Potential Utilisation of Secretome from Ascorbic Acid-Supplemented Stem Cells in Combating Skin Aging: Systematic Review of A Novel Idea

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Abstract
The secretome of stem cells consists of a spectrum of bioactive factors secreted by stem cells grown in culture media-cytokines, chemokines, and growth factors in addition to extracellular vesicles (exosomes and microvesicles). Ease of handling and storage of secretomes along with their bioactivity towards processes in skin aging and customizability makes them an appealing prospective therapy for skin aging. This systematic review aims to investigate the potential usage of ascorbic acid (AA)-supplemented stem cell secretomes (SCS) in managing skin aging. We extracted articles from three databases: PubMed, Scopus, and Cochrane. This review includes *in vitro*, *in vivo*, and clinical studies published in English that discuss the correlation of AA-supplemented-SCS with skin aging. We identified 1111 articles from database and non-database sources from which nine studies met the inclusion criteria. However, the study results were less specific due to the limited amount of available research that specifically assessed the effects of AA-supplemented SCS in skin aging. Although further studies are necessary, the AA modification of SCS is a promising potential for improving skin health.

Keywords: Ascorbic Acid, Secretome, Skin Aging, Stem Cells

Introduction

Stem cells are a group of cells characterised by their versatility; they can differentiate into a variety of cell lineages. Thus, they have a significant role in the facilitation of tissue repair. The stem cell "secretome" refers to the collective spectrum of bioactive factors secreted by stem cells grown in culture media, and it is responsible for the cellular actions of stem cells. The secretome includes soluble proteins such as cytokines, chemokines, and growth factors in addition to extracellular vesicles such as exosomes and microvesicles (1). While both secretomes and stem cells confer regenerative and anti-inflammatory properties, secretomes have several advantages in comparison to cell-based therapies, such as ease of manufacturing, handling, and storage. They also have less immunogenicity (2, 3). This, together with the precise tackling of mechanisms by which skin aging occurs, such as oxidative stress, DNA damage, chronic inflammation, microRNA dysregulation, and cellular senescence, makes secretome an appealing prospective therapy for cutaneous aging (4, 5). Previous studies have demonstrated anti-aging activities from the secretomes of mesenchymal stem cells (MSCs) sourced from adipose (6) and cell-free blood cell secretome (7). Other studies reported the use of secretome from human Wharton's jelly for antiviral activity (8) and from bone marrow MSCs for neuroprotective effects in a rat model of Parkinson's disease (9).

One well-documented feature of secretomes is the ability to modify their compositions and this benefit grants secretomes the flexibility to adjust themselves to specific treatment goals (2). Ascorbic acid (AA) supplementation is proposed to suppress stem cell senescence via its antioxidant property and promote the proliferation rate of MSCs, which ultimately leads to a more effective preparation (10). Furthermore, AA has the potential to optimise secretomes. A preclinical study of human adipose-derived MSCs (hADMSCs) demonstrated supplementation of basal media with AA increased type I collagen mRNA expression. Type I collagen is a structural protein with major implications in skin aging (11).

Although there are published studies about the utilisation of secretomes in dermatology, fewer studies have used AA to modulate the secretome of stem cells in skin aging. This systematic review aims to explore the impact of AA supplementation on stem cell secretomes (SCS) in terms of skin aging.

Materials and Methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) (12). The protocol for this review was registered as Open Science Framework Registries ID: 8s9n7 (13).

Search strategy

The search was conducted on 20 September 2022 for articles that pertained to the secretome of stem cells grown in AA-supplemented media. The following keywords were used during the search: (secretome) AND ((vitamin c) OR (ascorbic acid) OR (ascorbate). Table S1 (See Supplementary Online Information at www.celljournal. org) lists detailed keywords used for each database. Because the term "skin aging" did not produce any results in all of the databases, we expanded the search query by omitting this term. The five investigators (KAW, WP, IWW, IGRW, IAIW) independently searched for studies in the PubMed, Scopus, and Cochrane databases. Any discrepancies were discussed together, and the decisions were recorded in a Google Sheets spreadsheet program.

Study eligibility criteria

This review included *in vitro*, *in vivo*, and clinical studies. Studies that included secretomes of stem cells that correlated with the process of skin aging were included.

Review articles, studies published in languages other than English, studies that did not mention the AA concentration used, and studies that did not study the specifically study the effect of AA supplementation were excluded.

Data extraction

A predetermined outcome sheet was used to include the following data to be extracted: i. Author and year of publication, ii. Stem cell line used, iii. Growth media, iv. Concentration of AA supplemented, v. Other compounds present in the media, vi. Incubation period, and vii. Secretome-associated outcome. Four researchers (WP, IWW, IGRW, AIAW) extracted the data and the fifth researcher (KAW) verified the accuracy of the extracted data. Any disputes were resolved by discussion between the reviewers.

Results

Search selection and characteristics

Figure 1 details the flow of the literature search. We identified 1111 records from the databases and six from non-database sources. We eliminated four duplicates and 514 studies were marked as ineligible by the spreadsheet program. Thus, 593 titles and their abstracts were screened; ultimately, 55 studies were retrieved and assessed. After application of the exclusion criteria, we included nine studies in this review (11, 14-21).
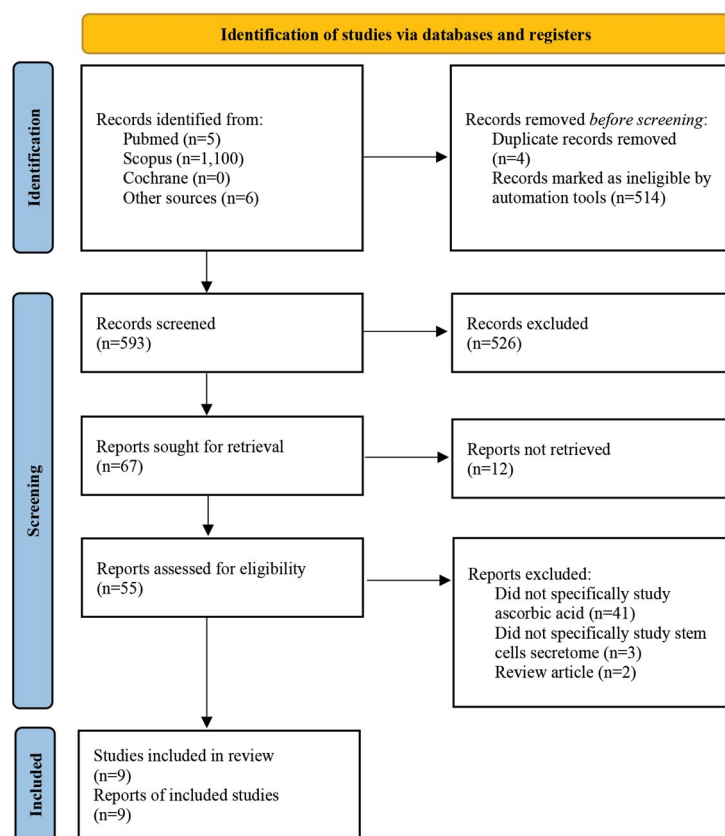

Fig.1: Details the flow of literature search based on the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) criteria.

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Table 1: Study characteristics of the included studies

Author (reference) (Year) Citation*	Stem cell		Induction of differentiation					Post-culture or post-induction treatment
	Lineage	Growth media	Duration	Target type	Media	Ascorbic Acid concentration	Induction period	
Wei et al. (14)(2012) Citations: 263	Human periodontal ligament stem cells (hPDLSCs) and minipig-PDLSCs	a-MEM (Gibco) with other compounds at 37°C in 5% CO_2	Not mentioned	N/A	N/A	N/A	N/A	PDLSCs was treated with various doses of AA: 0, 5, 10, 20 and 50 µg/mL
Lui et al. (15)(2016) Citations: 79	Sprague-Dawley rat tendon-derived stem cells	Low glucose DMEM (Gibco) with other compounds	Not mentioned	Tenogenic	Complete culture medium (Invitrogen) at 37°C and 5% CO_2	N/A	14 days	Addition of CTGF and 25 µmol/L AA during induction
Diomede et al. (16) (2019) Citations: 3	Human gingival mesenchymal stem cells	MSCGM-CD; changed every 2 days	2 weeks	Undifferentiated Osteogenic	Undifferentiated MSCGM-CD, MSCGM-CD + AA Osteogenic Osteogenic differentiation medium (Lonza)	Undifferentiated MSCGM-CD: 0 µg/mL MSCGM-CD +AA: 60 µg/mL, 90 µg/mL Osteogenic Not mentioned	21 days	None
Diomede et al. (17) (2019) Citations: 34	Human dental pulp stem cells	MSCGM-CD at 37°C and 5% CO_2	Not mentioned	Osteogenic Adipogenic	Osteogenic Osteogenic culture media kit (Lonza) Adipogenic Adipogenic medium kit (Lonza)	Osteogenic Not mentioned Adipogenic None	Osteogenic 21 days Adipogenic 28 days	HEMA: 2 mM AA: 50 µg/mL Cells grouped to: (1) Control (2) HEMA only (3) AA only (4) HEMA + AA (5) All are treated for 24 hours
Wahyuningsih et al. (11) (2020) Citations: 6	Post-thawed human adipose-derived stem cells	Expanded with in-house media Subsequently cryopreserved thawed, and cultured in either DMEM, DMFA or DMFA + AA	Not mentioned	N/A	N/A	N/A	N/A	Cells grouped to: (1) DMEM (2) DMFA + 0 AA (3) DMFA + 50 µg/mL AA (4) DMFA + 100 µg/mL AA
Bhandi et al. (18) (2021) Citations: 15	Stem cells from human exfoliated deciduous tooth	FBS (Gibco) at 37°C and 5% CO_2 Maintained in DMEM (Invitrogen) with other compounds at 37°C, 5% CO_2. Culture medium replenished 2x weekly	Initial incubation is 24 hours	Adipogenic Osteogenic Chondrogenic	Adipogenic Adipogenic Media (Sigma-Aldrich) Osteogenic Osteogenic Induction Medium (Sigma-Aldrich) Chondrogenic Chondrogenic Induction Medium (Sigma-Aldrich)	Adipogenic None Osteogenic 50 µM Chondrogenic 50 µg/mL	Adipogenic 21 days Osteogenic 21 days Chondrogenic 28 days	Each target type was grouped to: (1) 10 µM AA group (2) No AA added

Table 1: Continued

Author (reference) (Year) Citation[*]	Stem cell		Induction of differentiation					Post-culture or post-induction treatment
	Lineage	Growth media	Duration	Target type	Media	Ascorbic Acid concentration	Induction period	
Marconi et al. (19) (2021) Citations: 28	Human periodontal ligament stem cells	MSCGM-CD at 37°C and 5% CO_2; changed every 2 days	2 weeks	Adipogenic Osteogenic	Adipogenic MSCBM-CD with other compounds Osteogenic MSCBM-CD with other compounds	Adipogenic None Osteogenic 50 mmol/L	Adipogenic 28 days Osteogenic 21 days	LPS-G: 5 µg/mL AA: 50 µg/mL Cells grouped to: (1) hPDLSCs vs (2) hPDLSCs + LPS-G vs (3) hPDLSCs + AA vs (4) hPDLSCs + LPS-G + AA
Pizzicannella et al. (20) (2021) Citations: 6	Human gingiva-derived mesenchymal stem cells (hGMSCs) and endothelial-hGMSCs	MSCGM-CD at 37 °C and 5% CO_2; changed every 2 days	2 weeks	hGMSCs Adipogenic, osteogenic e-hGMSC Endothelial	Adipogenic MSCBM-CD with other compounds Osteogenic MSCBM-CD with other compounds Endothelial Endothelial growth medium (Lonza) composed of various compounds	Adipogenic None Osteogenic 50 mmol/L Endothelial Not mentioned	Adipogenic 28 days Osteogenic 21 days Endothelial 10 days	LPS-G: 5 µg/mL AA: 50 µg/mL Cells grouped to: (1) hGMSCs vs (2) hGMSCs + LPS-G vs (3) hGMSCs + AA vs (4) hGMSCs + LPS-G + AA (1) e-hGMSCs vs (2) e-hGMSCs + LPS-G vs (3) e-hGMSCs + AA vs (4) e-hGMSCs + LPS-G + AA
Pranskunas et al. (21) (2021) Citations: 4	Rabbit periosteum-derived mesenchymal stem cells	DMEM with other compounds at 37°C and 5% CO_2	Not mentioned	Undifferentiated Osteogenic	Undifferentiated DMEM with other compounds Osteogenic osteogenic-inducing media (in-house)	Undifferentiated None Osteogenic 25 µg/mL	24 days	None

*; As of 2 June 2023, α-MEM; Alpha-modified Eagle's medium, FBS; Fetal bovine serum, AA; Ascorbic acid, MSCGM-CD; Chemically-defined mesenchymal stem cell growth medium (Lonza, Switzerland), DMEM; Dulbecco's Modified Eagle's Medium (Gibco, USA), DMFA; DMEM+10% fetal bovine serum + antibiotic-antimycotic, HEMA; 2-hydroxyethylmethacrylate, LPS-G; P, gingivalis lipopolysaccharide, and ROS; Reactive oxygen species.

The studies included were published from 2012-2021. All were either conducted *in vitro* or *in vivo*, and the cell lineages varied from periodontal stem cells to tendon-derived stem cells from human or animal sources. The studies had different protocols and target type of cell differentiation, such as osteogenic or adipogenic cells. Some studies did not perform induction for cell differentiation. The studies also varied by AA concentration and timing of supplementation (during induction, after culture, or after induction). Table 1 provides details of the study characteristics.

Study outcomes

Table 2 lists the summary of the outcomes of each included study. Increased telomerase activity, higher expressions of structural protein such as collagen, modulation of anti-inflammatory and proinflammatory cytokines, regulation of NF-κB, and influences on other proteins were the various secretome-associated outcomes from AA supplementation of stem cells. Lower amounts of reactive oxygen species (ROS) were also observed in studies that included ROS levels as a study outcome.

Table 2: Outcome of included studies

Author (reference) (Year)	Outcome
Wei et al. (14) (2012)	Increased telomerase activity Higher expression of COL I, fibronectin and β1 integrin mRNAs
Lui et al. (15) (2016)	Improved tendon healing Better collagen arrangement (tightly packed fibrils) due to increased tendon-related mRNA expression (COL1A1)
Diomede et al. (16) (2019)	Increased upregulation of COL1A1 mRNA in Osteogenic Differentiation Medium (Lonza) and in AA 90 µg/mL medium compared to control
Diomede et al. (17) (2020)	AA significantly downregulated NF-κB in HEMA treated cells AA significantly decreased mean ROS production in HEMA treated cells
Wahyuningsih et al. (11) (2020)	Supplementation of AA increased type 1 collagen expression compared to control. No significant difference in collagen expression between 50 and 100 µg/mL AA group.
Bhandi et al. (18) (2021)	Increased secretion of: VEGF, SCF, IGF-1, HGF, bFGF, Ang-1 and EGF Increased secretion of anti-inflammatory cytokines: NO, IDO, PGE-2, IL-10, IL-6 Decreased secretion of inflammatory cytokines: CCL2, TGF-b1
Marconi et al. (19) (2021)	hGMSCs+LPS-G group: increased levels of NF-κB, MyD88, p300, and ROS hGMSCS+LPS-G+AA group: attenuated levels of NF-κB, MyD88, p300, and ROS
Pizzicannella et al. (20) (2021)	hGMSCs+LPS-G group: increased p300 and ROS; decreased DNMT1 hGMSCs+LPS-G+AA group: physiological expression of p300 and DNMT1; reduced ROS
Pranskunas et al. (21) (2021)	Supplementation of AA in osteogenic group resulted in: - Promotion of collagen alpha-1(I) chain, AE binding protein 1, and stanniocalcin-1 - Suppression of fibrillin-2 and cathepsin K Proteins expressed in both groups include collagen alpha-2(I) chain, alpha-1(XII) chain, and fibrillin-1

AA; Ascorbic acid, HEMA; 2-hydroxyethylmethacrylate, and ROS; Reactive oxygen species.

Discussion

Stem cells and secretomes

Stem cells are cells that self-renew and have the capability to differentiate into other cell lineages to repair tissue damage. Hence, they are extensively used in regenerative medicine. Stem cells can be procured from various sources and classified as either embryonic or somatic. Somatic stem cells are commonly used in research and medicine, and they include MSCs and hematopoietic stem cells (1). Somatic stem cells are commonly sourced from perinatal or postnatal sources; they can be procured from the placenta, amniotic fluid, bone marrow, fat, blood, skin, and other sources (1, 22). The most frequently used stem cells in clinical trials are MSCs as they are easily sourced from various adult tissues. The aforementioned capacity to differentiate and heal injured tissues are attributed to secretomes, a collection of substances released by stem cells that can mediate cell-to-cell communication as well as modulate immunity and regeneration (2). Some texts may also refer to secretomes as "conditioned medium" (5).

The secretome of a stem cell is composed of various substances. For instance, the secretome of mesenchymal cells may include peptides that have anti-microbial and immunomodulatory activities such as interleukin (IL)-1β, IL-6, IL-10, and TGF-β; growth factors such as vascular endothelial growth factor, fibroblast growth factor-2, platelet-derived growth factor, and epidermal growth factor that are responsible for stimulating angiogenesis and growth of other tissues; extracellular matrix (ECM) proteins such as matrix metalloproteinases (MMPs), elastin, and collagen that promote ECM production and remodelling; and other compounds enclosed in exosomes such as miRNAs, lipids, and noncoding RNAs that may regulate inflammatory pathways (2, 23, 24). The

therapeutic potentials of MSC secretomes have been studied in models of cutaneous wound healing, psoriasis, and muscle injuries. Its anti-photoaging, antioxidative, and hair growth properties have been reported (5, 23-25).

Mechanism of skin aging

Skin aging is described as a gradual process where the skin's appearance changes due to intrinsic and extrinsic aging from environmental damage (26). Some of the more prominent signs of aging are dyspigmentation, sagging, and telangiectasia. However, wrinkling is a key sign of aging in many validated and non-validated aging scales (27). Wrinkles mainly occur when there is a decrease in the production of collagen, which helps anchor the dermal-epidermal junction (11). Histological studies of aging skins have shown progressive reduction of dermis vasculature, thinning of collagen fibres, and lysis and thickening of the fibres in the deep dermis. Elastic fibres in the skin are mainly composed of elastin, and they also tend to diminish. They are also non-functioning, amorphous, curled, thickened, and fragmented. These pathological changes in elastic skin fibre are characteristic of photoaging and termed "solar elastosis" (28, 29).

The multifaceted process of skin aging is thought to involve various mechanisms such as oxidative stress, changes in genetic material, and others. These processes could occur intrinsically as a result of genetic factors and natural processes such as structural and functional changes in the components of the ECM. However, it could also occur extrinsically with environmental factors such as ultraviolet (UV) light and various free radical exposure (4, 30).

Reactive oxygen species and oxidative stress

Oxidative stress is a phenomenon that could occur in the body's cells and tissues when there is an imbalance between free radicals, specifically ROS, and the ability of the human body to neutralize them. Under normal circumstances cells produce ROS for certain physiological roles and as a by-product of oxygen-related metabolism (4, 31). ROS produced by the body are quickly stabilised through the body's antioxidant defence mechanism (31). Failure to do so causes an increase in oxidative stress that consequently leads to various problems such as accelerated skin aging. Excess ROS production in the skin can be induced by UV light and result in "photoaging". ROS generation is mainly brought about by UVA (320-400 nm) since UVB is unable to pass through the deeper section of the epidermis. The energy of UVA which penetrates the skin is absorbed by cellular chromophores that causes it to enter a singlet excited state. In this condition, the chromophore can partake in two possible pathways - either the chromophore falls back into its ground state and releases energy as heat or it can enter an intermediate triplet excited state. As this state is intermediary, the energy is further transferred into a reaction that involves DNA and molecular oxygen, which results in

DNA modification or ROS production that increases the oxidative stress of a cell. ROS produced by this mechanism includes superoxide, hydroxyl radicals, singlet oxygen or hydrogen peroxide. Meanwhile the modification of DNA, especially 4977 bp deletion of the mtDNA, leads to an increase in ROS production in the mitochondria (30). There is also evidence for UVB involvement of in photoaging. Wahyono previously demonstrated decreased expression of type I collagen and increased expression of MMP-1 in white rats exposed to 130-150 mJ/cm^2 of UVB (32). Aside from photoaging, there are other mechanisms or conditions which may generate excess ROS; one example is a surplus of D-galactose. This reducing sugar readily reacts with free amines of amino acids through nonenzymatic glycation to form advanced glycation end products. This oxidative metabolism of D-galactose and glycation of the end product reaction generates ROS as a side-product (33).

Nuclear factor kappa B signalling pathway in skin aging

An increase in oxidative stress due to ROS accumulation may lead to skin inflammation and the formation of wrinkles. Induction of skin inflammation by ROS may be mediated by the induction of the nuclear factor kappa B (NF-κB) pathway, typically due to stimulation of IGF1 (34, 35). This consequently leads to the expression of inflammatory cytokines and activation of tumour necrosis factor-α (TNF-α) and MMPs that are responsible for the degradation of various connective tissues, such as the ECM, and this can speed up the skin aging process (34). Degradation of the ECM will lead to expression and activation of activator protein 1 (AP-1), a transcription factor that promotes inflammation and collagen degradation, and consequently increases skin aging (30). The effect of NF-κB inhibition in skin aging has been previously reported by Rui et al. (36) Inhibition of the NF-κB pathway reduced downstream expressions of TNF-α and MMPs in the skin of mice. Hydroxyproline quantification and histopathological assessments also supported these results, and showed that inhibition of NF-κB attenuated MMP-mediated UV-induced photoaging. UV irradiation can decrease type VII collagen, which are anchoring fibrils responsible for stability at the dermal-epidermal junction (4). Li et al. (37) also reported that inhibition of NF-κB could reduce NF-κB-mediated gene expression and production of bFGF and MMP-1. Blockade of NF-κB also reduced UVB-induced proliferation of mice keratinocytes.

DNA/RNA damage and telomere shortening

In addition to ROS and oxidative stress, changes in genetic materials are large contributors toward skin aging. Phenomena such as DNA/RNA damage and telomere shortening are responsible for skin aging. UV irradiation

is one of the mechanisms that result in DNA/RNA damage. Damage in the *COL1A1*, *COL1A2*, *COL3A1*, *COL4A1*, and *COL7A* genes can disrupt collagen synthesis and may accelerate skin aging. The *COL1A1* and *COL1A2* genes are especially important in the maintenance of the skin's mechanical properties, as they encode type I collagen, which is a vital extracellular protein of the skin. Type I collagen does not work alone; it requires the support of type III collagen, which is encoded by *COL3A1*. Both type I and III collagen play a key role in maintaining the elasticity of human skin; therefore, downregulation or damage to their respective genes is thought to cause premature skin aging (38, 39).

Damaged DNA materials are usually repaired through the nucleotide excision repair pathway, such as single-stranded DNA-binding protein RPA, clamp loader PCNA, and polymerase (40). Normally, these proteins are present in the skin, but under certain circumstances they may be deficient. This deficiency contributes to premature skin aging. DNA in human cells is condensed into chromosomes with telomeres that serve as caps to protect the chromosomes from degradation and vital DNA from deletion. The telomeres protect the chromosomes by exposing themselves to degradation and they naturally shorten throughout the aging process. This shortening is balanced by telomerase activity, which extends the telomere ends of a chromosome. It is also thought that deficiency in this enzyme can lead to accelerated aging due to various defects in tissue regeneration and suppression of epidermal stem cell proliferation capacity (4).

Inflammaging

Inflammaging is a phenomenon where chronic, low-intensity inflammation is identified as one of the major characteristics of aging. The mechanism of inflammaging is attributed to oxidised lipid damage of cells. The two play a key role in an inflammatory response by activating macrophages, which cause them to release MMPs that degrade the ECM. One of the contributing factors of inflammaging is repeated exposure to UV radiation. This exposure causes an overdrive in the complement system that overworks the macrophages. These overworked macrophages release ROS and proinflammatory cytokines. This process damages the dermis and, consequently, the skin (4).

Immunosuppression

Chronic low-grade inflammation that may be caused by UV radiation can eventually stimulate immunosuppression of T regulatory cells, regulatory dendritic cells, and myeloid-derived suppressor cells. This immunosuppression causes immunosenescence, which not only decreases immune cell activity but also stimulates degeneration of neighbouring cells (41). Immune cell activity decreases due to the catabolism of L-arginine and tryptophan caused by the activation of the arginase 1 and indoleamine 2,3-dioxygenase enzymes. Degeneration of neighbouring cells are mainly due to impairment of host tissue homeostasis, such as TGF-β signalling, which may lead to degradation of the ECM (34). The immunosuppressed condition leads to a higher risk of infection; since the skin is exposed to a vast array of pathogens, immunosuppressed patients are thought to have higher risk of contracting *tinea pedis* fungal infections, pyoderma bacterial infections, and herpes zoster infection. Normal skin flora such as *Staphylococcus* sp. and *Streptococcus* sp. can become pathologic under immunosuppressed conditions. These opportunistic infections are considered to be a contributing factor towards skin aging because they cause disruptions to the skin's structure (42).

Cellular senescence

Cellular senescence refers to the gradual decrease of a cell's proliferative ability until it enters a state of irreversible cell cycle arrest. Other characteristics of senescent cells are the development of resistance to apoptosis, release of proinflammatory factors, and tissue deterioration factors (43). As many as 20 different cell types in the skin have different proliferative capacities. However, as the skin ages, they turn into senescent skin cells and accumulate over time. Senescent skin cells are formed normally as humans age; they are created by provoking senescence-inducing stimuli. However, the exact mechanism of how the diverse types of skin cells respond to these stimuli are not fully understood. Senescent skin cells can also form under pathologic circumstances, such as ones caused by extrinsic stress signals and oncogene expression (44). This cellular senescence can affect all skin layers and result in changes in their structure and functionality, such as the aging of the epidermal layer which results in decreased barrier and restoration function (43). Various age-related skin pathologies such as hyperpigmented lesions, diabetic wounds, and psoriasis are strongly associated with senescent skin cells (44).

Autophagy

Autophagy is defined as "a starvation-induced cellular recycling pathway" wherein cytoplasmic components undergo lysosomal degradation. It helps maintain the skin's health and plays a key role in controlling skin aging. The main function of autophagy in the aforementioned process involves the elimination of senescent subcellular organelles and proteins as well as regulation of the functions of various skin cells (keratinocytes, dermal fibroblasts, and melanocytes) (45). Physiologically, autophagy has been shown to correlate with the response of keratinocytes to various types of stress, the majority of which contribute to accelerated skin aging. UVB irradiation is the best example among the stressors that can cause inflammation and DNA damage. It has been said to instigate autophagy as primary keratinocyte response to

recognize DNA damage, aiding the process of nucleotide repair (46). A downregulation in autophagy may eventually give rise to unregulated hyperinflammatory skin reactions and lead to accelerated skin aging. Genes that play a major role in modulating autophagy are *BECN1, MAP1LC3B, ATG5, ATGJ,* and *mTOR* (45).

Optimising secretome production

Secretome production is influenced by various factors; intervention in this process can aid in optimising its production. It has been reported that ultrafiltration and freeze-drying techniques can enhance the quality of MSC-secretome production by isolating and purifying the secretomes (47). Molecular priming, tissue engineering, modification of growth medium composition, and hypoxic preconditioning are strategies proposed to increase the production of specific secretomes. Molecular priming with FGF, IL-1, and IFN-ɣ could increase G-CSF, IL-6, TSG-6, and VEGF production. Hypoxic preconditioning and tissue engineering with hydrogels or scaffolds has been shown to increase production of FGF2, HGF, IGF, TGF-β, and VEGF. Changing serum content of the growth medium caused stem cells to enhance the production of BDNF, HGF, IL-6, NGF, PEG2, TGF-β, and VEGF (48). Although these studies showed ways to optimise the production of secretome, they were not specifically applied to skin aging.

Secretomes affected by ascorbic acid and implications in skin aging

Promotion of collagen expression by stem cells has been reported (14-16). The promotion of collagen expression was observed in MSCs derived from human gingival MSCs and rabbit periosteum MSCs. We previously observed increased collagen expression in post-thawed hADMSCs supplemented with 50 and 100 µg/mL of AA. The 50 µg/mL AA demonstrated the greatest increase in collagen expression, although the difference was not statistically significant compared to the 100 µg/mL AA group (11). Collagen expression also increased in periodontal ligament stem cells and tendon-derived stem cells. In Sprague-Dawley rat tendon-derived stem cells, addition of 25 µmol/L of AA resulted in superior collagen arrangement, which was attributed to the greater COL1A1 mRNA expression (15). As previously mentioned, both the decline and degradation in collagen are heavily implicated in skin aging. Decreased collagen content and increased collagen degradation are observed in photoaged skin. Hence, remodelling and replacement of skin collagen along with promotion of collagen expression is hypothesised as a beneficial treatment for skin aging (4).

Fibronectin and β1 integrin expression activity also increased after AA supplementation of periodontal ligament stem cells (14). Fibronectin fibrils form scaffolds that enable proteins such as collagen, elastin, and proteoglycans to bind. Fibronectin is an important part of the ECM that plays a role in ECM maturation and attachment, signalling, and cellular migration (49, 50). Fibronectin matrices also can sequester growth factors, and release them in a timely manner to regulate cell growth and morphogenesis (51). The importance of fibronectin to prevent aging through the loss of stem cells has been reported (52). β1 integrin is a proliferative marker of stem cells, and there is a reduction in epidermal β1 integrin in skin aging (53). AA supplementation may induce stem cell proliferation as evidenced by the increased expression of β1 integrin in periodontal ligament stem cells (14). The result of the author's previous study showed that 75 µg/ml of AA supplementation resulted in the lowest cell spread size, highest cell yield, and fastest doubling time (54).

Pranskunas et al. (21) found that production of cathepsin K was suppressed in rabbit periosteum-derived MSCs supplemented with 25 µg/mL of AA. Cathepsins are elastolytic proteases that cleave elastin fibres. The elastolytic activity of cathepsins is thought to be significantly higher than MMPs. Cathepsins can release elastokines and ECM-degrading enzymes with exacerbation of local tissue damage and aging. As such, cathepsins are also a prospective target for treatment of skin aging (55). The same study also reported suppression of fibrillin-2 in AA-supplemented MSCs (21). Remodelling, reduction, and truncation of fibrillin-rich microfibrils (FRMs) were found to play a role in various pathologies, including photoaging (56). These changes might be caused by its photoreactive nature, which is attributed to the chromophoric amino acid constituents of microfibrils (57). Physiologically, FRMs play a role in the modulation of TGF-β signalling and skin remodelling. A disturbance in the TGF-β signalling pathway may result in aberrant elastin deposition (58). FRMs may also fragment under certain UV doses, which consequently upregulate MMPs and further degrade the ECM (56). UV exposure of the photoreactive FRMs might lead to microfibril exhaustion with simultaneous damage and degradation of the skin ECM. This would explain the concurrent observed phenomena of microfibril reduction and skin aging.

NF-κB is an inflammatory mediator that was elevated in cells treated with 2-hydroxyethylmethacrylate HEMA and in cells treated with *Porphyromonas gingivalis* (*P. gingivalis*) polysaccharide (LPS-G) (17, 20). However, cells treated with AA had decreased NF-κB levels, which was observed through inhibition of the MyD88 and p300 pathway (19). In another study, inhibition of NF-κB translocation was achieved through blockage of NF-κB translocation (17). As previously mentioned, initiation of the NF-κB signalling pathway could lead to skin inflammation and wrinkle formation through the activation of MMPs (34). Overexpression of NF-κB has been observed in aged human fibroblasts. Increased NF-κB levels also decreased dermal fibroblast *COL1A1* gene expression and type I collagen secretion *in vitro* (59).

While telomere shortening is crucial for prevention of abnormal cellular proliferation, it may result in cellular

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senesce and skin aging. As such, telomerase activation should theoretically improve skin aging. Telomeres in skin cells may be especially prone to accelerated shortening due to rapid proliferation and damaging agents such as ROS (60). Increased telomerase activity has been observed in human periodontal ligament stem cells (hPDLSCs) treated with AA (14). Nevertheless, it is not known if very long telomeres would be beneficial for cells.

The addition of AA to stem cells also reduced ROS production (17, 19, 20). Irradiation by UVA or UVB generates ROS and activates cell surface receptors that lead to the activation of MAP-kinase p38, JNK, and ERK. The transcription AP-1 is consequently expressed, which results in ECM degradation through the expression of various MMPs in fibroblasts and keratinocytes. AP-1 also has an inhibitory effect on TGF-β, which plays a part in collagen production (30). Yang et al. (60) have shown that AA slowed down MSC senesce via inhibition of the ROS-activated AKT/mTOR signalling pathway. They pretreated bone marrow-derived MSCs with AA followed by subsequent exposure to D-galactose and AA. D-galactose was administered to induce ROS production and activate the Akt/mTOR signalling pathway. The results showed that AA reduced p16 and SA-β-gal expression, a marker of cellular senescence and premature senescence, respectively. While no study exists on the effect of secretome from AA-treated stem cells on autophagy, there is evidence that secretomes from hypoxia-preconditioned hPDLSCs may activate the PI3K/Akt/mTOR signalling pathway, a regulator of the autophagy process (61). mTOR is thought to play an important role in triggering autophagy by its role as a pivotal upstream effector of the PI3K/Akt pathway. Hence, a balance in the PI3K/Akt/mTOR may be essential to achieve optimal skin conditions.

AA-treated human exfoliated deciduous tooth stem cells showed increased secretion of anti-inflammatory cytokines and decreased secretion of proinflammatory cytokines (18). While IL-6 is considered to be both an inflammatory and anti-inflammatory cytokine, it does not exhibit any direct inflammatory activity in the skin, even though its level increases in inflammatory conditions such as rheumatoid arthritis. Under damaging conditions such as exposure to UV radiation, the skin keratinocytes are stimulated to increase IL-6 production because of its ability to assist with keratinocyte proliferation. However, IL-6 also plays a role in the formation of skin wrinkles (62). In the same study, the AA-treated stem cells had increased production of growth factors that possess the ability to assist with ECM repair (19). EGF is a growth factor that has anti-aging properties and the ability to promote skin wound repairs (63). Another growth factor that plays an important role in preventing skin aging is FGF. It is directly correlated with the induction of collagen and elastin synthesis, two compounds essential in skin resistance and elasticity (64).

There is evidence that AA may not only affect the secretomes of stem cells, it may also indirectly modulate epigenetic changes in these stem cells. Human gingiva-derived MSCs treated with LPS-G had evidence of increased p300 levels and decreased DNMT1 levels. Addition of AA prior to exposure to LPS-G facilitated the cells to express physiological levels of p300 and DNMT1 (20). p300 is a histone acetyltransferase that may be implied in the process of skin aging via activation of UV-triggered MMP-1. Overexpression of p300 increased the activity of basal and MMP-1 promoter (65). DNMT1 is a major enzyme involved in DNA methylation. DNMT expression was higher in young human skin fibroblasts compared to their older counterparts. A knockout model of DNMT1 in young human skin fibroblasts induced senescence (66).

Potential for application in humans

Stem cells and their products are extensively used in medical practices as cell-based therapy and as SCS therapy, respectively. A study conducted in 2018 concluded that while cell-based stem cell therapy is helpful, there are a few notable safety issues. Some patients who received cell-based therapy as a treatment of inflammatory bowel disease experienced serious adverse effects and worsening of this disease (67). Another study reported that usage of ADSCs in treating age-related macular degeneration precipitated vision loss in three patients. The vision loss was thought to be caused by the undesired differentiation of the stem cells into myofibroblast-like cells (68). The biggest concern of cell-based therapy is the possibility of stem cell differentiation into undesired tissues such as bones, cartilages and even cancerous tissues. Stem cells, such as MSCs, exhibit an ability to suppress anti-tumour responses as well as the ability to form new blood vessels; these factors may promote tumour growth and metastasis in cancer. Migration of the stem cells towards a primary tumour may accelerate and worsen the cancer (67). To summarise, unpredictability and increased risk of malignancy are some of the primary drawbacks of cell-based therapy.

On the other hand, numerous evidence suggests that SCS are a better method of therapy compared to its cell-based counterpart. The absence of DNA in SCS therapy has overcome the increased risk of cancer found in cell-based therapy; the lack of DNA greatly reduces the risk of mutation and tumour formation in the host. Moreover, secretome production can be controlled; the type that is required can be produced by modifying the condition of the cell culture (69). For instance, cells cultured in a bioreactor secreted secretomes more efficiently (70).

The dosage and safety of SCS are also considered to be more flexible in comparison to cell-based therapy (69). Preclinical studies on the use of SCS in inflammatory bowel disease, an antigen-induced model of arthritis, and Sjögren's syndrome proved to have some therapeutic potential (71). The SCS exhibits anti-inflammatory and anti-fibrotic

activity; it is suggested that SCS likely has the ability to support cell differentiation and could assist damaged tissue repair (1). A clinical case report showed that secretomes of MSCs, specifically the growth factors and cytokines, have great potential in alveolar bone regeneration. The application of the secretome was considered safe, as it showed minimal inflammatory signs and did not present any systemic or local complications in this study (72).

Secretomes are currently being studied for their use in human health. An Indonesian clinical trial assessed secretomes from hypoxia-MSC as treatment for severe COVID-19 patients (73). The evidence collectively suggest that SCS therapy is safer compared to cell-based therapy and has great potential for managing certain diseases.

None of the studies included were designed for secretomes from skin cells. We considered the absence of these studies to be a limitation. Although the sources of the secretomes included in were from non-skin cell sources, the benefit was not limited to stem cells that received AA. Kerscher et al. demonstrated intradermal administration of secretome from non-skin cells to rejuvenate aging skin (7). Wang et al. (6) also reported encouraging results in human dermal fibroblasts cells treated with secretomes from human adipocyte-derived stem cells. This highlights the versatility and potential that SCS have for treatment of skin aging.

To the best of our knowledge, this is the first systematic review that studied the potential application of secretomes from AA-supplemented stem cells as treatment of skin aging. Due to the limited amount of research that specifically studied the effect of AA-supplemented SCS in the context of skin aging, adjustments to the query was made. Thus, the study results were less specific. Because of the decreased amount of literature, we also did not limit our search to a specific stem cell line. However, the scarcity of studies reflects the novelty of this topic.

Conclusion

While the results of our systematic review are promising, further studies are warranted. Modification of SCS with AA may render them better suited for treatment of skin aging. Various methods of secretome administration for skin aging should be studied. The secretome of skin cells treated with AA is necessary to be studied in the context of skin aging. Lastly, we recommend that the potential of secretomes from stem cells supplemented with AA should be confirmed in well-designed *in vivo* and clinical studies.

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Authors' contributions

K.A.W.; Conceptualization and Data curation. K.A.W.,

W.I.P., I.W.W., I.G.R.W.; Investigation. K.A.W., W.I.P., I.W.W., I.G.R.W., I.A.I.W.; Wrote the original draft. K.A.W., I.A.I.W.; Wrote, Reviewed, and Edited the manuscript. All authors read and approved the final manuscript.

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