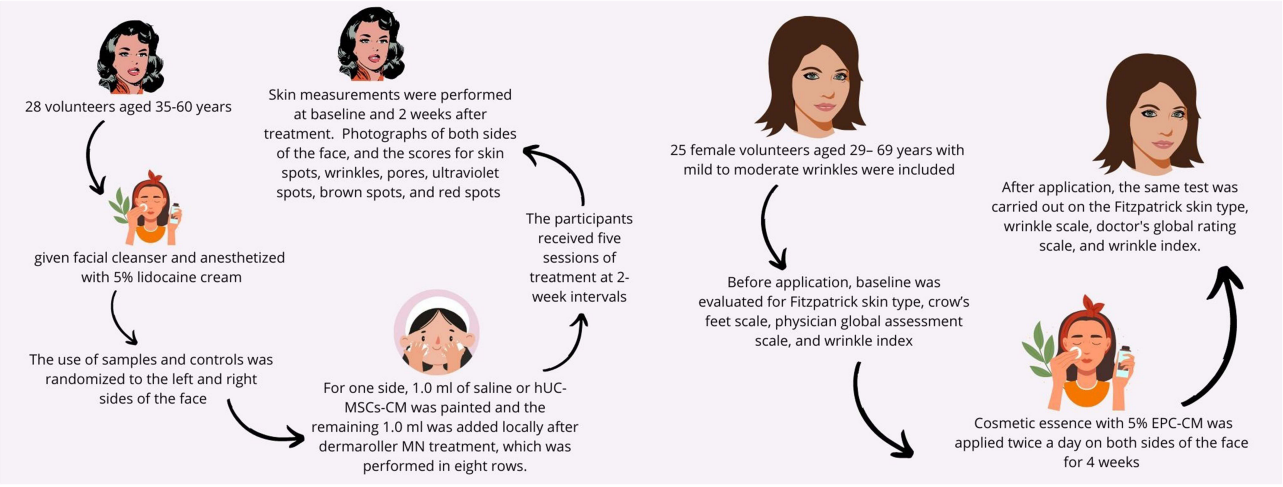

Figure 3 Test Results of Conditioned Medium as Clinical Anti-Aging. Previous research indicates that the integration of human umbilical cord mesenchymal stem cell-conditioned medium (hUC-MSCs-CM) with Microneedling (MN) resulted in noteworthy enhancements in brightness and pore size, as evidenced by comprehensive assessments conducted before and after treatment. These improvements were found to be significantly superior to those observed with MN as a standalone therapy. Another investigation emphasizes the practical results, wherein a cosmetic formulation comprising 5% EPC-CMs was administered to the facial skin of 25 female participants twice a day over a duration of four weeks. In this clinical investigation, EPC-CMs demonstrated improvements in the signs of skin aging, potentially through the activation of the cellular defense system, as substantiated by in vitro findings.

Mechanism of Stem Cell Condition Medium as Anti-Aging

Zuk et al published the first analysis of adipose-derived stem cells (ASCs) in 2001.[99] Numerous research investigations have demonstrated the beneficial impacts of these cells, such as anti-apoptotic, immunomodulatory, and wound repair impacts, either through paracrine effects or differentiation.[99,100] Recent research has indicated that ASCs have the potential to improve the emergence of wrinkles resulting from photoaging is accompanied by a simultaneous enhancement in collagen synthesis within fibroblasts cultured in vitro.[29,101]

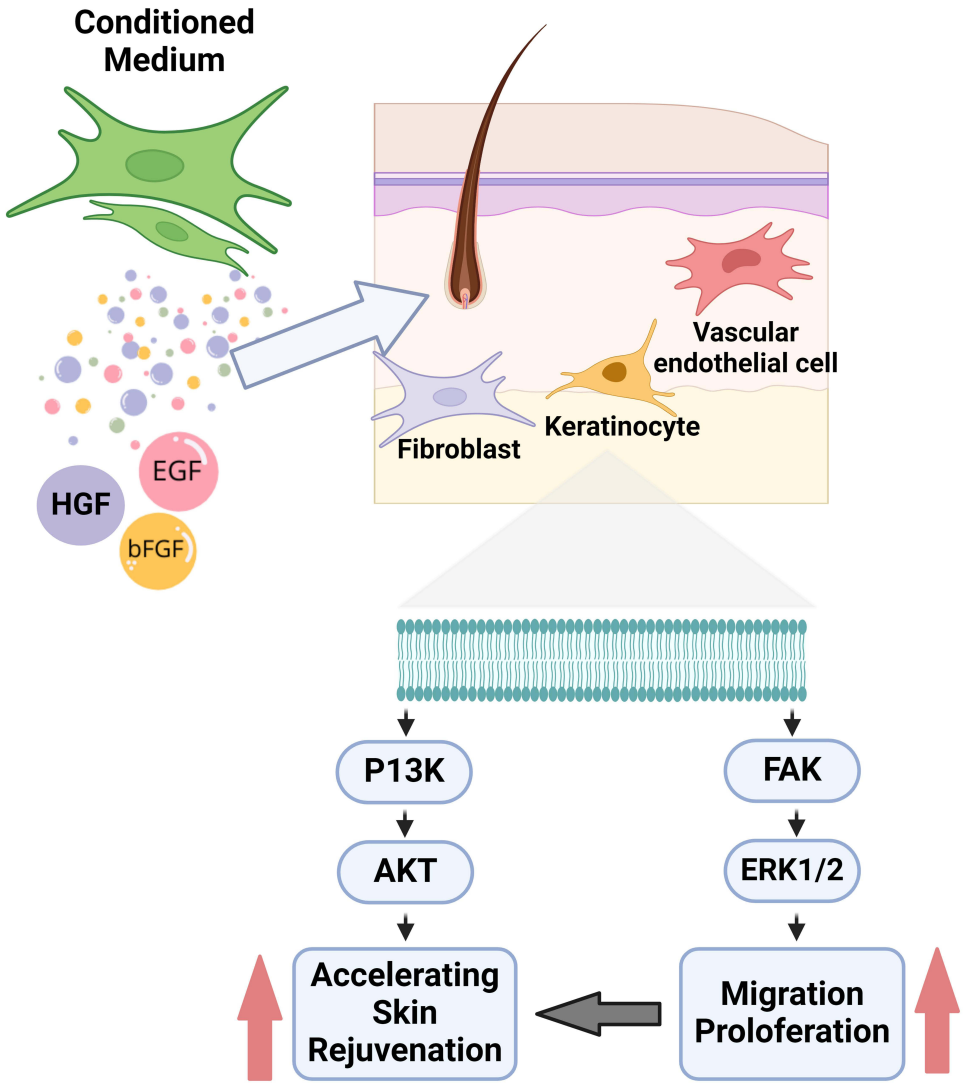

Figure 4 Mechanism of Stem Cell Condition Medium as Anti-Aging.

In a separate study conducted by Wang et al in 2015,[85] HDFs at different points in the aging process underwent UVB exposure followed by cultivation in conditioned medium derived from adipose-derived stem cells (ADSC-CM), which contains the whole crude secretions and offers more convenient storage and safer application compared to ADSCs. Various biochemical markers were assessed to comprehensively observe the aging characteristics of HDFs and evaluate the anti-aging effects of ADSC secretions.

Moreover, it was observed that mesenchymal stem cells obtained from umbilical cord blood (UCB-MSCs) exhibit elevated expressions of wound healing mediators in contrast to various other MSCs.[102] UCB-MSCs promote collagen synthesis, proliferation, and fibroblast migration.[103] Additionally, conditioned media from UCB-MSCs have demonstrated the ability to improve wound closure and re-epithelialization in animals when administered via subcutaneous injection.[104] The visual representation of the mechanism of conditioned media in helping to prevent skin aging is shown in Figure 4.

Conclusion and Future Perspective

Skin aging is a problem that arises from natural (intrinsic) and external (extrinsic) factors. The challenge of reducing these skin aging factors has attracted significant attention from researchers today. Conditioned media from stem cells

have tremendous potential in reducing skin aging factors. In addition, conditioned media stimulate skin regeneration and recovery while showing low cytotoxicity. The description above provides examples of various types of conditioned media from different stem cell sources, as well as research results demonstrating their anti-aging effects on the skin. Many tests have been conducted to prove the benefits of conditioned media for anti-aging, including in vitro, in vivo, and clinical studies. In the future, numerous areas require further exploration to advance the use of conditioned media, particularly for anti-aging applications. Current research still has gaps that need to be addressed, such as the potential use of methotrexate,[105] mechanistic studies,[106] ECM development and Its influence on scar formation after glaucoma filtration surgery through the p53/Sp1/miR-29b pathway,[107] the impact of endogenous klotho deficiency on the potential for stem cell differentiation during blood vessel calcification,[108] and the interaction between macrophages and MSCs to generate ideas for developing macrophage activators to promote tendon-bone healing.[109] Advances in the exploration and utilization of conditioned media are expected to offer solutions to skin aging problems.

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References

1. Wagner W, Roderburg C, Wein F, et al. Molecular and Secretory Profiles of Human Mesenchymal Stromal Cells and Their Abilities to Maintain Primitive Hematopoietic Progenitors. *Stem Cells*. 2007;25(10):2638–2647. doi:10.1634/stemcells.2007-0280
2. Bhang SH, Lee S, Shin JY, Lee TJ, Jang HK, Kim BS. Efficacious and clinically relevant conditioned medium of human adipose-derived stem cells for therapeutic angiogenesis. *Mol Ther*. 2014;22(4):862–872. doi:10.1038/mt.2013.301
3. Devidasrao Ramdasi S, Ramdasi S *Growth Factors and Cytokines Secreted in Conditioned Media by Mesenchymal Stem Cells-Promising Possible Therapeutic Approach for Hair Regeneration*. Vol 6. 2016. Available from: http://www.bellafigurahair.co.nz/blog/previous/2. Accessed Sept 7, 2024.
4. Yoon BS, Moon JH, Jun EK, et al. Secretory Profiles and Wound Healing Effects of Human Amniotic Fluid–Derived Mesenchymal Stem Cells. *Stem Cells Dev*. 2009;19(6):887–902. doi:10.1089/scd.2009.0138
5. Kim WS, Park BS, Park SH, Kim HK, Sung JH. Antiwrinkle effect of adipose-derived stem cell: activation of dermal fibroblast by secretory factors. *J Dermatol Sci*. 2009;53(2):96–102. doi:10.1016/j.jdermsci.2008.08.007
6. Park BS, Kim WS, Choi JS, et al. Hair Growth Stimulated by Conditioned Medium of Adipose-Derived Stem Cells Is Enhanced by Hypoxia: evidence of Increased Growth Factor Secretion. *Biomed Res*. 2010;31:27–34.
7. Muthu S, Bapat A, Jain R, Jeyaraman N, Jeyaraman M. Exosomal therapy—a new frontier in regenerative medicine. *Stem Cell Investig*. 2021;8. doi:10.21037/sci-2020-037
8. El-Domyati M, Attia S, Saleh F, et al. Intrinsic aging vs. photoaging: a comparative histopathological, immunohistochemical, and ultrastructural study of skin. *Exp Dermatol*. 2002;11(5):398–405. doi:10.1034/j.1600-0625.2002.110502.x
9. Jenkins G. Molecular mechanisms of skin ageing. *Mech Ageing Dev*. 2002;123(7):801–810. doi:10.1016/S0047-6374(01)00425-0
10. Chen S, He Z, Xu J. Application of adipose-derived stem cells in photoaging: basic science and literature review. *Stem Cell Res Ther*. 2020;11(1):491. doi:10.1186/s13287-020-01994-z
11. Amirkhani MA, Mohseni R, Soleimani M, Shoae-Hassani A, Nilforoushzadeh MA. A rapid sonication based method for preparation of stromal vascular fraction and mesenchymal stem cells from fat tissue. *BioImpacts*. 2016;6(2):99–104. doi:10.15171/bi.2016.14
12. Konno M, Hamabe A, Hasegawa S, et al. Adipose-derived mesenchymal stem cells and regenerative medicine. *Dev Growth Differ*. 2013;55(3):309–318. doi:10.1111/dgd.12049
13. Gold M, Goldman M, Biron J. Efficacy of novel skin cream containing mixture of human growth factors and cytokines for skin rejuvenation. *J Drugs Dermatol*. 2007;6:197–201.
14. Sohn SJ, Yu JM, Lee EY, et al. Anti-aging Properties of Conditioned Media of Epidermal Progenitor Cells Derived from Mesenchymal Stem Cells. *Dermatol Ther*. 2018;8(2):229–244. doi:10.1007/s13555-018-0229-2
15. Pawitan JA. Prospect of Stem Cell Conditioned Medium in Regenerative Medicine. *Biomed Res Int*. 2014;2014:965849. doi:10.1155/2014/965849
16. Russell-Goldman E, Murphy GF. The Pathobiology of Skin Aging: new Insights into an Old Dilemma. *Am J Pathol*. 2020;190(7):1356–1369. doi:10.1016/j.ajpath.2020.03.007
17. Bhat S. Human-Mesenchymal-Stromal-Cells-Derived-Conditioned-Medium-Based-Formulation-for-Advanced-Skin-Care. *J Stem Cells Res Dev Ther*. 2019;5:1–8. doi:10.24966/SRDT-2060/100012
18. Kim HJ, Jung MS, Hur YK, Jung AH. A study on clinical effectiveness of cosmetics containing human stem cell conditioned media. *Biomed Dermatol*. 2020;4(1). doi:10.1186/s41702-020-0056-9

19. Rando TA, Chang HY. Aging, rejuvenation, and epigenetic reprogramming: resetting the aging clock. *Cell*. 2012;148(1–2):46–57. doi:10.1016/j.cell.2012.01.003
20. Ma W, Wlaschek M, Tantcheva-Poór I, et al. Chronological ageing and photoageing of the fibroblasts and the dermal connective tissue. *Clin Exp Dermatol*. 2001;26(7):592–599. doi:10.1046/j.1365-2230.2001.00905.x
21. Besaratinia A, Kim S, Pfeifer GP. Rapid repair of UVA-induced oxidized purines and persistence of UVB-induced dipyrimidine lesions determine the mutagenicity of sunlight in mouse cells. *THE FASEB Journal*. 2008;22(7):2379–2392. doi:10.1096/fj.07-105437
22. Schuch AP, Moreno NC, Schuch NJ, Menck CFM, Garcia CCM. Sunlight damage to cellular DNA: focus on oxidatively generated lesions. *Free Radic Biol Med*. 2017;107:110–124. doi:10.1016/j.freeradbiomed.2017.01.029
23. GILCHREST BA. A review of skin ageing and its medical therapy. *Br J Dermatol*. 1996;135(6):867–875. doi:10.1046/j.1365-2133.1996.d01-1088.x
24. Widowati W, Noverina R, Ayuningtyas W, et al. Reactive Oxygen Species and Aging Mechanism *Biomedical Engineering*. 2018;1:101–134.
25. Glady A, Tanaka M, Moniaga CS, Yasui M, Hara-Chikuma M. Involvement of NADPH oxidase 1 in UVB-induced cell signaling and cytotoxicity in human keratinocytes. *Biochem Biophys Rep*. 2018;14:7–15. doi:10.1016/j.bbrep.2018.03.004
26. Jia Y, Qin Q, Fang CP, et al. UVB induces apoptosis via downregulation of CALML3-dependent JNK1/2 and ERK1/2 pathways in cataract. *Int J Mol Med*. 2018;41(5):3041–3050. doi:10.3892/ijmm.2018.3478
27. Cui H, Kong Y, Zhang H. Oxidative Stress, Mitochondrial Dysfunction, and Aging. *J Signal Transduct*. 2012;2012:1–13. doi:10.1155/2012/646354
28. Fagot D, Asselineau D, Bernerd F. Direct role of human dermal fibroblasts and indirect participation of epidermal keratinocytes in MMP-1 production after UV-B irradiation. *Arch Dermatol Res*. 2002;293(11):576–583. doi:10.1007/s00403-001-0271-1
29. Kim WS, Park BS, Sung JH. Protective role of adipose-derived stem cells and their soluble factors in photoaging. *Arch Dermatol Res*. 2009;301(5):329–336. doi:10.1007/s00403-009-0951-9
30. Quan T, He T, Kang S, Voorhees JJ, Fisher GJ. Solar ultraviolet irradiation reduces collagen in photoaged human skin by blocking transforming growth factor-β type II receptor/Smad signaling. *Am J Pathol*. 2004;165(3):741–751. doi:10.1016/S0002-9440(10)63337-8
31. Kim S, Chung JH. Berberine prevents UV-induced MMP-1 and reduction of type I procollagen expression in human dermal fibroblasts. *Phytomedicine*. 2008;15(9):749–753. doi:10.1016/j.phymed.2007.11.004
32. Buechner N, Schroeder P, Jakob S, et al. Changes of MMP-1 and collagen type Iα1 by UVA, UVB and IRA are differentially regulated by Trx-1. *Exp Gerontol*. 2008;43(7):633–637. doi:10.1016/j.exger.2008.04.009
33. Moon HJ, Lee SH, Ku MJ, et al. Fucoidan inhibits UVB-induced MMP-I promoter expression and down regulation of type I procollagen synthesis in human skin fibroblasts. *Eur J Dermatol*. 2009;19(2):129–134. doi:10.1684/ejd.2008.0611
34. Prasad S, Gupta SC, Tyagi AK. Reactive oxygen species (ROS) and cancer: role of antioxidative nutraceuticals. *Cancer Lett*. 2017;387:95–105. doi:10.1016/j.canlet.2016.03.042
35. Suharyani I, Suhandi C, Rizkiyan Y, et al. Molecular docking in prediction of α-mangostin/cyclodextrin inclusion complex formation. *AIP Conf Proc*. 2023;2706(1). 10.1063/5.0120782.
36. Ho YS, So KF, Chang RCC. Anti-aging herbal medicine-How and why can they be used in aging-associated neurodegenerative diseases? *Ageing Res Rev*. 2010;9(3):354–362. doi:10.1016/j.arr.2009.10.001
37. Asgary S, Rastqar A, Keshvari M. Functional Food and Cardiovascular Disease Prevention and Treatment: a Review. *J Am Coll Nutr*. 2018;37(5):429–455. doi:10.1080/07315724.2017.1410867
38. Wathoni N, Suhandi C, Purnama MFG, et al. Alginate and chitosan-based hydrogel enhance antibacterial agent activity on topical application. *Infect Drug Resist*. 2024:17. 10.2147/IDR.S456403.
39. Papakonstantinou E, Roth M, Karakiulakis G. Hyaluronic acid: a key molecule in skin aging. *Dermatoendocrinol*. 2012;4(3). doi:10.4161/derm.21923
40. El-Kadiry AEH, Rafei M, Shammaa R. Cell Therapy: types, Regulation, and Clinical Benefits. *Front Med Lausanne*. 2021;8. doi:10.3389/fmed.2021.756029
41. Amirthalingam M, Seetharam RN *Issue 2 Article 10 12-1-2016 Part of the Medicine and Health Sciences Commons Recommended Citation Recommended Citation Amirthalingam*. Vol 1. 2016. Available from: https://impressions.manipal.edu/mjmsAvailableat:https://impressions.manipal.edu/mjms/vol1/iss2/10. Accessed Sept 7, 2024.
42. Shidie C, He Z, Xu J. Application of adipose-derived stem cells in photoaging: basic science and literature review. *Stem Cell Res Ther*. 2020;11. doi:10.1186/s13287-020-01994-z
43. Suhandi C, Mohammed AFA, Wilar G, El-Rayyes A, Wathoni N. Effectiveness of Mesenchymal Stem Cell Secretome on Wound Healing: a Systematic Review and Meta-analysis. *Tissue Eng Regen Med*. 2023. doi:10.1007/s13770-023-00570-9
44. Wong SP, Rowley JE, Redpath AN, Tilman JD, Fellous TG, Johnson JR. Pericytes, mesenchymal stem cells and their contributions to tissue repair. *Pharmacol Ther*. 2015;151:107–120. doi:10.1016/j.pharmthera.2015.03.006
45. Kim KH, Blasco-Morente G, Cuende N, Arias-Santiago S. Mesenchymal stromal cells: properties and role in management of cutaneous diseases. *J Eur Acad Dermatol Venereol*. 2017;31(3):414–423. doi:10.1111/jdv.13934
46. Guo S, Wang T, Zhang S, et al. Adipose-derived stem cell-conditioned medium protects fibroblasts at different senescent degrees from UVB irradiation damages. *Mol Cell Biochem*. 2020;463:1–12. doi:10.1007/s11010-019-03630-8
47. Warren R, Gartstein V, Kligman AM, Montagna W, Allendorf RA, Ridder GM. Age, sunlight, and facial skin: a histologic and quantitative study. *J Am Acad Dermatol*. 1991;25(5, Part 1):751–760. doi:10.1016/S0190-9622(08)80964-4
48. Montagna W, Kirchner S, Carlisle K. Histology of sun-damaged human skin. *J Am Acad Dermatol*. 1989;21(5, Part 1):907–918. doi:10.1016/S0190-9622(89)70276-0
49. Eaglstein WH. Wound Healing and Aging. *Dermatol Clin*. 1986;4(3):481–484. doi:10.1016/S0733-8635(18)30811-8
50. Farage MA, Miller KW, Elsner P, Maibach HI. Characteristics of the Aging Skin. *Adv Wound Care*. 2013;2(1):5–10. doi:10.1089/wound.2011.0356
51. Beatty S, Koh HH, Phil M, Henson D, Boulton M. The Role of Oxidative Stress in the Pathogenesis of Age-Related Macular Degeneration. *Surv Ophthalmol*. 2000;45(2):115–134. doi:10.1016/S0039-6257(00)00140-5

52. Beauséjour C, Krtolica A, Galimi F, et al. Reversal of human cellular senescence: roles of the p53 and p16 pathways. *EMBO J*. 2003;22:4212–4222. doi:10.1093/emboj/cdg417
53. Nickoloff BJ, Chaturvedi V, Bacon P, Qin JZ, Denning MF, Diaz MO. Id-1 Delays Senescence but Does Not Immortalize Keratinocytes*. *J Biol Chem*. 2000;275(36):27501–27504. doi:10.1074/jbc.C000311200
54. Ansari MM. Therapeutic Potential of Canine Bone Marrow Derived Mesenchymal Stem Cells and its Conditioned Media in Diabetic Rat Wound Healing. *J Stem Cell Res Ther*. 2013;3(3). doi:10.4172/2157-7633.1000141
55. Balasubramanian S, Thej C, Walvekar A, et al. Evaluation of the Secretome Profile and Functional Characteristics of Human Bone Marrow Mesenchymal Stromal Cells-Derived Conditioned Medium Suggest Potential for Skin Rejuvenation. *J Cosmet Dermatological Sci Appl*. 2017;07(01):99–117. doi:10.4236/jcdsa.2017.71010
56. Gunawardena TNA, Rahman MT, Abdullah BJJ, Abu Kasim NH. Conditioned media derived from mesenchymal stem cell cultures: the next generation for regenerative medicine. *J Tissue Eng Regen Med*. 2019;13(4):569–586. doi:10.1002/term.2806
57. Solursh M, Meier' S. A Conditioned Medium (CM) Factor Produced by Chondrocytes That Promotes Their Own Differentiation. *Dev Biol*. 1973;30:279–289.
58. Kichenbrand C, Velot E, Menu P, Moby V. Dental Pulp Stem Cell-Derived Conditioned Medium: an Attractive Alternative for Regenerative Therapy. *Tissue Eng Part B Rev*. 2019;25(1):78–88. doi:10.1089/ten.teb.2018.0168
59. Maguire G. Stem cell therapy without the cells. *Commun Integr Biol*. 2013;6(6). doi:10.4161/cib.26631
60. Konala VBR, Mamidi MK, Bhonde R, Das AK, Pochampally R, Pal R. The current landscape of the mesenchymal stromal cell secretome: a new paradigm for cell-free regeneration. *Cytotherapy*. 2016;18(1):13–24. doi:10.1016/j.jcyt.2015.10.008
61. Kasper G, Mao L, Geissler S, et al. Insights into mesenchymal stem cell aging: involvement of antioxidant defense and actin cytoskeleton. *Stem Cells*. 2009;27(6):1288–1297. doi:10.1002/stem.49
62. Broekman W, Roelofs H, Zarcone MC, Taube C, Stolk J, Hiemstra PS. Functional characterisation of bone marrow-derived mesenchymal stromal cells from COPD patients. *ERJ Open Res*. 2016;2(2). doi:10.1183/23120541.00045-2015
63. Chuang TJ, Lin KC, Chio CC, Wang CC, Chang CP, Kuo JR. Effects of secretome obtained from normoxia-preconditioned human mesenchymal stem cells in traumatic brain injury rats. *J Trauma Acute Care Surg*. 2012;73(5):1161–1167. doi:10.1097/TA.0b013e318265d128
64. Porzionato A, Patrizia Zaramella X, Dedja A, et al. Intratracheal administration of clinical-grade mesenchymal stem cell-derived extracellular vesicles reduces lung injury in a rat model of bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol*. 2019;316:6–19. doi:10.1152/ajplung.00109.2018.-Mesenchymal
65. Ingato D, Lee JU, Sim SJ, Kwon YJ. Good things come in small packages: overcoming challenges to harness extracellular vesicles for therapeutic delivery. *J Control Release*. 2016;241:174–185. doi:10.1016/j.jconrel.2016.09.016
66. Liu SH, Huang JP, Lee RKK, et al. Paracrine factors from human placental multipotent mesenchymal stromal cells protect endothelium from oxidative injury via STAT3 and manganese superoxide dismutase activation. *Biol Reprod*. 2010;82(5):905–913. doi:10.1095/biolreprod.109.081828
67. Li Q, Chen Y, Ma K, Zhao A, Zhang C, Fu X. Regenerative and reparative effects of human chorion-derived stem cell conditioned medium on photo-aged epidermal cells. *Cell Cycle*. 2016;15(8):1144–1155. doi:10.1080/15384101.2016.1158376
68. Son WC, Yun JW, Kim BH. Adipose-derived mesenchymal stem cells reduce MMP-1 expression in UV-irradiated human dermal fibroblasts: therapeutic potential in skin wrinkling. *Biosci Biotechnol Biochem*. 2015;79(6):919–925. doi:10.1080/09168451.2015.1008972
69. Robert A, Gomes F, Rode M, et al. The skin regeneration potential of a pro-angiogenic secretome from human skin-derived multipotent stromal cells. *J Tissue Eng*. 2019;10:204173141983339. doi:10.1177/2041731419833391
70. Liu N, Matsumura H, Kato T, et al. Stem cell competition orchestrates skin homeostasis and ageing. *Nature*. 2019;568:7752):344–350. doi:10.1038/s41586-019-1085-7
71. MACKENZIE JC. Ordered Structure of the Stratum Corneum of Mammalian Skin. *Nature*. 1969;222:5196):881–882. doi:10.1038/222881a0
72. Fernández-Gallego N, Sánchez-Madrid F, Cibrian D. Role of ahr ligands in skin homeostasis and cutaneous inflammation. *Cells*. 2021;10(11). doi:10.3390/cells10113176
73. Wang X, Wang Q, Yin P, et al. Secretome of human umbilical cord mesenchymal stem cell maintains skin homeostasis by regulating multiple skin physiological function. *Cell Tissue Res*. 2023;391(1):111–125. doi:10.1007/S00441-022-03697-8
74. Zou X, Zou D, Li L, et al. Multi-omics analysis of an in vitro photoaging model and protective effect of umbilical cord mesenchymal stem cell-conditioned medium. *Stem Cell Res Ther*. 2022;13(1). doi:10.1186/S13287-022-03137-Y
75. Liang X, Li J, Yan Y, et al. Efficacy of Microneedling Combined With Local Application of Human Umbilical Cord-Derived Mesenchymal Stem Cells Conditioned Media in Skin Brightness and Rejuvenation: a Randomized Controlled Split-Face Study. *Front Med Lausanne*. 2022:9. 10.3389/fmed.2022.837332.
76. Park YM, Lee MJ, Jeon SH, Hrůzová D. In vitro effects of conditioned medium from bioreactor cultured human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) on skin-derived cell lines. *Regen Ther*. 2021;18:281–291. doi:10.1016/J.RETH.2021.08.003
77. Kim YJ, mi YS, Park HH, et al. Exosomes derived from human umbilical cord blood mesenchymal stem cells stimulates rejuvenation of human skin. *Biochem Biophys Res Commun*. 2017;493(2):1102–1108. doi:10.1016/J.BBRC.2017.09.056
78. Hong YK, An S, Lee YH, et al. Potential anti-ageing effects of probiotic-derived conditioned media on human skin cells. *Acta Pharm*. 2022;72(3):359–374. doi:10.2478/acph-2022-0027
79. Widowati W, Noverina R, Ayuningtyas W, et al. Potential of Conditioned Medium of hATMSCs in Aging Cells Model. *Hayati*. 2022;29(3):378–388. doi:10.4308/hjb.29.3.378-388
80. Noverina R, Widowati W, Ayuningtyas W, et al. Growth factors profile in conditioned medium human adipose tissue-derived mesenchymal stem cells (CM-hATMSCs). *Clin Nutr Exp*. 2019;24:34–44. doi:10.1016/j.yclnex.2019.01.002
81. Putri WE, Endaryanto A, Tinduh D, Rantam F, Notobroto HB, Prakoeswa CRS. Skin barrier before and after topical adipose stem cell-conditioned medium (Asc-cm) treatment in photoaging. *Bali Med J*. 2021;10(2):688–691. doi:10.15562/bmj.v10i2.2589
82. Go YY, Lee CM, Ju WM, Chae SW, Song JJ. Extracellular Vesicles (Secretomes) from Human Trophoblasts Promote the Regeneration of Skin Fibroblasts. *Int J Mol Sci*. 2021;22(13). doi:10.3390/IJMS22136959
83. Ohta H, Liu X, Maeda M. Autologous adipose mesenchymal stem cell administration in arteriosclerosis and potential for anti-aging application: a retrospective cohort study. *Stem Cell Res Ther*. 2020;11(1). doi:10.1186/s13287-020-02067-x

84. Li L, Ngo HTT, Hwang E, et al. Conditioned medium from human adipose-derived mesenchymal stem cell culture prevents uvb-induced skin aging in human keratinocytes and dermal fibroblasts. *Int J Mol Sci*. 2020;21(1). doi:10.3390/ijms21010049
85. Wang T, Guo S, Liu X, Xv N, Zhang S. Protective effects of adipose-derived stem cells secretome on human dermal fibroblasts from ageing damages. *Int J Clin Exp Pathol*. 2015;8(12):15739–15748.
86. Lee SE, Kwon TR, Kim JH, et al. Anti-photoaging and anti-oxidative activities of natural killer cell conditioned medium following UV-B irradiation of human dermal fibroblasts and a reconstructed skin model. *Int J Mol Med*. 2019;44(5):1641–1652. doi:10.3892/ijmm.2019.4320
87. Chowdhury SR, Jing LS, Zolkafli MNHB, et al. Exploring the potential of dermal fibroblast conditioned medium on skin wound healing and anti-ageing. *Sains Malays*. 2019;48(3):637–644. doi:10.17576/jsm-2019-4803-17
88. Yang L, Xing S, Wang K, Yi H, Du B. Paeonol attenuates aging MRC-5 cells and inhibits epithelial–mesenchymal transition of premalignant HaCaT cells induced by aging MRC-5 cell-conditioned medium. *Mol Cell Biochem*. 2018;439(1–2):117–129. doi:10.1007/s11010-017-3141-7
89. Pan S, Gong S, Zhang J, et al. Anti-aging effects of fetal dermal mesenchymal stem cells in a D-galactose-induced aging model of adult dermal fibroblasts. *Vitro Cell Dev Biol Anim*. 2021;57(8):795–807. doi:10.1007/s11626-021-00624-z
90. Sun B, Meng X, Li Y, Li Y, Liu R, Xiao Z. Conditioned medium from human cord blood mesenchymal stem cells attenuates age-related immune dysfunctions. *Front Cell Dev Biol*. 2023;10. doi:10.3389/fcell.2022.1042609
91. Feehan R, Shantz L. Molecular signaling cascades involved in nonmelanoma skin carcinogenesis. *Biochem J*. 2016;473:2973–2994. doi:10.1042/BCJ20160471
92. Tomas D. Apoptosis, UV-radiation, precancerosis and skin tumors. *Acta Med Croatica*. 2009;63(2):53–58.
93. Calcinotto A, Kohli J, Zagato E, Pellegrini L, Demaria M, Alimonti A. Cellular Senescence: aging, Cancer, and Injury. *Physiol Rev*. 2019;99 (2):1047–1078. doi:10.1152/physrev.00020.2018
94. CARRUTHERS A, CARRUTHERS J, Hardas B, et al. A Validated Grading Scale for Crow's Feet. *Dermatologic Surg*. 2008:34(s2):S173–S178. doi:10.1111/j.1524-4725.2008.34367.x.
95. Jiang LI, Stephens TJ, Goodman R. SWIRL, a clinically validated, objective, and quantitative method for facial wrinkle assessment. *Skin Res Technol*. 2013;19(4):492–498. doi:10.1111/srt.12073
96. Kichenbrand C, Velot E, Menu P, Moby V. Dental Pulp Stem Cell-Derived Conditioned Medium: an Attractive Alternative for Regenerative Therapy. *Tissue Eng Part B Rev*. 2018;25(1):78–88. doi:10.1089/ten.teb.2018.0168
97. Rosochowicz MA, Lach MS, Richter M, Suchorska WM, Trzeciak T. Conditioned Medium – is it an Undervalued Lab Waste with the Potential for Osteoarthritis Management? *Stem Cell Rev Rep*. 2023;19(5):1185–1213. doi:10.1007/s12015-023-10517-1
98. Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R. Mesenchymal stem cell secretome: toward cell-free therapeutic strategies in regenerative medicine. *Int J Mol Sci*. 2017;18(9). doi:10.3390/ijms18091852
99. Zuk P, Zhu M, Mizuno H, et al. Multilineage Cells from Human Adipose Tissue. *Tissue Eng*. 2001;7:211–228. doi:10.1089/107632701300062859
100. Meliga E, Strem BM, Duckers HJ, Serruys PW. Adipose-Derived Cells. *Cell Transpl*. 2007;16(9):963–970. doi:10.3727/096368907783338190
101. Kim WS, Park BS, Sung JH, et al. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. *J Dermatol Sci*. 2007;48(1):15–24. doi:10.1016/j.jdermsci.2007.05.018
102. Doi H, Kitajima Y, Luo L, et al. Potency of umbilical cord blood- and Wharton's jelly-derived mesenchymal stem cells for scarless wound healing. *Sci Rep*. 2016;6(1):18844. doi:10.1038/srep18844
103. Luo G, Cheng W, He W, et al. Promotion of cutaneous wound healing by local application of mesenchymal stem cells derived from human umbilical cord blood. *Wound Repair Regener*. 2010;18(5):506–513. doi:10.1111/j.1524-475X.2010.00616.x
104. Kim J, Lee JH, Yeo SM, Chung HM, Chae JI. Stem cell recruitment factors secreted from cord blood-derived stem cells that are not secreted from mature endothelial cells enhance wound healing. *Vitro Cell Dev Biol Anim*. 2014;50(2):146–154. doi:10.1007/s11626-013-9687-0
105. Tian H, Li S, Jia W, Yu K, Wu G. Risk factors for poor hemostasis of prophylactic uterine artery embolization before curettage in cesarean scar pregnancy. *J Int Med Res*. 2020;48(10). doi:10.1177/0300060520964379
106. Qin W, Liu K, Su H, et al. Tibial cortex transverse transport promotes ischemic diabetic foot ulcer healing via enhanced angiogenesis and inflammation modulation in a novel rat model. *Eur J Med Res*. 2024;29(1). doi:10.1186/s40001-024-01752-4
107. Li N, Wang Z, Yang F, Hu W, Zha X, Duan X. MiR-29b Downregulation by p53/Sp1 Complex Plays a Critical Role in Bleb Scar Formation After Glaucoma Filtration Surgery. *Transl Vis Sci Technol*. 2023;12(12). doi:10.1167/tvst.12.12.5
108. Yu L, Li M. Roles of klotho and stem cells in mediating vascular calcification (Review). *Exp Ther Med*. 2020;20(6):1. doi:10.3892/etm.2020.9252
109. Chen Z, Jin M, He H, et al. Mesenchymal stem cells and macrophages and their interactions in tendon-bone healing. *J Orthop Translat*. 2023;39:63–73. doi:10.1016/j.jot.2022.12.005