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Review Article

Mesenchymal Stem Cells-Conditioned Medium (SECRETOME) in Skin Aging: A Systematic Review

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ABSTRACT

Background: The skin aging is the most important factor that constitutes the general "well-being" and perception of "health" in humans. The mesenchymal stem cells (MSCs) have a therapeutic effect on anti-apoptosis, angiogenic, immunomodulatory and chemo-existing activity, especially in its treatment. However, the use of mesenchymal stem cell-conditioned medium (MSC-CM) as skin aging therapy requires further investigation.

Methods: The systematic literature search have the following keywords; conditioned media or conditioned medium or secretome as well as photoaging or skin aging. A total of 283 articles were reviewed from Pubmed, Wiley Online Library, Science Direct and The Cochrane Library, and only 11 articles were relevant for this systematic review.

Results: The results show that Secretome is promising in improving the skin aging process. However, due to the high heterogeneity, a meta-analysis cannot be performed.

Conclusion: The use of secretome was promising in improving skin aging process, as shown by the available preclinical studies. However, further clinical studies are needed to confirm the benefits and effects of secretome.

Keywords: mesenchymal stem cell, secretome, conditioned medium, skin aging.

INTRODUCTION

Skin aging is a complex biological process that is influenced by a combination of intrinsic and extrinsic factors¹. Furthermore, it is associated with decreased skin function, increased skin fragility, and impaired skin wound healing. In contrast to thin, atrophic, finely wrinkled and dry intrinsically aged skin, premature photoaged skin typically shows thickened epidermis, mottled discoloration, deep wrinkles, laxity, dullness and roughness¹⁻⁵. The increase in the aging of the population and the changes in skin aging that affect self-esteem leads many anti-aging therapies to be developed^{6,7}.

Stem cells (SC) therapy, which contains different types of growth factors, is commonly used for skin aging. Furthermore, they are unique populations of cells that are undifferentiated, able to renew themselves, and differentiate into different cell lineage. The mesenchymal SC (MSC) is a multipotent stromal cell that can differentiate into

various types of cells such as adipocytes, osteoblasts, chondrocytes, fibroblasts, and myoblasts. There are many types of MSC, which includes bone marrow stem cell (BMSC), umbilical cord (UC) -MSC, placenta (P) -MSC, umbilical cord blood (UCB) -MSC, amniotic fluid (AF) -MSC, and adipose stem cell (ASC)⁸. In addition, Mesenchymal stem cell produces various secreted factors like cytokines, growth factors and chemokines, which may trigger intracellular mechanism. The main functions of MSC are tissue replacement through multipotent differentiation, immunomodulatory and anti-inflammatory effects, and molecular secretion, which helps tissue repair^{9,10}. This SC is a good choice for regenerative medicine because it is easily obtained (bone marrow, adipose and UCB) and has low immunogenicity⁹.

In recent years, conditioned medium (CM) or SC-derived secretome has received more attention in the field of regenerative medicine and has generally

shown good results. The use of secretome has several advantages when compared to SC, as CM can be manufactured, freeze-dried, packaged, and transported more easily. Moreover, as it is devoid of cells, there is no need to match the donor and the recipient to avoid rejection problems¹¹. The secretome contains many growth factor, cytokines, chemokines, angiogenic factors, microvesicle and exosome. Therefore, SC-derived CM have a promising prospect to be produced as pharmaceuticals for regenerative medicine^{11,12}. In addition, its potential role as a cosmetic ingredient is being explored¹³.

A previous *in vitro* study showed that medium conditioned with adipose stem cells (ASC-CM) can increase the expression of collagen type I mRNA, type III collagen and elastin in senescence human dermal fibroblast (HDF). However, this ability were reduced, as the passage of HDF increased¹⁴. Another study conducted by Li et al showed anti photoaging activities of this ASC-CM on human keratinocytes and fibroblasts exposed to UVB light. Inhibition of phosphorylation of the MAPK/ AP-1 pathway, decrease in NF- κ B activation and downregulation of HO-1 activation as well as increase in expression of TGF- β and Smad 2/3 proteins were reported¹⁵. Therefore, the purpose of this study literature is to know the effects and

benefits of MSC-CM or secretome for skin aging therapy.

METHODS

Eligibility criteria

The inclusion criteria used an experimental study in English with human and animal study groups with skin aging (intrinsic and extrinsic factors), given the intervention in the form of the application of MSC-CM/secretome. The expected outcome was the clinical and histological outcome as the primary outcome. Non-English studies, duplicates, review studies, and irrelevant articles as exclusion criteria.

Literature search and study selection

The integrated search process was carried out in accordance with the instructions of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The article was identified from searches of PubMed (MEDLINE), the Cochrane Library, Science Direct, and Wiley Online Library on June 9th, 2020. The terms used as the search keywords contains at least two words: "conditioned media" or "conditioned medium" or secretome and skin aging or photoaging. The authors (W.E.P and C.R.S.P) then reviewed the feasibility of the article on the basis of inclusion and exclusion criteria, the due diligence was conducted by the author in a discussion and independent manner.

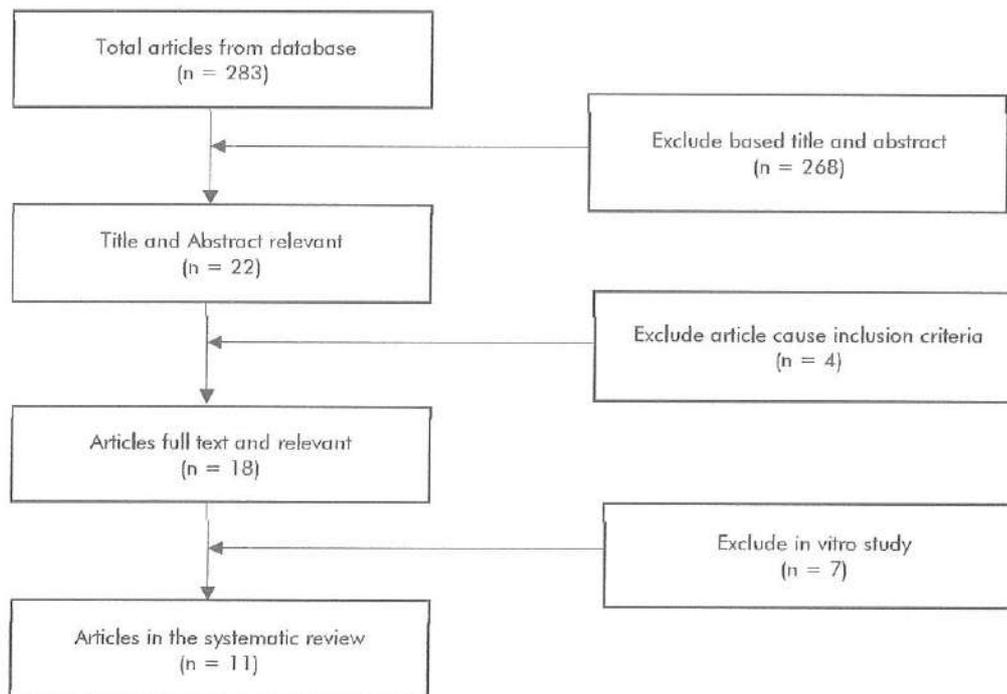


Fig.1: Prisma Flowchart

Methodological quality assessment and risk of bias

Two authors (W.E.P and C.R.S.P) in determining the assessment of methodological quality and risk of bias by combining animal testing guidelines: Reporting on in vivo Experiments (ARRIVE) guidelines with Consolidating Reporting of Trials¹⁶.

Data extraction and synthesis

The data extraction was performed by two authors (W.E.P and C.R.S.P) that were independent of each study and were in agreement with all included studies. Then, the following data were extracted: study design, human model and type of animal model for in vivo studies; establishment process for

animals or human in the included studies; type and specific donor of MSCs; the isolation process for the CM; interventions; comparison; duration of follow-up; main outcome for the in vivo studies and the results; any significant differences from control or baseline; and other outcomes. For in vivo studies, any adverse reactions were reviewed, clinical outcomes, and histological outcomes. The authors agreed to classify types of MSCs into BMSC, ASC, UCB-MSC, AF-MSC and AMSC. The data collection of in vivo study and clinical study outcomes are shown in Table 1. A meta-analysis could not be carried out due to high heterogeneity data.

Table 1: The studies overview

Author	Type of MSC	Level CM	Population	Type of Study	Sample	Intervention	Controlled
Sohn et al ¹⁷	EPC	CM	Human	Clinical study In vitro	25 Korean women (29-69 years old) with mild to moderate wrinkles. MSCs were differentiated to EPCs for 10-21 days. Conditioned medium was also collected from undifferentiated MSC.	5% EPC-CM was applied twice a day on both sides of the face for 4 weeks. NHDF cells were pretreated with each CM for 24 h, followed by exposure to 600 μ m H ₂ O ₂ for 24 h.	N/A HDF cells were exposed to 600 μ m H ₂ O ₂ for 24 h.
Prakoeswa et al ¹⁸	AMSC	CM	Human	Clinical study	48 subjects aged 41-60 years old and receive priming with 0,025% tretinoin cream for 2 weeks.	subjects were treated with microneedling plus 3 mL of AMSC-CM for 3 times with an interval of 2 weeks.	subjects were treated with microneedling plus 3 mL of NS; for 3 times with an interval of 2 weeks.

Author	Type of MSC	Level CM	Population	Type of Study	Sample	Intervention	Controlled
Kim et al ¹⁴	AD, BM, and UCB	CM	Human	Clinical study	22 volunteers (18–55 years-old women).	USC-CM in cream base were treated daily to their skin.	N/A
				In vitro	Conditioned media (CM) of MSCs and HDFs were collected. The conditioned media were measured with GDF-11 ELISA kit.	HDFs (1 × 10 ³ cells/well) were seeded in 96-well plates and cultured for 24 h in KSB-3 medium. After washing, the medium was replaced by HDF-CM, AD-MSC-CM and UCB-CM.	KSB-2 media.

Author	Type of MSC	Level CM	Population	Type of Study	Sample	Intervention	Controlled
Yan Xu et al ²⁰	ASCs and PCs	SC	Human	Clinical Study	18 young volunteers were randomly divided into 3 groups with 6 in each group: ASC-CM group, PSC-CM group, and control group.	The CM of ASC or PSC was dissolved into injectable hyaluronic acid (HA)	Injected with HA
				In vitro	100 mL of adipose tissue was harvested from a 45-year-old female cosmetic surgery patient. The final concentration from ASC-CM and PSC-CM were 70 mL of 5.989±0.07 mg/mL in freeze dry powder.	Characterization of ASC and PSC with FACS analysis and multipotency analysis. Characterization of CM with MALDI-TOF/TOF analysis.	N/A
Yang Xu et al ²¹	DA	CM	Mice	In vivo	8 immunodeficient nude BALB/C mice (female, 8 weeks old); UVB induced photoaging mice (5x/weeks for 8 weeks). Adipose tissue was taken by liposuction from healthy adult. HDFs derived from the foreskin of a 20-year-old donor.	200 µL of 10-fold-concentrated DA-CM was subcutaneously injected at two places on one side of dorsal skin, once a week for a total of three times. DA-CM-treated HDFs (HDFs cultured in DACM),	200 µL of 10-fold-concentrated DMEM was subcutaneously injected at other side as control, once a week for a total of three times.

Author	Type of MSC	Level CM	Population	Type of Study	Sample	Intervention	Controlled
				In vitro	It was irradiated with UVB source, with dosage 10 mJ/cm ² , once a day for 5 days.	and DA-CM-treated SIPS-HDFs (HDFs cultured in DA-CM and exposed to UVB irradiation).	HDFs (HDFs cultured in serum-free DMEM), SIPS-HDFs (HDFs cultured in serum-free DMEM and exposed to UVB irradiation).
El-Domyati et al ²²	AF-MSC	CM	Human	Clinical Study	10 volunteers (3 males and 4 females) age 41-60 years old with facial aging	5 sessions of skin needling, 2 weeks apart, AF-MSC-CM was added topically to the right side only.	Left side, only skin needling.
Lee et al ²³	ESC-EPC	CM	Human	Clinical study	25 participants were 41-64 years old and had Fitzpatrick Skin Type III or IV.	5 treatment sessions of microneedle with hESC-EPC CM were repeated at 2-week intervals.	5 treatment sessions of microneedle with saline were repeated at 2-week intervals.
Amirthalingam et al ¹¹	BMSC	CM	Mice	In vivo	6 to 8 weeks old nude mice (NU/NU)	All mice were irradiated with UVB at a dose of 150 mJ/cm ² , once daily, for seven days and given CM.	Not irradiated with UVB
				In vitro	HFF cells were photo-damaged by UVB irradiation (300 mJ/cm ²).	HFF treated with 0.25%, 0.5% and 1% for 48 h.	
Kim et al ¹⁹	ASC	CM	Human	Clinical Study	Female adults aged 30-60 years were selected.	Cream containing 3D cultured ADMSCs-CM was applied on: Left lower forearm Left upper forearm after treated with SLS Face HDF was treated with 2D or 3D cultured ASC-CM	
				In vitro	HDF were obtained from males of 20 years old or under		Negative control was cultured with basal medium without serum. Positive control was cultured in a basal

Author	Type of MSC	Level CM	Population	Type of Study	Sample	Intervention	Controlled
							medium supplemented with 0.04%.
Wang et al ²⁴	ASC	Protein extract of CM	Human	Clinical Study	30 females between 40 and 63 years (skin type III and IV),	Protein extracts from ADSC-CM were applied via microneedles into the skin with interval 2 weeks (W0, W2, W4, W6, W8, W10).	Ultrapure water was applied via microneedles in control group.
Kwon et al ²²	BMSC	CM	Mice	In vivo	48 photoaged female SKH-1 hairless mice, 6 weeks old	Topical BMSC-CM was applied on dorsal skin mice 3x/week for 8 weeks.	Vehicle solution (polyethylene glycol: ethanol 7:3) was applied on dorsal skin mice 3x/week for 8 weeks
				In vitro	Human dermal fibroblasts	Treated by MSC-CM (0,1%, 1% and 10%) for 24 and 48 h	NA

Table 2: In vivo studies and clinical studies results

Author	Assessment of main outcome	Main outcome measure(s)	Results	Significant difference from baseline	Significant difference between groups	Other evaluation	Other outcome measure(s)
Sohn et al ¹⁷	4 weeks	Crow's feet. The levation of skin surface on the right cheek. The texture small (Ra) values of the right cheek.	Improved significantly during clinical test period.	Yes ($p < 0.001$)	N/A	N/A	N/A
Prakoeswa et al ¹⁸	4 weeks and 8 weeks	Pore (baseline)	NS: 49.63 ± 11.193 AMSC-CM: 53.17 ± 4.565	Yes	N/A	Side effect	Erythema, urticaria (resolved after 2 days with topical 1% hydrocortisone cream).
		Pore 4 weeks	NS: 49.58 ± 6.903 AMSC-CM: 51.42 ± 4.745		N/A		
		Pore 8 weeks	NS: 50.29 ± 4.467 AMSC-CM: 48.46 ± 6.171		N/A		
		Wrinkle (baseline)	NS: 12.13 ± 7.011 AMSC-CM: 13.92 ± 6.639	Yes	N/A		
		Wrinkle 4 weeks	NS: 12.29 ± 6.196 AMSC-CM: 12.42 ± 6.413		N/A		
		Wrinkle 8 weeks	NS: 11.67 ± 5.498 AMSC-CM: 9.29 ± 5.279		N/A		
		Spot (polarized) (baseline)	NS: 32.79 ± 8.968 AMSC-CM: 34.96 ± 6.557	Yes	N/A		
		Spot (polarized) 4 weeks	NS: 34.46 ± 7.852 AMSC-CM: 32.71 ± 7.474		N/A		
		Spot (polarized) 8 weeks	NS: 34.08 ± 9.016 AMSC-CM: 30.46 ± 8.335		N/A		
		Spot (UV) baseline	NS: 14.54 ± 8.748	Yes	N/A		

Author	Assessment of main outcome	Main outcome measure(s)	Results	Significant difference from baseline	Significant difference between groups	Other evaluation	Other outcome measure(s)
			AMSC-CM: 17.17 ± 8.646				
		Spot (UV) 4 weeks	NS: 13.50 ± 5.934 AMSC-CM: 13.67 ± 5.880		N/A		
		Spot (UV) 8 weeks	NS: 13.04 ± 6.196 AMSC-CM: 12.46 ± 7.581		N/A		
		Skin tone (baseline)	NS: 38.58 ± 8.717 AMSC-CM: 39.54 ± 7.028	No	N/A		
		Skin tone 4 weeks	NS: 38.58 ± 8.677 AMSC-CM: 38.96 ± 7.624		N/A		
		Skin tone 8 weeks	NS: 36.67 ± 7.750 AMSC-CM: 36.92 ± 6.788		N/A		
Kim et al ¹⁹	2 weeks and 4 weeks	Dermal density baseline 2 weeks 4 weeks	14.20 ± 0.09 14.33 ± 0.08 14.55 ± 0.10	- No Yes	N/A	Adverse reaction	No adverse reaction
		Skin wrinkle Ra baseline Ra 2 weeks Ra 4 weeks	13.62 ± 0.56 13.40 ± 0.50 13.08 ± 0.45	- No No	N/A		
		Rmax baseline Rmax 2 weeks Rmax 4 weeks	94.93 ± 4.31 92.97 ± 3.34 90.35 ± 3.44	- No Yes	N/A		
		Rz baseline Rz 2 weeks Rz 4 weeks	68.87 ± 2.49 67.10 ± 2.08 66.04 ± 2.15	- No No	N/A		
		Rp baseline Rp 2 weeks Rp 4 weeks	42.66 ± 1.19 41.70 ± 1.20 40.35 ± 1.02	- No Yes	N/A		
		Rv baseline Rv 2 weeks Rv 4 weeks	57.23 ± 3.43 56.00 ± 2.54 54.59 ± 2.70	- No No	N/A		

Author	Assessment of main outcome	Main outcome measure(s)	Results	Significant difference from baseline	Significant difference between groups	Other evaluation	Other outcome measure(s)
Yan Xu et al ²⁰	15 days	Erythema index	N/A	N/A	Control group > ASC-CM (p<0.01) Control group > PSC-CM (p>0.05)	N/A	N/A
		Melanin index	N/A	N/A	Control group > ASC-CM (p<0.01) Control group > PSC-CM (p<0.01) ASC-CM < PSC-CM (P<0.01)		
		Glossymer index	N/A	N/A	Control group < ASC-CM (p<0.01) Control group < PSC-CM (p<0.01)		
		TEWAmeter index	N/A	N/A	Control group > ASC-CM (p<0.05) Control group > PSC-CM (p<0.01)		
		Corneometer index	N/A	N/A	Control group < ASC-CM (p<0.01) Control group < PSC-CM (p<0.01)		
Yang Xu et al ²¹	4 weeks and 8 weeks	Collagen amount 4 weeks	control: 19.78% ± 2.01% DA-CM: 34.31% ± 3.44%	Yes (p<0,05)	N/A	Immunohistochemistry	MMP-1 MMP-3
		Collagen amount 8 weeks	control: 19.35% ± 2.53% DA-CM: 49.33% ± 4.78%	Yes (p< 0.05)	N/A		
		Collagen Type I 4 weeks	control: 19.01%± 2.22% DA-CM: 23.14%± 2.43%	Yes (p <0.05)	N/A		
		Collagen Type I 8 weeks	control: 22.02% ± 3.41% DA-CM: 45.37% ± 3.07%	Yes (p< 0.05)	N/A		
		Collagen Type III	control: 23.31% ± 1.34% DA-CM: 45.93% ± 3.50%	Yes (p< 0.05)	N/A		

Author	Assessment of main outcome	Main outcome measure(s)	Results	Significant difference from baseline	Significant difference between groups	Other evaluation	Other outcome measure(s)
		4 weeks Collagen Type III 8 weeks MMP-1 MMP-3	control: 23.02% ± 2.42% DA-CM: 56.94% ± 6.63% N/A N/A	Yes (p< 0.05) N/A N/A	N/A N/A N/A		
El-Damyati et al ²²	One month	Clinical Improvement Left side Right side Score Left side Very good Good Moderate Mild Right side Very good Good Moderate Mild Histological evaluation Collagen fibers Before After Elastic fiber Before After	33.3 ± 8.95 60.6 ± 9.77 0 (0%) 0 (0%) 7 (70%) 3 (30%) 1 (10%) 7 (70%) 2 (20%) 0 (0%) Disorganized collagen bundles with increased intercellular spaces Increased and more organized collagen bundles with decreased interfibrillary spaces Dense elastotic material Elastotic material decreased, fine and well-arranged new elastic fibers	Yes (p=0.026) Yes (p= 0.019)	Yes (p<0.001) Yes (p=0.003)	Side effects	Erythema Edema Pigmentary change Ecchymosis Crusting

Author	Assessment of main outcome	Main outcome measure(s)	Results	Significant difference from baseline	Significant difference between groups	Other evaluation	Other outcome measure(s)
		Mean epidermal thickness					
		Left side	47.55 ± 5.52	Yes (p<0.001)	No (p=0.78)		
		Before	62.18 ± 5.69				
		After					
		Right side	48.19 ± 4.36	Yes (p<0.001)	No (p=0.41)		
		Before	64.08 ± 4.30				
		After					
Lee et al ²²	12 weeks	Clinical assessment: Overall satisfaction scores	Control: 2.72 ± 1.45 hESC-EPC: 3.25 ± 1.26	N/A	Yes (p<0.05)	Adverse event	Mild pain and temporary erythema, mild desquamation
		Assessment for pigmentation	Control: 1.32 ± 0.62 hESC-EPC: 1.54 ± 0.57	N/A	Yes (p<0.05)		
		Assessment for wrinkle	Control: 1.49 ± 0.48 hESC-EPC: 1.92 ± 0.42	N/A	Yes (p<0.05)		
		Non-invasive measurement					
		Pigmentation					
		Baseline	Control: 143 ± 11.1 hESC-EPC: 138 ± 14.2		Yes (p<0.05)		
		Two weeks	Control: 136 ± 12.8 hESC-EPC: 113 ± 12.1	No (p= 0.052) Yes (p<0.05)			
		Erythema					
		Baseline	Control: 268 ± 25.1 hESC-EPC: 271 ± 24.2		Yes (p<0.05)		
		Two weeks	Control: 243 ± 21.1 hESC-EPC: 255 ± 29.8	No (p>0.05) Yes (p<0.05)			
		Wrinkle					
		R2 value	Control: 0.52 ± 0.07 hESC-EPC: 0.58 ± 0.1		Yes (p<0.05)		
		Baseline					
		Two weeks	Control: 0.50 ± 0.1	No (p>0.05)			

Author	Assessment of main outcome	Main outcome measure(s)	Results	Significant difference from baseline	Significant difference between groups	Other evaluation	Other outcome measure(s)
		R3 value Baseline	hESC-EPC: 0.46 ± 0.09 Control: 0.38 ± 0.06 hESC-EPC: 0.4 ± 0.1	Yes ($p < 0.05$)	Yes ($p < 0.05$)		
		Two weeks	Control: 0.34 ± 0.1 hESC-EPC: 0.31 ± 0.06	No ($p = 0.51$) Yes ($p < 0.05$)			
Amirhalingam et al ¹²	8 days	skin moisture control group UVB exposed group Formulation treated	49.6 ± 3.7 - 55.80 ± 1.2 54.60 ± 4.7 - 14.5 ± 1.3 47.8 ± 3.2 - 37.4 ± 2.5	N/A	Yes ($p < 0.05$)	N/A	N/A
		Erythema Index control group UVB exposed group Formulation treated	N/A 286.8 ± 12.9 - 366.2 ± 14.4 265 ± 14.3 - 271.4 ± 15.9	N/A	Yes ($p < 0.05$)		
		histological Epidermal thickness Control UVB-irradiated Formulation treated	3.06 ± 0.41 14.25 ± 1.19 10.98 ± 2.05	N/A			
		Dermal thickness Control UVB-irradiated Formulation treated	55.18 ± 3.64 99.21 ± 8.73 76.38 ± 4.60				
		Epidermal hyperplasia Control UVB-irradiated Formulation treated	- +++ +				
		Hyperkeratosis Control UVB-irradiated Formulation treated	- +++ +				
		Infiltration of inflammatory					

Author	Assessment of main outcome	Main outcome measure(s)	Results	Significant difference from baseline	Significant difference between groups	Other evaluation	Other outcome measure(s)
		cells in dermis Control UVB-irradiated Formulation treated	- +++ ++				
Kim et al ¹⁹	3 weeks	Skin irritation degree after 0.5 h 24 h 48 h	0 0 0	N/A	N/A		
	2 weeks and 4 weeks	Skin texture damaged by external stimuli	Control: 3.74% Application site: 11.94%	N/A	Yes (p<0.05)		
		Deep skin elasticity Ur/Ue value 2 weeks 4 weeks	5.97% 9.34%	Yes (p<0.05)	N/A		
		Wrinkle assessment 2 weeks 4 weeks	5.01% 6.23%	Yes (p<0.05)	N/A		
		Dermal density 2 weeks 4 weeks	6.97% 12.5%	Yes (p<0.05)	N/A		
	Right after 1 time use and after 20 minutes of artificial cool breeze	Skin moisture retention effect in artificial cold wind environment Right after 1 time use 20 minutes after cool breeze	543.60% 452.38%	Yes (p<0.05)	N/A		
Wang et al ²⁴	12 weeks	Melanin index	N/A	Yes (p<0,001) week 10 and week 12	Yes p<0.01 week 2 p<0.001 week 6, 10, 12	Facial photograph Adverse effect	N/A
		Skin color	N/A	No	Yes p<0.01 week 10, 12		Erythema Dryness Desquamation Burning Itching Stinging

Author	Assessment of main outcome	Main outcome measure(s)	Results	Significant difference from baseline	Significant difference between groups	Other evaluation	Other outcome measure(s)
		Skin radiance	N/A	Yes p<0.05 week 2 p<0.01 week 6, 12	Yes p<0.001 week 6, 10, 12		
		Skin surface topography SEsm	N/A	Yes AAPE: p<0.01, week 6 p<0.001, week 10, 12 Control: p<0.05, week 12	No		
		SEsc	N/A	Yes AAPE: p<0.05, week 10 p<0.01, week 12 Control: p<0.05, week 10 p<0.01, week 12			
		Sew	N/A N/A	Yes AAPE: p<0.05, week 2, 6 p<0.001, week 10, 12 Control: p<0.01, week 10, 12			
		Value at each time	N/A	Yes AAPE: p<0.01, week 2 p<0.001, week 6, 10, 12 Control: p<0.05, week 10 p<0.01, week 12			
		Skin elasticity R2 value	N/A	Yes AAPE:	Yes p<0.05 week 2 p<0.01 week 6		

Author	Assessment of main outcome	Main outcome measure(s)	Results	Significant difference from baseline	Significant difference between groups	Other evaluation	Other outcome measure(s)
		Forehead		p<0.001, week 6, 10, 12 Control: p<0.01, week 10, 12	p<0.001 week 10, 12		
		Crow's feet		Yes AAPE: p<0.01, week 2 p<0.001, week 6, 10, 12 Control: p<0.05, week 10 p<0.01, week 12	Yes p<0.01 week 2, 12 p<0.001 week 6, 10		
		Periorbital skin relief	AAPE week 12:	Yes AAPE: p<0.05, week 2 p<0.01, week 6,10,12	Yes p<0.05 week 12		
		Ra	-3.08±2.61	Yes AAPE: p<0.05, week 2, 10 p<0.01, week 6,12	Yes p<0.01 week 12		
		Rq	-3.83±3.15	Yes AAPE: p<0.05, week 2, 6, 10 p<0.001, week 12	Yes p<0.01 week 12		
		Rz	-11.48±10.01	Yes AAPE: p<0.05, week 2, 6, 10 p<0.01, week 12	Yes p<0.01 week 12		
		Rmax	-21.66±21.90	N/A	Yes p<0.05 week 12		

Author	Assessment of main outcome	Main outcome measure(s)	Results	Significant difference from baseline	Significant difference between groups	Other evaluation	Other outcome measure(s)
				N/A			
		Self-evaluation questionnaire		N/A	Yes p<0.01 week 2, 6, 10, 12		
		Anti aging Wrinkles	N/A		Yes p<0.05, week 2 p<0.01 week 6, 10, 12		
		Wrinkles around eyes	N/A	N/A			
		Skin more resilient	N/A	N/A	Yes p<0.05, week 2, 6 p<0.01 week 10, 12		
		Skin more elastic	N/A	N/A	Yes p<0.05, week 2, 10 p<0.01 week 6, 12		
		Lifting effect	N/A		Yes p<0.05, week 2, 6 p<0.01 week 10, 12		
		Fine wrinkles reduced	N/A	N/A	Yes p<0.05, week 2, 10, 12 p<0.01 week 10, 6		
		Whitening and moisturizing Feels rejuvenated	N/A	N/A	Yes p<0.05, week 10 p<0.01, week 6, 12 p<0.001, week 2		
		Skin more hydrated	N/A	N/A	Yes p<0.01, week 2, 6, 10		

Author	Assessment of main outcome	Main outcome measure(s)	Results	Significant difference from baseline	Significant difference between groups	Other evaluation	Other outcome measure(s)
		Skin more radiant	N/A	N/A	p<0.001, week 12 Yes p<0.05, week 2 p<0.01, week 10 p<0.001, week 6, 12		
		Skin healthier	N/A	N/A	Yes p<0.01, week 2, 6, 10 p<0.001, week 12		
		Skin more fair	N/A		Yes p<0.01, week 2, 6, 10 p<0.001, week 12		
		Overall satisfaction	N/A		Yes p<0.01, week 2, 6, 10 p<0.001, week 12		
					Yes p<0.05, week 2 p<0.01, week 6, 10 p<0.001, week 12		
Kwon et al ²⁵	8 weeks	TEWL	N/A	N/A	Yes p<0.001, normal group, adenosine and 10% MSC-CM group p<0.01, UVB, 1% MSC-CM	Immunohistochemistry Histological evaluation	Type I collagen Epidermal thickening Collagen fiber
		Skin hydration	N/A	N/A	Yes p<0.001, normal group p<0.01, 1% MSC-CM		
		Keratin content	N/A	N/A	Yes p<0.001, normal group, adenosine, 1% MSC-CM and 10% MSC-CM group		
		Wrinkle area	N/A	N/A	Yes		

Author	Assessment of main outcome	Main outcome measure(s)	Results	Significant difference from baseline	Significant difference between groups	Other evaluation	Other outcome measure(s)
		Clinical score	N/A	N/A	<p>p<0.001, normal group, adenosine, 1 % MSC-CM and 10% MSC-CM group</p> <p>Yes</p> <p>p<0.001, normal group, adenosine, 1 % MSC-CM and 10% MSC-CM group</p>		

Assessment of methodology quality

Study characteristics: Table 1 presented an overview of the study, which describes the type of MSC and CM level used, study design, human or animal model for in vivo study, cell preparation for in vitro study, and the intervention. Table 2 mostly describes the study's outcomes.

Out of 11 studies, 8 were human-model clinical studies, and 2 studies mentioned the Fitzpatrick skin type and only 1 mentioned Glogau classification for photoaging. Others did not mention the classification. Furthermore, 3 studies in this review used mice, including nude BABL/C and, SKH-1 hairless, and nude mice (NU/NU). The aging model in animal was established by irradiating with UVB light.

The most common type of MSC used was adipose tissue (five studies), followed by bone marrow (two studies), amnion tissue, epidermal progenitor cell, human umbilical cord blood, placental tissue and amniotic fluid. Topical MSC was used in 5 studies, while injection was used in 2. Prakoeswa et al, Lee et al, Wang et al and Domyati et al used microneedle and MSC-CM compared between treatment group and the control group. Study carried out by Xu et al compared between ASC-CM or PSC-CM, which was combined with hyaluronic acid.

In vivo study outcomes: Table 2 presented In vivo and clinical study outcomes, including the main outcomes measures, the scores or results, significant difference, and also other outcome measures. Out of 11 studies, clinical outcomes were mostly reported. Sohn et al, Prakoeswa et al, Kim et al reported the significant clinical improvement from baseline, which includes Crow's feet, skin surface, skin texture; pore, wrinkle, spot, skin tone; dermal density, Rmax, Rp; and pore, wrinkle, pigment, moisture, skin elasticity, sebum U zone, and T zone, respectively¹⁷⁻¹⁹. Otherwise, Yan Xu et al only reported significance difference between the control group and the treatment group²⁰. Index of Melanin, Glossymer, Tewameter and Corneometer of ASC-CM and PSC-CM group showed improvement than control group for 15 days. However, Erythema index, reported significance difference from baseline only in ASC-CM group. Melanin index of ASC-CM group was significantly lower than PSC-CM group. Lee et al showed that after treatment for 12 weeks with microneedle and hESC-EPC CM, clinical assessment such as overall satisfaction scores, assessment for pigmentation and wrinkle were significantly improved between control and hESC-EPC CM group. Pigmentation, wrinkle and erythema were significantly decreased in 12 weeks. The overall satisfaction reported by Wang

et al was significantly improved between group in week 2, week 6 and week 12²⁴. Xu et al, Amirthalingam et al and Kwon et al, used photoaged mice model to observe the effect of MSC-CM^{13,19,21}. Amirthalingam et al and Kwon et al, that uses both BMSC-CM, reported an improvement in clinical and histological outcomes^{13,19,21}.

Histological outcomes were reported in 4 studies, all of which showed good improvements in the histological structure of aging skin, and 2 studies showed significance difference from baseline. Xu et al reported the significant increase of collagen amount, collagen type I and collagen type III in BABL/C mice treated with DA-CM, compared with control (DMEM) group for 4 weeks and 8 weeks. Domyati et al reported that after one-month treatment, mean epidermal thickness left side (microneedle only) and right side (microneedle and AF-MSC-CM) increased significantly; however, there is no significance difference between these group.

Other evaluations performed by the authors aim to monitor side effect or adverse reaction, facial photograph and immunohistochemistry. Lee et al reported mild pain, temporary erythema and mild desquamation with microneedle. Similar to Lee et al, Prakoeswa et al and Domyati et al also used microneedle and reported erythema. Only Xu et al conducted immunohistochemistry examination. All results supported the beneficial effects of MSC-CM in the treatment of skin aging.

DISCUSSION

The medium or secretome conditioned by mesenchymal stem cells contains many growth factors, cytokines, chemokines as well as extracellular matrix proteins and exosomes. These secreted factors can play a role in tissue repair and regeneration through their paracrine effect^{10,12,26,27}. This study showed that MSC-CM is always associated with significantly better clinical and histological outcomes. In addition, 11 clinical or in vivo studies showed improvement of clinical outcomes with or without histological outcomes. Some studies performed in vitro study to support in vivo studies.

An in vitro study performed by Xu et al, Sohn et al, and Pan et al, all reported an increase in superoxide dismutase (SOD). The combined laser therapy and ASC-CM conducted by Xu et al also showed a decrease in malondialdehyde (MDA) and increased in the expression of Wnt/ β -catenin, which plays a role in collagen formation for the improvement of skin aging²¹. Sohn et al did culture of HDF with H₂O₂ and reported that hEPC-CM and MSC-CM have similar protective effects on SOD and glutathione peroxidase (GPx),

which significantly increase type I procollagen also inhibited hydrogen peroxide-induced phosphorylation of proteins in mitogen activated protein kinase (MAPK) signalling and play a role in skin aging¹⁷. Parado et al²⁸ demonstrated that AMSC-CM increased catalase (CAT) and decreased malondialdehyde (MDA) also blocked cell cycle. These could delay oxidative stress-induced premature senescence²⁸. Kim et al reported that cell migration and collagen type I and type III synthesis were increased in the UCB- MSC CM group than in HDF-CM and ASC-CM. They also reported that GDF11 (rejuvenation factor) secretion was the highest in UCB- MSC CM, and therefore improving the skin wrinkles¹⁹. GDF-11 or bone morphogenetic protein-11 (BMP-11), member of TGF- β family, decreases with age. Although not high, ASC also secretes GDF-11, which can improve proliferation, differentiation of cell and migration and secretion of ECM that is important for skin aging²⁹.

An in vitro study performed by Balasubramanian et al demonstrated that BMSC-CM showed beneficial effects on the damaged fibroblasts caused by oxidative stress or UV radiation³⁰. The secretome was able to restore fibroblast proliferation and migration, also an increase in synthesis of ECM²⁶. They also reported an increase in the cyclin B1 levels, which decreases in senescence cell. Another study supported BMSC-CM as anti-aging, conducted both in vivo and invitro by Amirhalingam et al reported that the cyclobutene pyrimidine dimers (CPD) formation, which cause primary lesion in UV-irradiated DNA, was prevented by BMSC-CM treatment. They showed that these CM extensively microscopically and macroscopically protected mice skin from toxic UVB-irradiation¹³.

Sohn et al, Prakoeswa et al, and Kim et al agreed that MSC-CM can improve the appearance of wrinkle. A study by Prakoeswa et al using AMSC-CM with microneedling showed its capability to improve clinical photoaging and it was a promising option for rejuvenation therapy¹⁸. Another in vivo study conducted by Kim et al showed that topical treatment of UCB-CM showed anti-wrinkle effect and significantly increased dermal density in photoaged women¹⁹. A study by Xu et al showed that ASC-CM and PSC-CM improve skin aging for 20 days. Indeed, every type of MSC has shown improvement in skin aging. Secretome composition varies between donors depending on the type of cultured MSC²⁶. Side effects of the application of the CM were observed only in 4 studies.

This study had several limitations. First, out of 11 studies, only 2 reported clinical and histological results with randomized subjects. Second, these

studies used different cell sources, research subject, and delivery methods, which clearly leads to different outcomes. The studies used in our review varies considerably in terms of MSC sources (epidermal progenitor cell, amniotic membrane, adipose, bone marrow, umbilical cord blood, placental, and amniotic fluid), research subjects (human, mice), and delivery methods (with or without device-microneedle, laser, with or without additional material-hyaluronic acid). Finally, the clinical outcomes and the device used in measuring the same index also varies between studies. Therefore, it was impossible to perform a quantitative analysis with the included studies. Further research is needed to decide which type of MSC-CM will be the best choice for skin aging and to know more about mechanism of MSC-CM in skin aging improvement.

CONCLUSION

The use of secretome was promising in improving skin aging process, as shown by the available preclinical studies; however, further clinical studies are needed to choose which type of MSC is superior for the improvements of skin aging.

CONFLICT OF INTEREST

There is no conflict of interest in this study literature.

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