AD-MSC-CM EXOSOME

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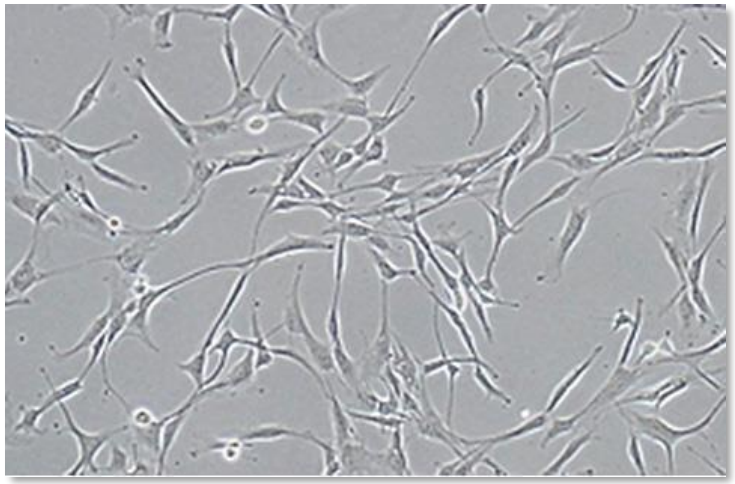
Acquire adipose tissue and isolate stem cells

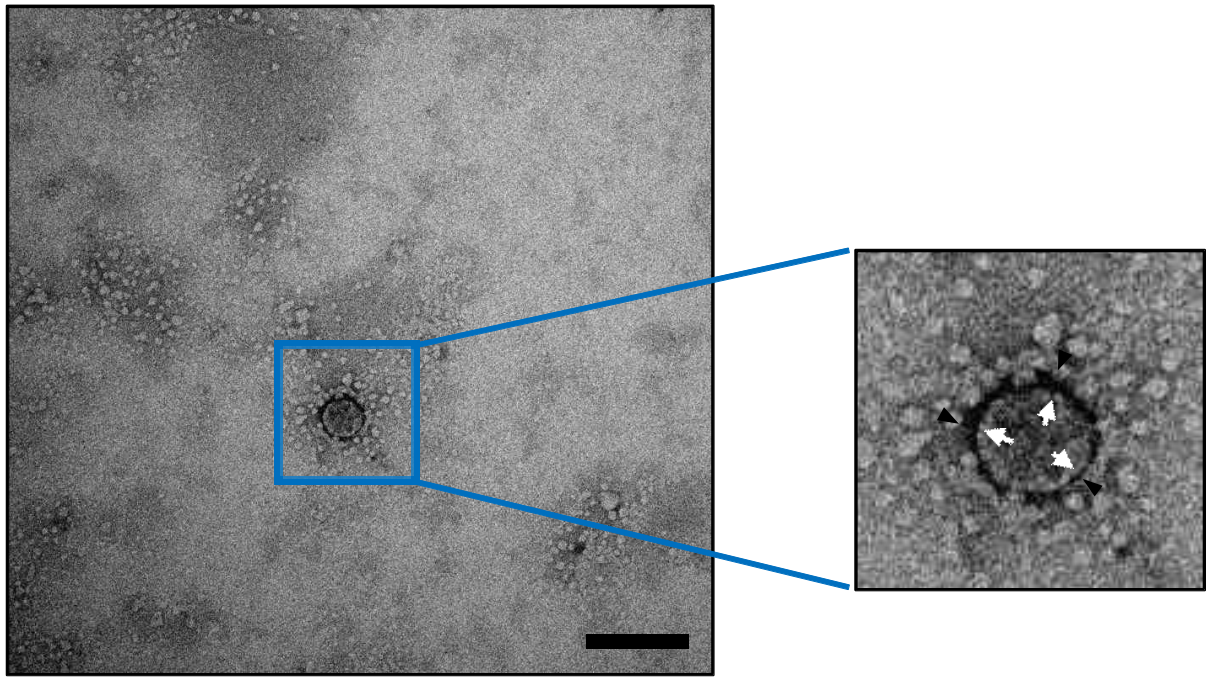
Acquire adipose tissue

Adipose-derived stem cells



Structure

Structure of AD-MSC-CM exosomes (TEM image)



- ✓ Human Adipose Stromal Cell Conditioned Media : circular structure
- ✓ Black line : Human Adipose Stromal Cell Conditioned Media membrane (Lipid bilayer)

How do we secure adipose-derived stem cells

Human Application Test Report

Completion of primary stimulation evaluation test through skin patch for 24 hours of human adipose stem cell-derived exosome mixture (IRB approval)

"Obtain human safety data"

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How do we secure adipose-derived stem cells

Confirmation of Agreement for Eligibility of Donor safety

Confirmation of Agreement for Test of Congenital and Chronic Disease

Confirming of Agreement for Test of TSE

Consent of Agreement for Adipose tissue

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How do we secure adipose-derived stem cells

Completed the anti-aging efficacy evaluation test of the human adipose stem cell-derived exosome mixture

For a total of 4 weeks of testing, As a result of the test on 13 study subjects, the test site using the "AD-MSC-CM exosomes (1st agent) & Skin solvent (2nd agent)" sample showed wrinkles around the eyes, skin moisture, elasticity, and pigmentation compared to placebo. It was confirmed that there is an effect of improving density (anti-aging effect), and a statistically significant difference ($p < 0.05$) between groups was also observed in comparison with the control site using the control sample. Therefore, when judged comprehensively, it is judged that the test sample can help skin anti-aging.

"Prove anti-aging function in human skin"

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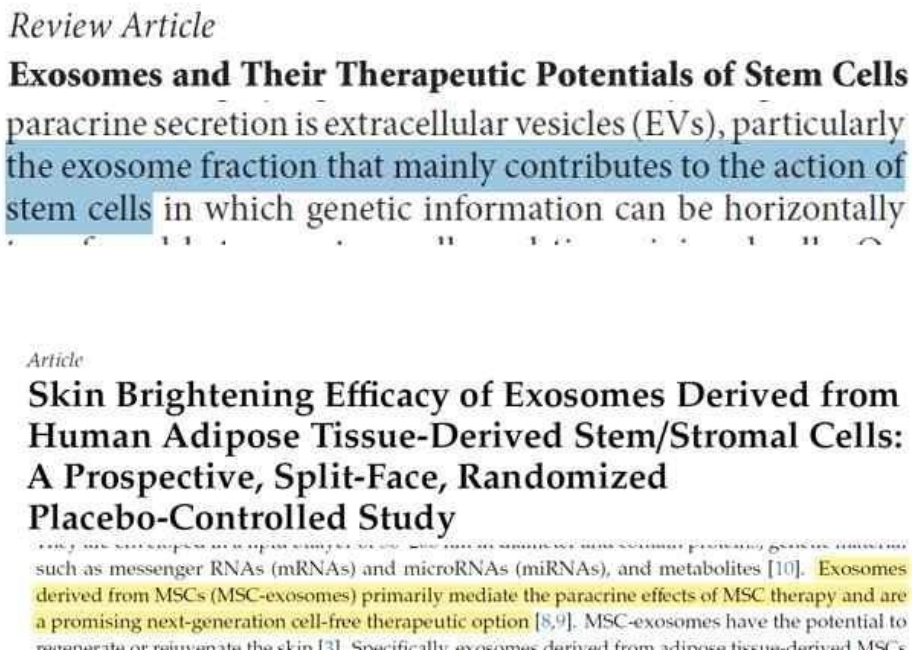
Exosome vs Stem cell

The hot Exosome Market

- **Mesenchymal stem cells (MSCs) are cells that have ability to differentiate into other types of cells by external environmental factors. They are known to have anti-inflammatory, immune-modulating, and regenerative abilities.**
- **Recent reports have shown that exosomes secreted from MSCs possess most of the abilities of MSCs and play a major role in various medical therapeutic effects.**
- **Exosomes can be combined with existing components or methods to develop new components and new products, so it is estimated that exosomes will replace more than 70% of the current stem cell market.**

Category	Exosome	Stem Cell
Size	30~200nm	Thousands ~ Tens of thousands
Efficacy	Excellent (Concentrating of effective ingredients)	Low (low engraftment <1%)
Enhance active ingredient	Possible	Difficult
Blood vessel injection	Easy	Difficult
BBB (Blood Brain Barrier)	Passable	Unpassable
Purity	High	Low
Storage stability	High (long-term storage)	Extremely Low (<6 hours)



Review Article

Exosomes and Their Therapeutic Potentials of Stem Cells

paracrine secretion is extracellular vesicles (EVs), particularly the exosome fraction that mainly contributes to the action of stem cells in which genetic information can be horizontally

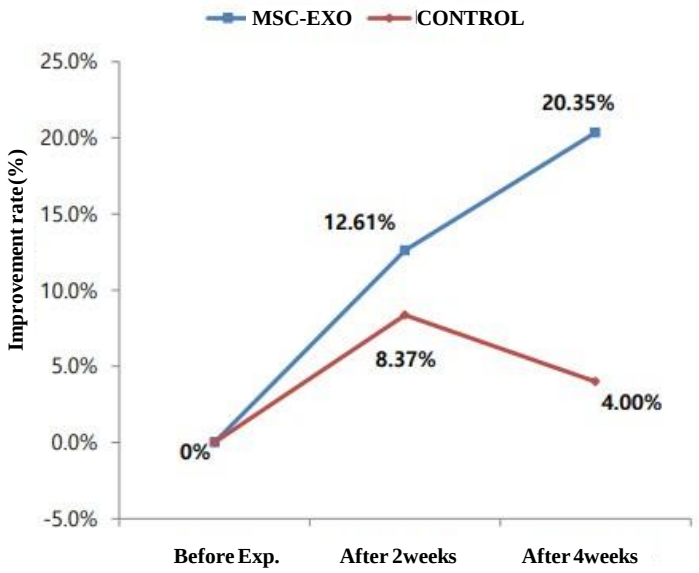
Article

Skin Brightening Efficacy of Exosomes Derived from Human Adipose Tissue-Derived Stem/Stromal Cells: A Prospective, Split-Face, Randomized Placebo-Controlled Study

such as messenger RNAs (mRNAs) and microRNAs (miRNAs), and metabolites [10]. Exosomes derived from MSCs (MSC-exosomes) primarily mediate the paracrine effects of MSC therapy and are a promising next-generation cell-free therapeutic option [8,9]. MSC-exosomes have the potential to

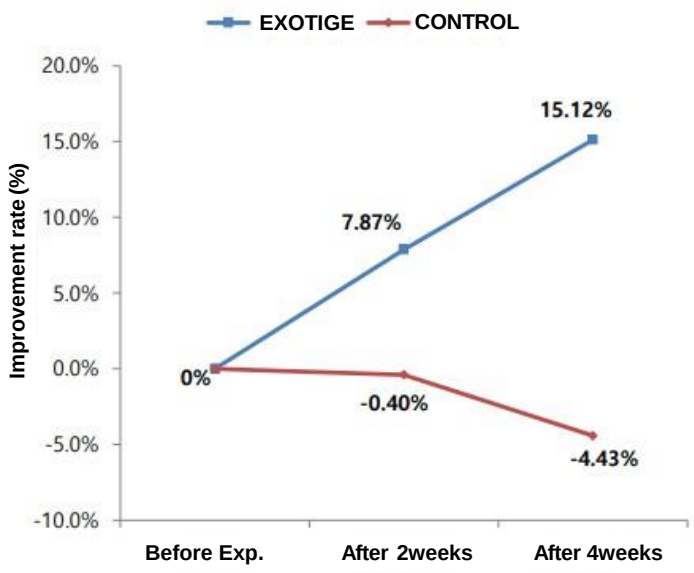
❖ **AD-MSC-CM exosomes** functional analysis

Increase skin moisture content



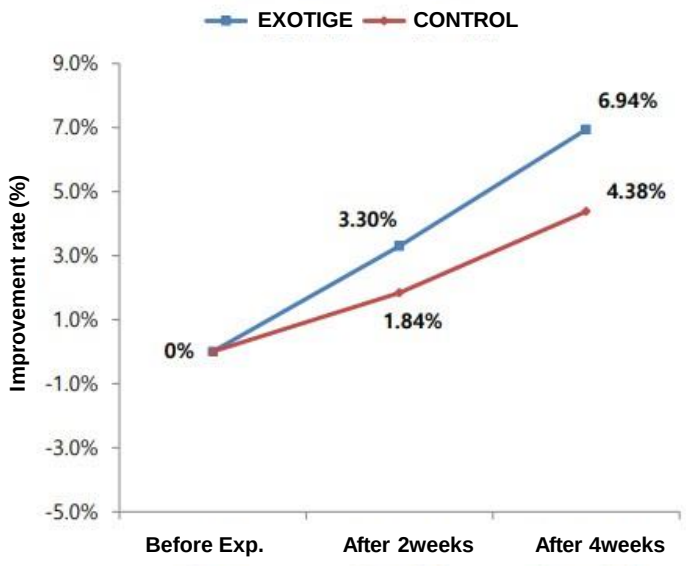
When applied to the skin for 4 weeks, it is confirmed that the skin moisture content increases by 20.35%

Increase skin elasticity



When applied to the skin for 4 weeks, skin elasticity increased by 15.12%

Skin whitening effect



When applied to the skin for 4 weeks,
Through inhibition of melanin content (6.94%)
Confirmation of increased skin whitening effect

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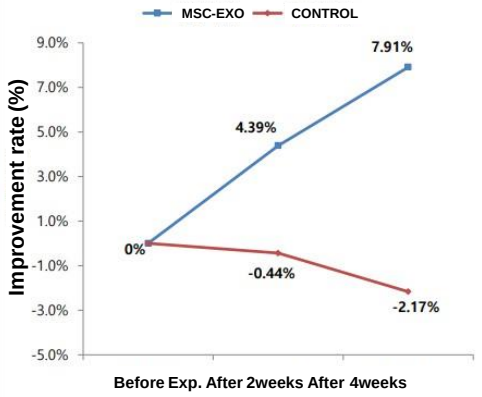
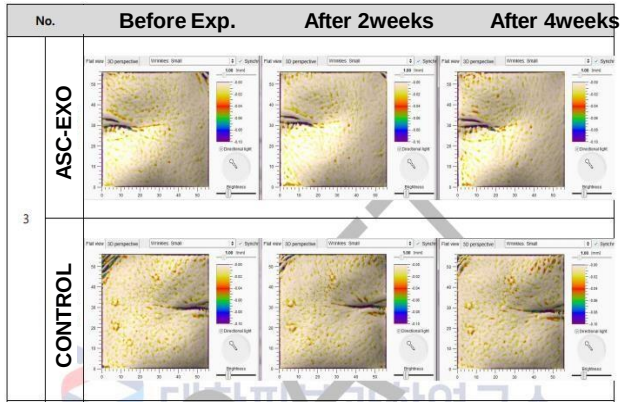
❖ **AD-MSC-CM exosomes** functional analysis

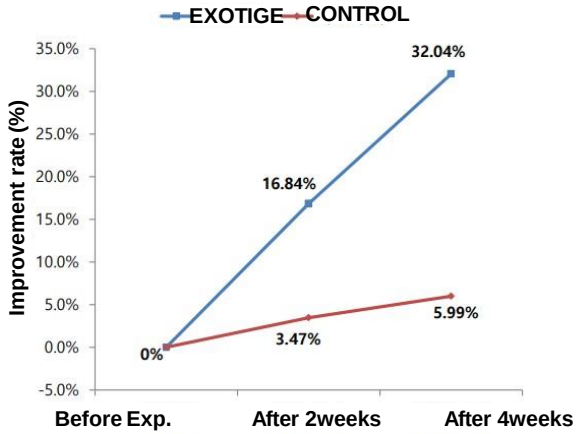
Anti-wrinkle improvement

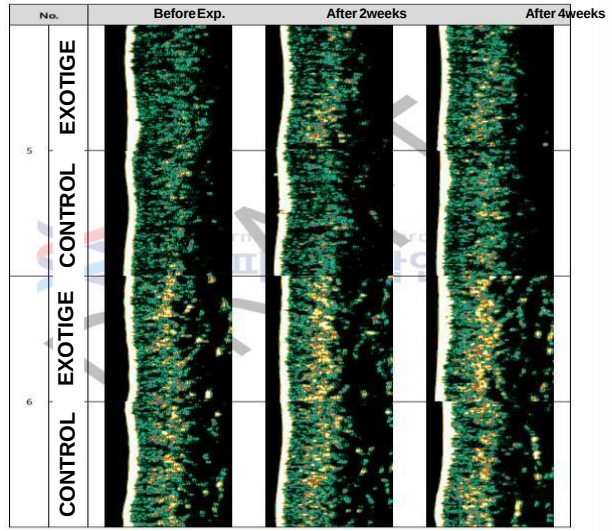




When applied to the skin for 4 weeks, the depth of wrinkles was reduced by 7.91%

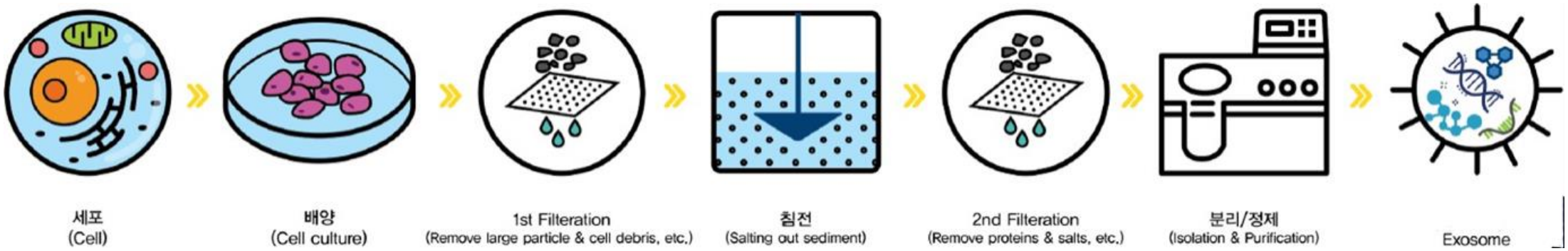
Improvement of skin density





When applied to the skin for 4 weeks, it is confirmed that the skin density increases by 32.04%

How to create exosomes.



Important tests before release

* Appearance: Confirmed visually.

* Identity Test: Qualitative analysis using liquid chromatography.

* Purity Test: Test for the absence of nucleic acids from the origin cells and confirmation of the absence of Bovine Serum Albumin (BSA) in the culture medium.

* Content Test: Test the total protein content according to the pharmaceutical standards and testing methods using Bradford's Method.

* Bacteria/Fungi/Mycoplasma/Virus Testing: Bacteria/Fungi/Mycoplasma negative test - confirmed by negative results without specified criteria. Virus testing is conducted by a contracted organization for verification.

- Deviation Handling: In case of inappropriate test results, the cause is investigated, and corrective actions are taken.
- In Vivo & In Vitro Test : This combined approach enhances the research process, optimizing both scientific outcomes and ethical considerations.
- NTA analysis: Measurement for the particle numbers of exosomes.
- Biomarker analysis (Western blot) : To see the amount of exosomes by the volume of tetraspanin protein CD9 & CD63

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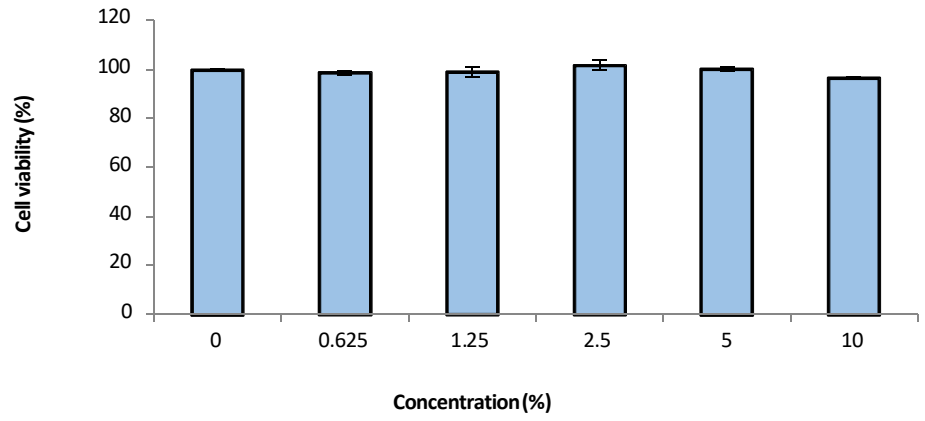
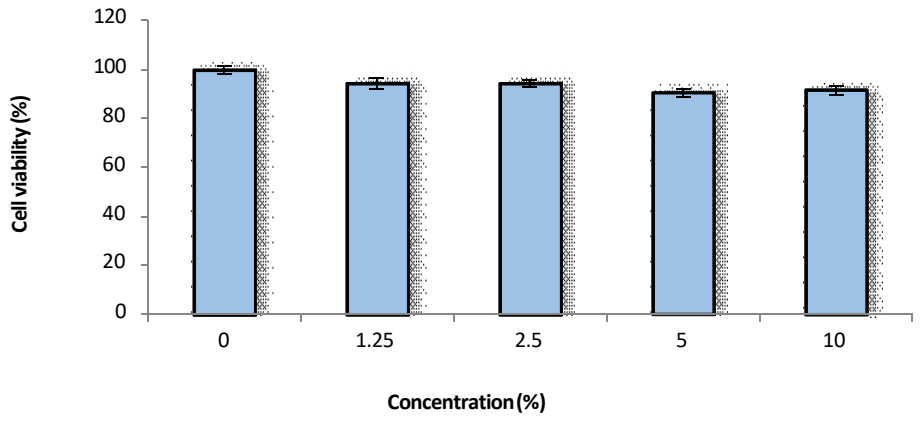
Efficacy in vivo

◎ Cell viability (Macrophage)



⇒ No cytotoxicity observed up to a 10% concentration.

◎ Cell viability (Fibroblast)



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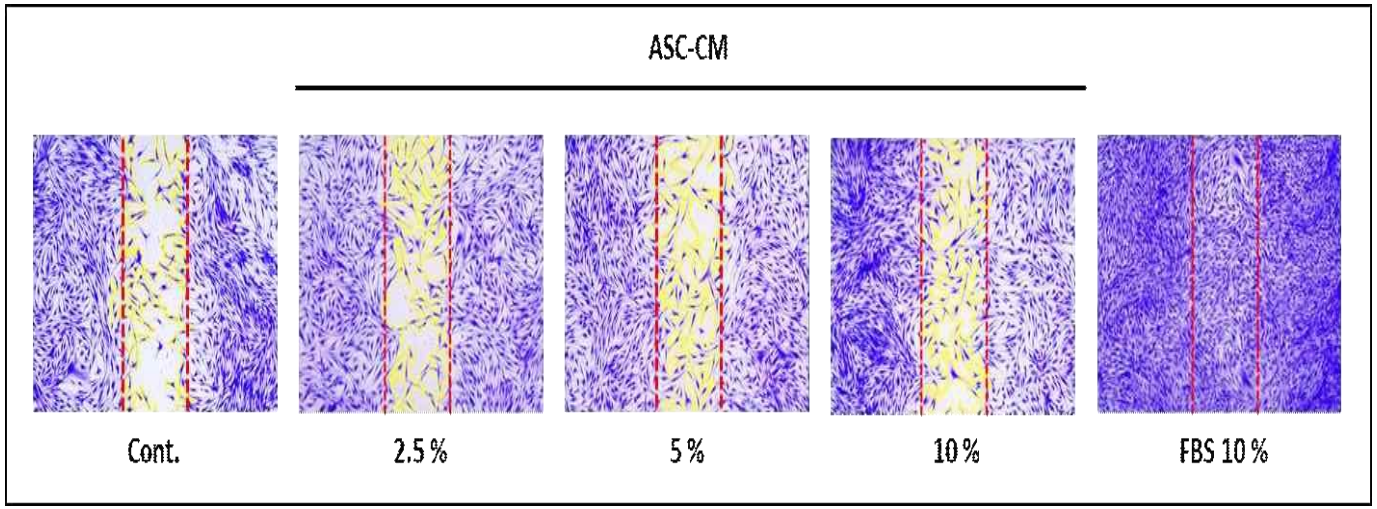
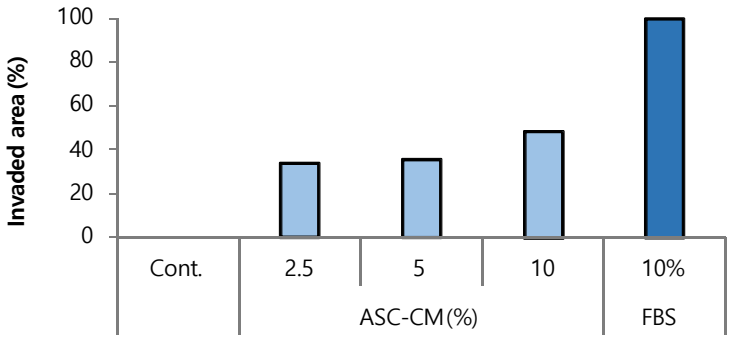
Efficacy in vivo

◎ **Wound healing assay**





⇒ **Cell gap reduced by 48.75% when processed with a 10%concentration.**

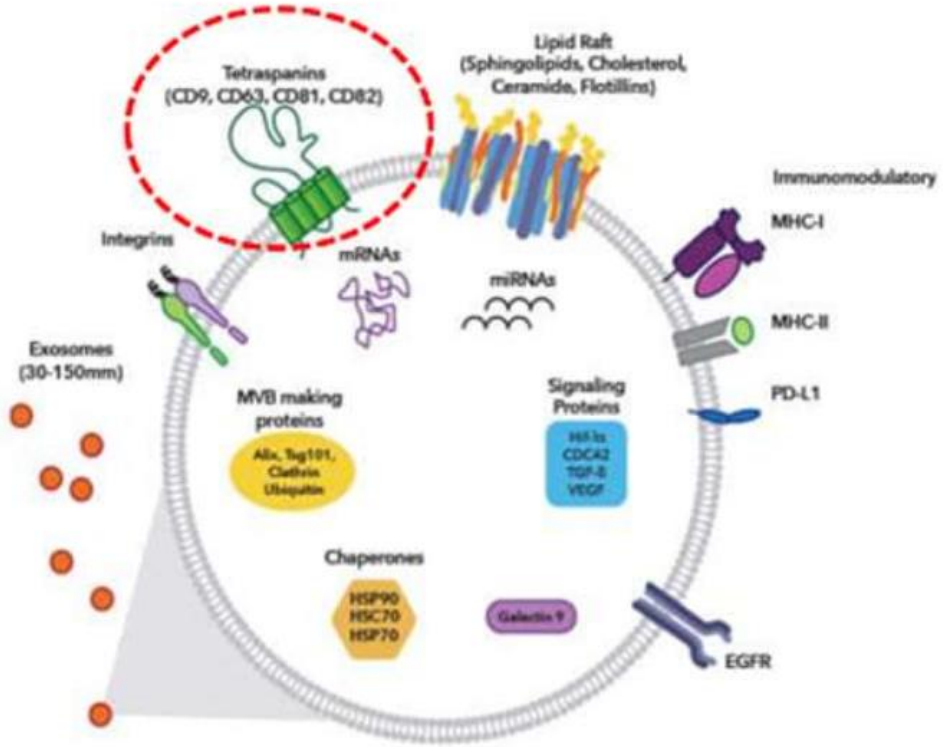
⇒ **Expected to be effective in healing wounds.**

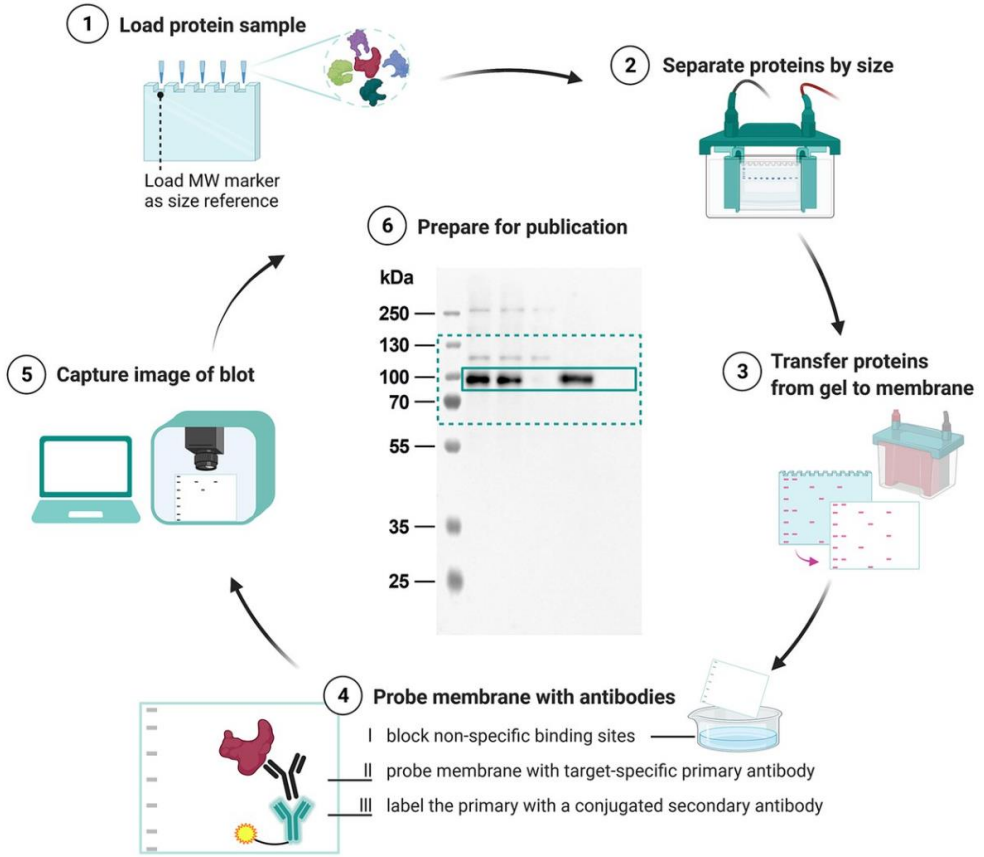
Biomarker analysis(Western Blot)

❖**Tetraspanin proteins : General Exosome Markers (CD9, CD63)**

-Associate with adhesion molecules, transmembrane receptors and intracellular signaling proteins to facilitate and regulate signaling events.

-Have four membrane spanning regions and are localized to tetraspanin enriched microdomains(TEM) in the plasma membrane.





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Biomarker analysis(Western Blot)

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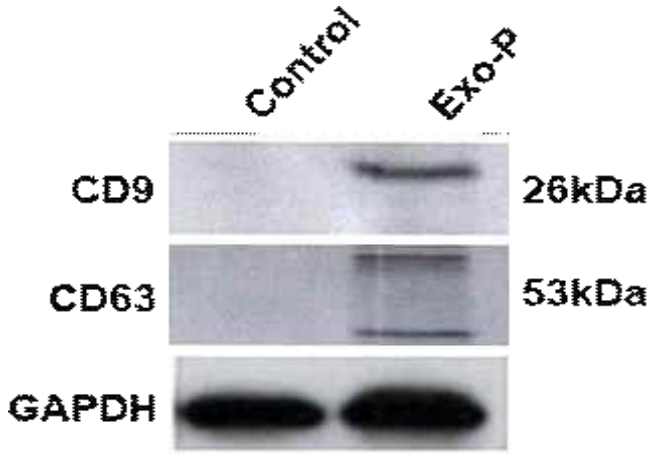
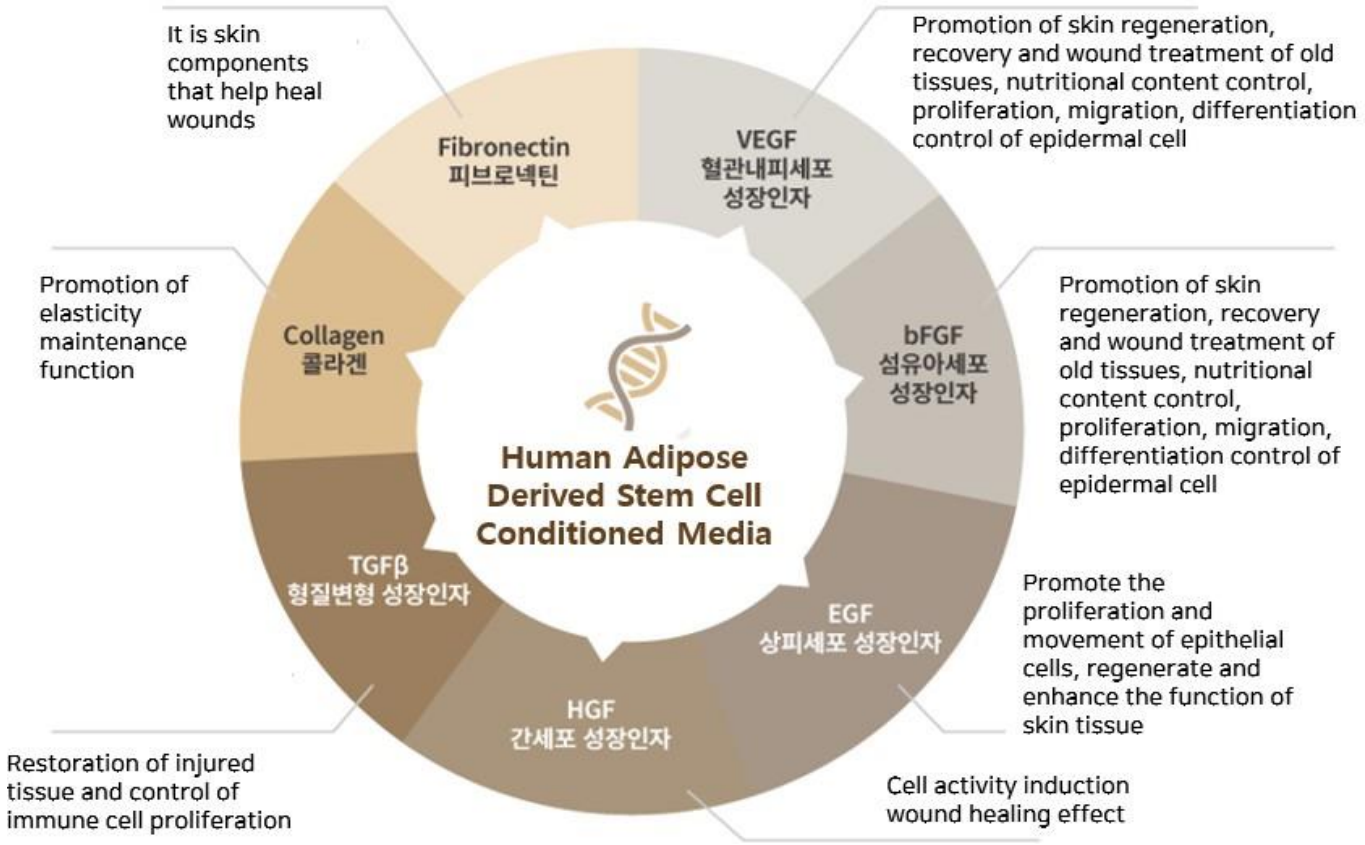


Control: Adipose-derived stem cells conditioned media

Exo-P: Duoexoti-Powder

What's in our 250 growth factor?



Contains around 250 growth factors
(Promotes cell division or growth and differentiation)

- ◎ HGF Hepatocellular Growth Factor : Efficacy of cell activity induction and surgical wound treatment
- ◎ VEGF Vascular Endothelial cell Growth Factor : Cell proliferation and angiogenesis promotion
- ◎ α-EGF Epithelial cell Growth Factor : Promotes epithelial cell proliferation and promotes the production of collagen, hyaluronic acid and SOD in fibroblasts
- ◎ β-FGF Fibroblast Growth Factor : Skin dermal regeneration factor which is effective in wound treatment
- ◎ EGF Epidermal cell Growth Factor : Promotes cell turnover by expressing epidermal cell growth signals
- ◎ KGF Keratinocyte Growth Factor : Promoting the growth of keratinocytes

- ◎ IGF-1 Insulin-like Growth Factor : Contains Exosome of regeneration and tissue growth promotion
- ◎ Exosome only exist 0.1% ratio in stem cell conditioned media
- ◎ Exosome is nano vesicles of 50~200nm size, containing various genetic information substance in cells

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* Composition of growth factors 파일 참고*

Classification	Factor name	Exosome (pg/Am	Function
Immune system (Proinflammatory / Anti-inflammatory)	CD40Ligand	65.7	CD40Ligand is a critical growth factor that plays Immune Response Enhancement, Regulation of Inflammatory Response, Tissue Regeneration Promotion.
	Eotaxin	94.8	Eotaxin is a critical growth factor involved in the immune system, particularly in allergic and parasitic infection responses.
	Flt-3Ligand	79.3	Flt-3 ligand (FL) is a critical growth factor that plays a pivotal role in hematopoiesis, the process of blood cell production. It exerts its effects by binding to its specific receptor, FLT3, expressed on the surface of hematopoietic progenitor cells.
	GranzymeB	0.91	Granzyme B (GrB) is a serine protease enzyme that plays a crucial role in the immune system, particularly in the cytotoxic response mediated by cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. GrB is stored in the cytotoxic granules of these effector cells and is released upon their activation to eliminate infected or abnormal cells.
	IFN-alpha2	3.7	IFN-alpha2 is a type of interferon that plays a crucial role in the immune system. It is used to treat various diseases, including viral infections, cancer, and autoimmune disorders.
	IFN-gamma	0.6	IFN-gamma is a type of interferon that plays a crucial role in the immune system. It is used to treat various diseases, including viral and bacterial infections, cancer, and autoimmune disorders.
	IL-1alpha	2.36	IL-1alpha is a pro-inflammatory cytokine that plays a crucial role in the immune system. It is involved in various inflammatory responses, autoimmune diseases, and cancer.
	IL-10	8.5	IL-10 is a critical anti-inflammatory cytokine that plays a pivotal role in the immune system. It modulates inflammatory responses, suppresses immune cell activation, and contributes to the prevention of autoimmune diseases.
	IL-12p70	17.1	IL-12P70 plays a pivotal role in the immune system, particularly in bridging innate and adaptive immunity
	IL-13	124.2	IL-13 is a pleiotropic cytokine that plays a critical role in various immune and inflammatory processes. It is primarily produced by activated Th2 cells and has a wide range of effects on immune cells
	IL-15	13.6	IL-15 is a cytokine that plays a critical role in the development, activation, and survival of various immune cells, particularly natural killer (NK) cells, cytotoxic T lymphocytes (CTLs), and memory T cells.
	IL-17A	0.6	IL-17A is a pro-inflammatory cytokine that plays a critical role in host defense against extracellular pathogens, particularly bacteria and fungi
	IL-1ra	144.2	IL-1ra plays a critical role in regulating the inflammatory response and preventing excessive inflammation.
	IL-2	6.6	IL-2 plays a pivotal role in the immune system, particularly in regulating the growth and activation of T cells, which are essential for cell-mediated immunity.
	IL-33	1.2	IL-33 plays a crucial role in initiating and amplifying immune responses,particularly in allergic inflammation and tissue repair.
	IL-4	0.34	IL-4 plays a critical role in various immune responses, particularly in regulating humoral immunity and allergic inflammation.
	IL-9	63.79	IL-9 plays a critical role in allergic inflammation and mast cell function.
	RANTES	11.51	RANTES is an immune-activating chemokine (β-chemokine) that plays a pivotal role in immune responses and inflammation regulation.
	TNF-alpha	3.45	TNF-alpha, also known as tumor necrosis factor-alpha, is a pivotal cytokine that plays a central role in inflammation and immune responses.
	TNF-beta	0.9	TNF-beta plays Activating Immune Cells, Inducing Cell Death, Regulating Cell Adhesion.
	TRAIL	39.9	TRAIL is a type II transmembrane protein belonging to the TNF superfamily. It plays a critical role in regulating cell death, particularly apoptosis (programmed cell death), and immune responses.
	G-CSF	7.67	G-CSF plays a pivotal role in granulopoiesis,the production of granulocytes,a type of white blood cell.
	IL-8	201.0	IL-8 plays a pivotal role in inflammation and neutrophil recruitment.
	IP-10	2.0	IP-10 plays Directing Immune Cell Migration, Modulating Immune Responses.
Apoptosis	MIP-1alpha	98.6	MIP-1alpha enhances white blood cells to improve their phagocytosis and microbial killing ability, enabling them to effectively eliminate pathogens.
	MIP-3alpha	1.3	MIP-3alpha&MIP-3beta play directing Immune Cell Migration to Inflammatory Sites, Activating Immune Cells and Enhancing Their Effector Functions, Stimulating the growth of new blood vessels, a process called angiogenesis, which is essential for supplying oxygen and nutrients to inflamed tissues.
	MIP-3beta	1.3	
	PD-L1/B7-H1	4.1	PD-L1/B7-H1 plays Inhibiting T Cell Activation. This downregulation of T cell activity prevents excessive immune responses that could lead to autoimmune diseases.
Proliferation and Regeneration (Woundhealing / Hairgrowth)	MCP-1	2,695.5	MCP-1 plays Directing Monocyte Migration to Inflammatory Sites, Activating Monocytes and Enhancing Their Effector Functions.
	PDGF-AA	107.1	PDGF-AA&PDGF-AB/BB play promoting cell proliferation, migration, and differentiation, guiding the formation of complex tissue structures, Attracting and activating fibroblasts, key cells responsible for producing collagen and other extracellular matrix components, essential for tissue repair, Vascular Remodeling.
	PDGF-AB/BB	2.2	
	VEGF	4,466.7	Crucial for the development of the vascular system, ensuring adequate oxygen and nutrient supply to growing tissues and organs.
	MMP-1	6,018.1	MMP-1&MMP-3&MMP-9 play a pivotal role in extracellular matrix (ECM) remodeling and tissue repair, facilitating the formation of new blood vessels by degrading ECM components that restrict blood vessel growth, involving in the inflammatory response by breaking down ECM components that release inflammatory mediators.
	MMP-3	3,078.9	
	MMP-9	5.2	
	Procollagen I alpha1	4,594,536.2	See separate note
	FGFbasic(FGF2)	117.7	Fibroblast is a potent mitogen and morphogen that plays a critical role in various cellular processes, including cell growth, proliferation, differentiation, and migration.
	GM-CSF	1.1	GM-CSF plays a pivotal role in the development, activation, and function of granulocytes and macrophages.
	GROalpha	509.4	GROalpha&GRObeta play a critical role in the recruitment and activation of neutrophils, the first line of defense against microbial infections and tissue injury.
	GRObeta	9.24	
Whitening	IL-6	7,689.7	IL-6 plays a pivotal role in inflammation, immune responses, and metabolic regulation. It is one of the most popular factors used for antibacterial and whitening purposes.
Haematopoiesis	IL-3	28.6	IL-3 plays a pivotal role in regulating immune responses and hematopoiesis, the process of blood cell formation.
	IL-7	0.8	IL-7 plays a pivotal role in the development,survival,and homeostasis of various immune cells,particularly lymphocytes.

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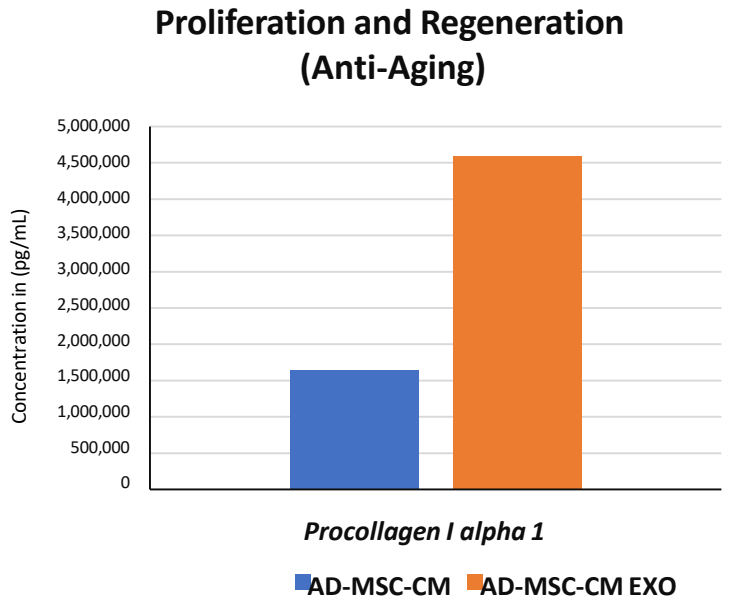
Multiplex Human Cytokine ELISA Analysis



- Overall, AD-MSC-CM showed high expression of most cytokines.
- Remarkably, in the case of Procollagen 1 alpha 1, the AD-MSC-CM Exo exhibited a staggering three-fold increase in comparison to AD-MSC-CM, underscoring the significant potential of AD-MSC-CM Exo in regenerative medicine.
- It is a precursor to the protein collagen, an essential factor in connective tissues.

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