
LXC
LACTO EXO COLLA
EXOSOME (LACTO, GINSENG, CICA, CAMELLIA) + PDRN + COLLAGEN

5th Generation Exosome Skin Booster

LACTO EXO COLLA

EXOSOME (LACTO, GINSENG, CICA, CAMELLIA) + PDRN + COLLAGEN

4 EXOSOMES

Lactobacillus vesicles
Panax Ginseng Callus
Centella Asiatica Callus
Camellia Japonica Callus

PDRN

Made by French
From Wild Alaskan salmon
High-Purity PDRN
30,000 ppm

Collagen & Peptides

Atelocollagen
Hydrolyzed Collagen
8 Amino Acid Peptides
Glutathione

5th Generation Exosome Skin Booster

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5th Generational Exosome Skin Booster

PRODUCT NAME | LACTO EXO COLLA

EXOSOME(Lacto, Ginseng, Cica, Camellia) + PDRN + Collagen + Peptides

EXOSOME(Lacto, Ginseng, Cica, Camellia)

- Lactobacillus Extracellular Vesicles
- Panax Ginseng Callus Extracellular Vesicles
- Centella Asiatica Callus Extracellular Vesicles
- Camellia Japonica Callus Extracellular Vesicles

PDRN

- Made by a French manufacturer derived from wild Alaskan salmon.
- Authorized by European Health Authorities (USA and Canada).

Collagen & Peptides

- Atelocollagen, Hydrolyzed Collagen
- 8 Amino Acid Peptides

PACKAGE

No. 1 EXOSOME, Freeze-Dried Ampoule : 100mg x 5vials
No. 2 PDRN + Collagen + Peptides, Solvent Ampoule : 6ml x 5vials

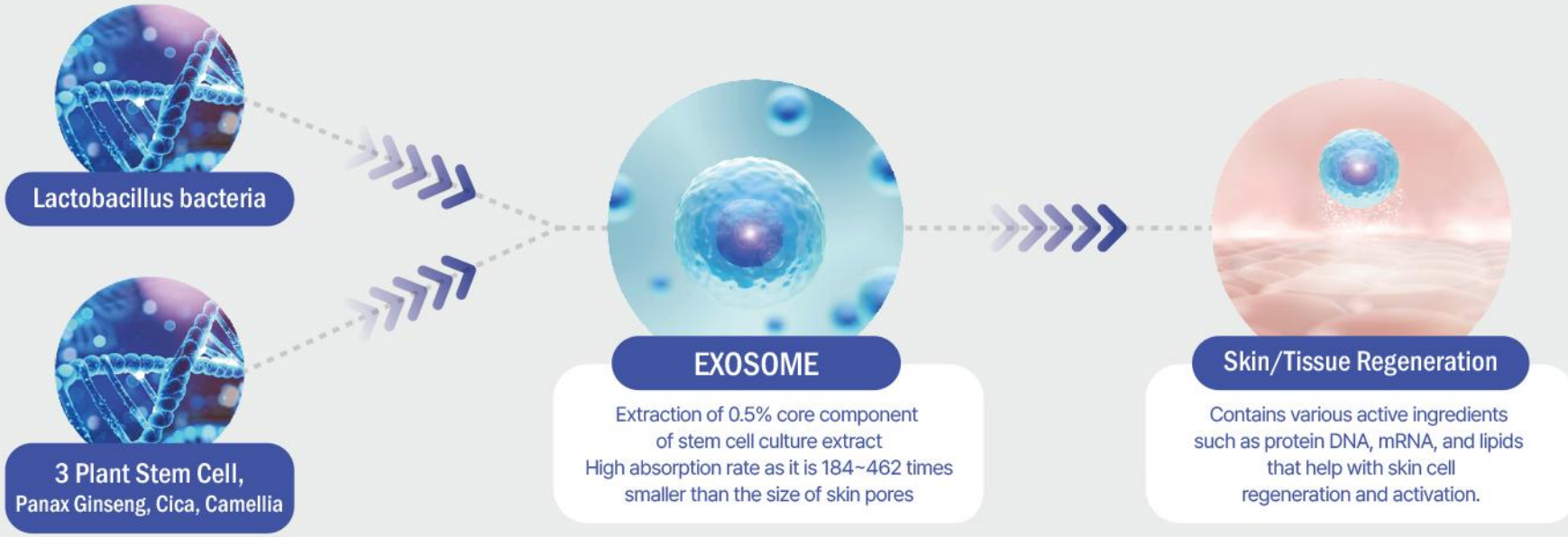
5th Generation Exosome Skin Booster

LACTO EXO COLLA

EXOSOME (LACTO, GINSENG, CICA, CAMELLIA)
PDRN + COLLAGEN

EXOSOME

LACTO EXO COLLA is a product that extracts exosomes, the core active ingredient, from Skin-beneficial Lactobacillus bacteria and 3 plant stem cell cultures and uses technology to increase the absorption of active ingredients into the skin through exosome nanoparticles. Also contains highly purified PDRN and 8 peptides.



LACTO EXO COLLA

EXOSOME (LACTO, GINSENG, CICA, CAMELLIA)
PDRN + COLLAGEN

4 EXOSOMES + PDRN + Collagen & Peptides

4 EXOSOMES

Technology extracts exosomes, a key active ingredient, from plant stem cell (callus) culture medium, from Skin beneficial Lactobacillus bacteria and exosome nanoparticles increase the absorption rate of the active ingredient.
Ultra-Nano Particles of about 14 billion/ml of purified High-Purity Exosomes alone.
(Lactobacillus : 1.1 billion, Ginseng : 11 billion, CICA : 1.1 billion, Camellia : 1.0 billion)
High-purity exosomes are directly isolated and purified.

Lactobacillus Exosome

Panax Ginseng Exosome

Cica Exosome

Camellia Japonica Exosome

4 EXOSOMES + PDRN + Collagen & Peptides

4 EXOSOMES

Purified high-purity exosomes isolated from only the core active ingredients Plant Stem Cell (callus) culture medium

Lactobacillus Exosome

A strong skin regeneration effect demonstrated by an increase in the number of split cells when **Special Lactobacillus Beneficial Bacteria cultured** from found only on the skin of people in their 20s were treated exosomes and placed into aging skin.

Panax Ginseng Exosome

Ginsenoside(Saponin) helps regenerate aged skin cells, promotes the synthesis of hyaluronic acid, a natural moisturizing factor in the dermal layer of the skin, provides skin elasticity (improves wrinkles), and prevents skin aging.

Cica Exosome

Phytosterol, Glycoside Tannin ingredients promote skin cell regeneration, antibacterial skin inflammation and wound healing action
Madecassic Acid, Asiaticoside ingredients help skin collagen synthesis, giving skin elasticity and preventing aging.

Camellia Japonica Exosome

Rich in **flavonoid, polyphenol, catechin, methionine**, etc. and have excellent antioxidant properties, which help protect the skin from irritation inside and outside the skin. They also have moisturizing effects, inhibiting inflammation, shrinking pores, and inhibiting excessive sebum secretion.

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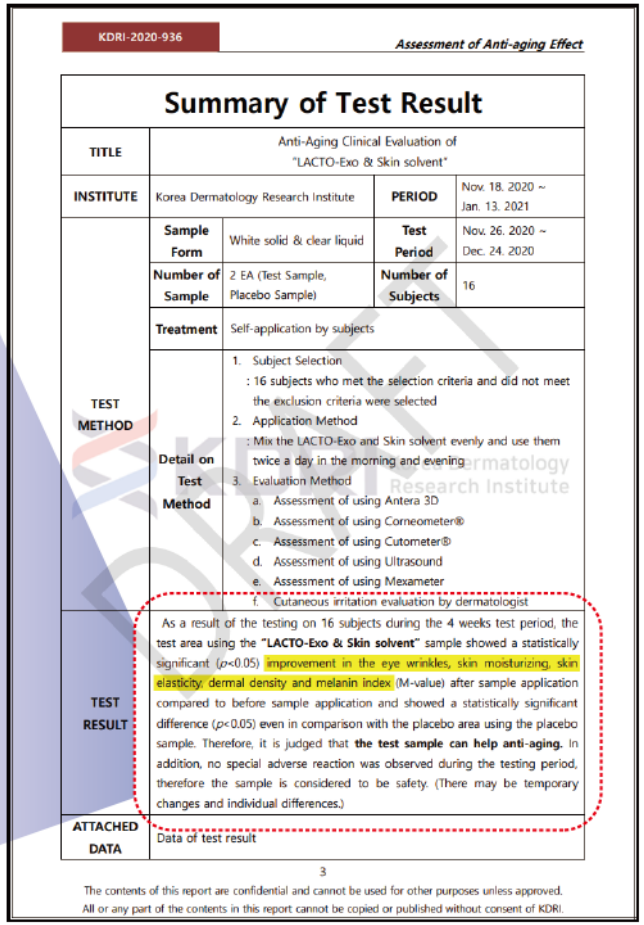
Clinic Report on Efficacy

As a result of the testing on 16subjects during the 4weeks test period, the test area using the "LACTO-Exo& Skin solvent" sample showed a statistically significant (p<0.05) **improvement in the eye wrinkles, skin moisturizing, skin elasticity, dermal density and melanin index** (M-value) after sample application compared to before sample applicationand showed a statistically significant difference (p<0.05) even in comparison with the placebo area using the placebo sample



KDRI-2020-936 *Assessment of Anti-aging Effect*

Summary of Test Result

TITLE	Anti-Aging Clinical Evaluation of "LACTO-Exo & Skin solvent"			
INSTITUTE	Korea Dermatology Research Institute		PERIOD	Nov. 18. 2020 ~ Jan. 13. 2021
TEST METHOD	Sample Form	White solid & clear liquid	Test Period	Nov. 26. 2020 ~ Dec. 24. 2020
	Number of Sample	2 EA (Test Sample, Placebo Sample)	Number of Subjects	16
	Treatment	Self-application by subjects		
	Detail on Test Method	1. Subject Selection : 16 subjects who met the selection criteria and did not meet the exclusion criteria were selected 2. Application Method : Mix the LACTO-Exo and Skin solvent evenly and use them twice a day in the morning and evening 3. Evaluation Method a. Assessment of using Antera 3D b. Assessment of using Corneometer® c. Assessment of using Cutometer® d. Assessment of using Ultrasound e. Assessment of using Mexameter f. Cutaneous irritation evaluation by dermatologist			
TEST RESULT	As a result of the testing on 16 subjects during the 4 weeks test period, the test area using the **"LACTO-Exo & Skin solvent"** sample showed a statistically significant (p<0.05) improvement in the eye wrinkles, skin moisturizing, skin elasticity, dermal density and melanin index (M-value) after sample application compared to before sample application and showed a statistically significant difference (p<0.05) even in comparison with the placebo area using the placebo sample. Therefore, it is judged that **the test sample can help anti-aging.** In addition, no special adverse reaction was observed during the testing period, therefore the sample is considered to be safety. (There may be temporary changes and individual differences.)			
ATTACHED DATA	Data of test result			

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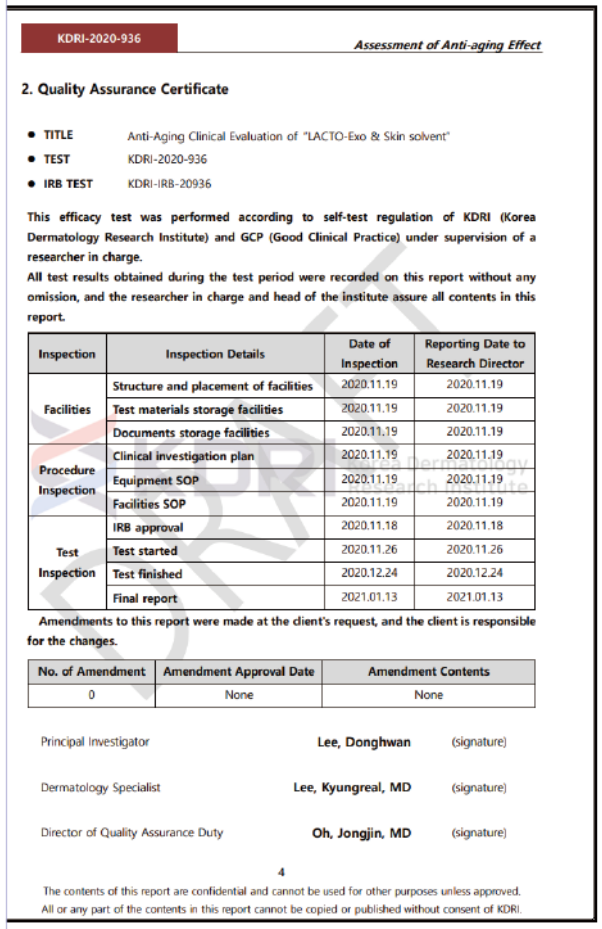

KDRI-2020-936 *Assessment of Anti-aging Effect*

2. Quality Assurance Certificate

- **TITLE** Anti-Aging Clinical Evaluation of "LACTO-Exo & Skin solvent"
- **TEST** KDRI-2020-936
- **IRB TEST** KDRI-IRB-20936

This efficacy test was performed according to self-test regulation of KDRI (Korea Dermatology Research Institute) and GCP (Good Clinical Practice) under supervision of a researcher in charge.
All test results obtained during the test period were recorded on this report without any omission, and the researcher in charge and head of the institute assure all contents in this report.

Inspection	Inspection Details	Date of Inspection	Reporting Date to Research Director
Facilities	Structure and placement of facilities	2020.11.19	2020.11.19
	Test materials storage facilities	2020.11.19	2020.11.19
	Documents storage facilities	2020.11.19	2020.11.19
Procedure Inspection	Clinical investigation plan	2020.11.19	2020.11.19
	Equipment SOP	2020.11.19	2020.11.19
	Facilities SOP	2020.11.19	2020.11.19
Test Inspection	IRB approval	2020.11.18	2020.11.18
	Test started	2020.11.26	2020.11.26
	Test finished	2020.12.24	2020.12.24
	Final report	2021.01.13	2021.01.13

Amendments to this report were made at the client's request, and the client is responsible for the changes.

No. of Amendment	Amendment Approval Date	Amendment Contents
0	None	None

Principal Investigator **Lee, Donghwan** (signature)

Dermatology Specialist **Lee, Kyungreal, MD** (signature)

Director of Quality Assurance Duty **Oh, Jongjin, MD** (signature)

4

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Thesis Papers on Clinical

current issues in molecular biology

MDPI

Article

The Effect of *Lactobacillus plantarum* Extracellular Vesicles from Korean Women in Their 20s on Skin Aging

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† These authors contributed equally to this work.

Abstract: Extracellular vesicles, which are highly conserved in most cells, contain biologically active substances. The vesicles and substances interact with cells and impact physiological mechanisms. The skin is the most external organ and is in direct contact with the external environment. Photoaging and skin damage are caused by extrinsic factors. The formation of wrinkles is a major indicator of skin aging and is caused by a decrease in collagen and hyaluronic acid. MMP-1 expression is also increased. Due to accruing damage, skin aging reduces the ability of the skin barrier, thereby lowering the skin's ability to contain water and increasing the amount of water loss. *L. plantarum* suppresses various harmful bacteria by secreting an antimicrobial substance. *L. plantarum* is also found in the skin, and research on the interactions between the bacteria and the skin is in progress. Although several studies have investigated *L. plantarum*, there are only a limited number of studies on extracellular vesicles (EV) derived from *L. plantarum*, especially in relation to skin aging. Herein, we isolated EVs that were secreted from *L. plantarum* of women in their 20s (*Lp*EVs). We then investigated the effect of *Lp*EVs on skin aging in CCD986sk. We showed that *Lp*EVs modulated the mRNA expression of ECM related genes in vitro. Furthermore, *Lp*EVs suppressed wrinkle formation and pigmentation in clinical trials. These results demonstrated that *Lp*EVs have a great effect on skin aging by regulating ECM related genes. In addition, our study offers important evidence on the depigmentation effect of *Lp*EVs.

Keywords: extracellular vesicles (EVs); exosome; skin aging; *Lactobacillus plantarum*; pigmentation

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Citation: Jo, C.S.; Myung, C.H.; Yoon, Y.C.; Ahn, B.H.; Min, J.W.; Seo, W.S.; Lee, D.H.; Kang, H.C.; Heo, Y.H.; Choi, H.; et al. The Effect of *Lactobacillus plantarum* Extracellular Vesicles from Korean Women in Their 20s on Skin Aging. *Curr. Issues Mol. Biol.* **2022**, *44*, 526–540. https://doi.org/10.3390/cimb44020036

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1. Introduction

Extracellular vesicles (EVs) are highly conserved lipid-membrane-enclosed vesicles found in most cells, including prokaryotes, eukaryotes, and archaea [1,2]. EVs contain a variety of biologically active substances such as proteins, lipids, nucleic acids, and metabolites. They reflect the state of the cells from the originating molecules, and communicate with neighboring or distant cells [3]. EVs include exosomes and micro-vesicles [4]. Exosomes are 30–200 nm vesicles secreted from multi-vesicular endosomes (MVEs), which are endosomes that make up numerous vesicles and undergo fusion with the plasma membrane [5]. Micro-vesicles are 100–1000 nm in size and are produced through budding with the plasma

Consequently, the results suggest that LpEVs, which are components of the skin microbiome, can be applied as an **effective anti-aging agent to improve skin aging, and also as an effective anti-pigmentation agent.**

LpEVs(Lactobacillus plantarum Extracellular Vesicles) **suppressed wrinkle formation and pigmentation in clinical trials.** These results demonstrated that **LpEVs have a great effect on skin aging** by regulating ECMrelated genes

suggests that the microbiome may be involved in skin aging [56]. The microbiome has direct contact with the outermost skin, and also interacts with the skin cells by secreting extracellular vesicles (EVs), such as exosomes, that contain biologically active molecules [4]. Therefore, we hypothesized that differences in the microbiome between women in their 20s and 50s, on average, would be related to skin aging.

*Lp*EVs have an effect on the cell proliferation of CCD986sk dermal fibroblasts (Figure 4A). It is known that many EVs have an anti-aging effect, and can increase skin density by restoring or increasing the proliferation of fibroblasts [57]. Likewise, the data showed that the EVs of *L. plantarum* increased cell proliferation. We then investigated the *Lp*EVs in this experiment-induced processes that inhibited ECM degradation (Figure 4B,C) and increased the expression of proteins related to ECM such as collagen, filaggrin and HAS2 (Figure 5). Based on our results, we suggest that *L. plantarum* could be applied to help prevent skin aging.

We conducted clinical assessments on women that were in their 50s women, on average, and confirmed the aging index, which is caused by a decrease in skin elasticity and wrinkle formation. We found that *Lp*EVs could reduce wrinkle formation (Figure 6). The loss of moisture content in the skin arises from damage to the skin barrier [48]. Interestingly, *Lp*EVs increased the moisture content of the skin (Figure 7). However, future studies are required to assess whether the *Lp*EVs restore the skin barrier or the moisture content is increased by the ECM improvements, such as collagen and hyaluronic acid. In addition, another characteristic caused by damage to the skin barrier is an increase in the amount of water loss in the skin. Therefore, it is important to also assess the amount of moisture loss in the skin.

We also determined that *Lp*EVs suppressed pigmentation caused by aging skin for women in their 50s, on average (Figure 8). Our results showed that the *Lp*EVs can influence aging-induced pigmentation. Further studies on the depigmentation effect can further elucidate the applications for *Lp*EVs.

In this study, we demonstrated that women in their 20s had a higher population of *L. plantarum* in their skin microbiome than women in their 50s, on average. Additionally, *Lp*EVs could suppress aging factors (Figure 9). Consequently, the results suggest that *Lp*EVs, which are components of the skin microbiome, can be applied as an effective anti-aging agent to improve skin aging, and also as an effective anti-pigmentation agent.

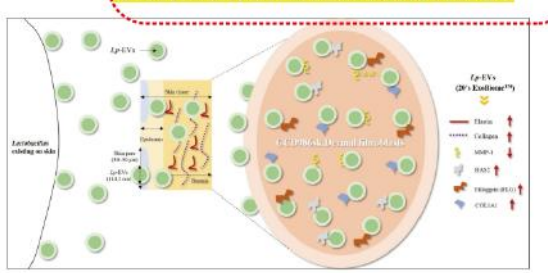

Figure 9. The anti-aging effects of extracellular vesicles derived from *Lactobacillus plantarum* isolated from the skin of women in their 20s.

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4 EXOSOMES + PDRN + Collagen & Peptides

Thesis Papers on Clinical Trials

Table 2. Subject information of clinical trials.

Subjects of Clinical Trials (IRB Number: KDRI-IRB-20936) *			
Gender	Age		Average Age
Female	40's	50's	Age 50
	n = 6	n = 10	
	Total n = 16		

*: Each data partner obtained the necessary Institutional Review Board (IRB) approval or exemption.

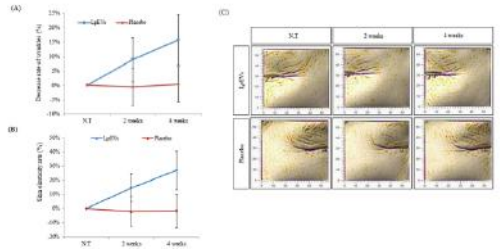

Figure 6. Evaluation of the effects of *Lp*EVs on wrinkle formation: (**A**) eye-wrinkle improvement assessments in clinical trials; (**B**) epidermidis elasticity improvement results in clinical trials; and (**C**) Antera 3D image (Wrinkle: Small) from clinical trials.

3.6. *LpEV Treatment Moisturizes Skin and Enhances Skin Density*

A major characteristic of skin aging is the change in moisture content and skin density. The moisture content of the skin decreases as the skin barrier weakens, and skin density is also reduced due to a decrease in the ECM [45,48,49]. Therefore, we tried to assess whether *Lp*EVs can affect and improve the water content and density in the skin. Unlike the placebo group, which had no significant effect, the *Lp*EVs increased water content by 10.79% at 2 weeks and 21.40% at 4 weeks (Figure 7A). We confirmed that skin density increased at the 2-week and 4-week assessments in both groups, using ultrasound (Figure 7B). Image quantification images showed an increase in skin density, but the increased rate of the density (39.30%) of the *Lp*EV group was higher than that of the placebo (15.19%) (Figure 7C). Therefore, we confirmed that *Lp*EVs suppress the reduction in skin moisture content and increase skin density.

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Therefore, these data showed that LpEVs have an effect on pigmentation caused by skin aging. Consequently, LpEVs have a great anti-aging effect.

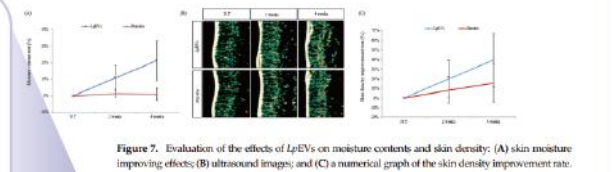


Figure 7. Evaluation of the effects of *Lp*EVs on moisture contents and skin density: (**A**) skin moisture improving effects; (**B**) ultrasound images; and (**C**) a numerical graph of the skin density improvement rate.

3.7. *LpEV Treatment Suppresses Skin Pigmentation Caused by Aging*

Another factor of skin aging is skin pigmentation [50]. We investigated whether the *Lp*EVs had a whitening effect that suppressed pigmentation caused by aging. As a result, for patients treated with *Lp*EVs, unlike the placebo, the pigmentation of the lesion site was decreased at the 2-week and 4-week assessments (Figure 8A,C). In the *Lp*EV treatment group, skin density improved by 3.87% at 2 weeks and 8.7% at 4 weeks (Figure 8B). Therefore, these data showed that *Lp*EVs have an effect on pigmentation caused by skin aging. Consequently, *Lp*EVs have a great anti-aging effect.

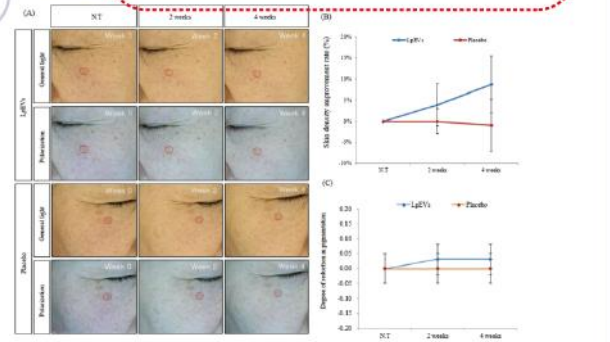


Figure 8. Evaluation of the effects of pigmentation reduction through image analysis (Mark Vu) with *Lp*EVs in clinical trials: (**A**) Mark Vu image; (**B**) the numerical graph of the skin density improvement rate of (**A**); (**C**) degree of reduction in pigmentation in visual reading.

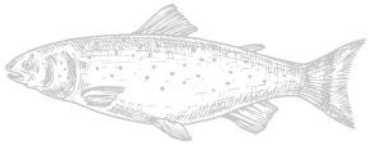
4. Discussion

Skin aging is caused by external factors such as UV rays and internal factors, which include telomere shortening [51–53]. Recently, studies on the skin microbiome have attracted considerable interest, because it has been identified as a factor that can impact skin aging [54,55]. The difference in the microbiome composition of young and aged skin

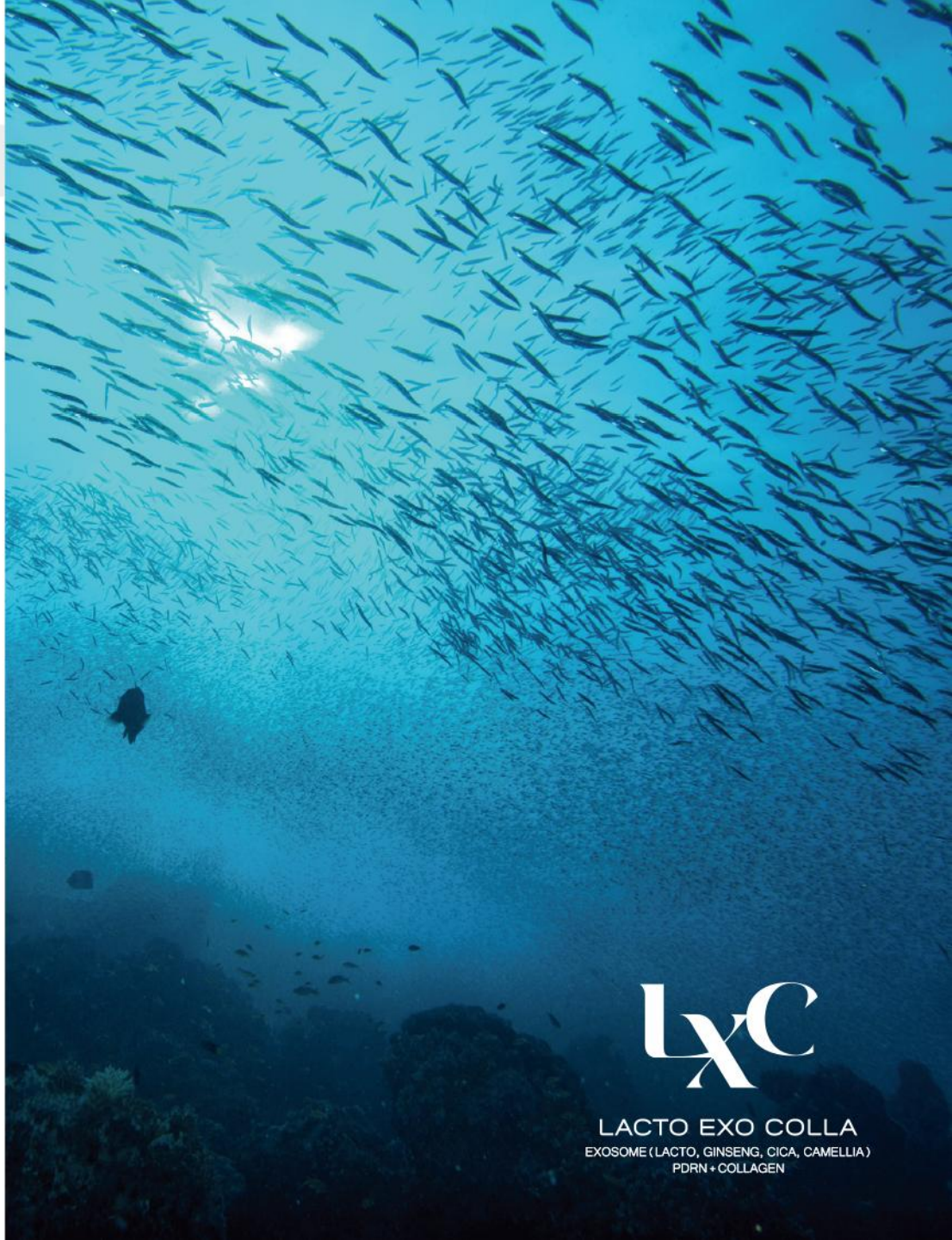
5th Generation Exosome Skin Booster

4 EXOSOMES + PDRN + Collagen & Peptides

PDRN



- High-purity PDRN 30,000 ppm
- PDRN is made by a French manufacturer derived from wild Alaskan salmon.
- PDRN is authorized by European Health Authorities (USA and Canada).
- Promotes and increases skin hydration
- OH ° free radical scavenger
- Reconstruction of damaged skin
- UV light protection
- Reinforces the antioxidant barrier of the skin



4 EXOSOMES + PDRN + Collagen & Peptides

Collagen & Peptides

EGF, FGF, IGF

Collagen & Elastin regeneration growth factors

Atelocollagen, Hydrolyzed Collagen

Promotes the synthesis of collagen and elastin,
Doubles the moisturizing effect compared to hyaluronic acid
Provide elasticity to the skin and prevent sagging

8 (Amino Acid) Peptides : made from 99% high purity powder raw materials

1. Galloyl Pentapeptide-74(SEATIDE) : Effective for Anti-wrinkle with high collagen mRNA expression
2. Nicotinoyl Tripeptide-1 : Effective powerful antioxidant, soothing and anti-inflammatory
3. Nonapeptide-1 : Helps improve bright clean skin tone by reducing multi-pigmentation
4. Acetyl Hexapeptide-8 : Effective for Anti-wrinkle by inhibiting skin muscle contraction
5. Palmitoyl Pentapeptide-4 : Increasing collagen and preventing elastin decomposition
6. Palmitoyl Tetrapeptide-7 : Increasing collagen and preventing elastin decomposition
7. Biotinoyl Tripeptide-1 : Preventing melanin production in the skin for whitening effect
8. Glycine Soja Peptide : Promote fibroblast proliferation, collagen production, wrinkle improvement

Essential Amino Acids for Skin

Glutathione

Excellent whitening effect due to melanin suppression effect
Helps delay skin aging with powerful antioxidant effect

Tranexamic Acid

Excellent in suppressing and reducing the production of melanin pigments.
Helps improve freckles by eliminating the factors that cause freckles
with anti-inflammatory/anti-allergic effects.

Adenosine

Helps to produce and promote collagen cells that prevent aging,
and also helps improve wrinkles.

Ascorbic Acid, Carnitine, Arginine ,Arbutin

Helps with whitening, anti-aging, moisturizing, and wrinkle improvement

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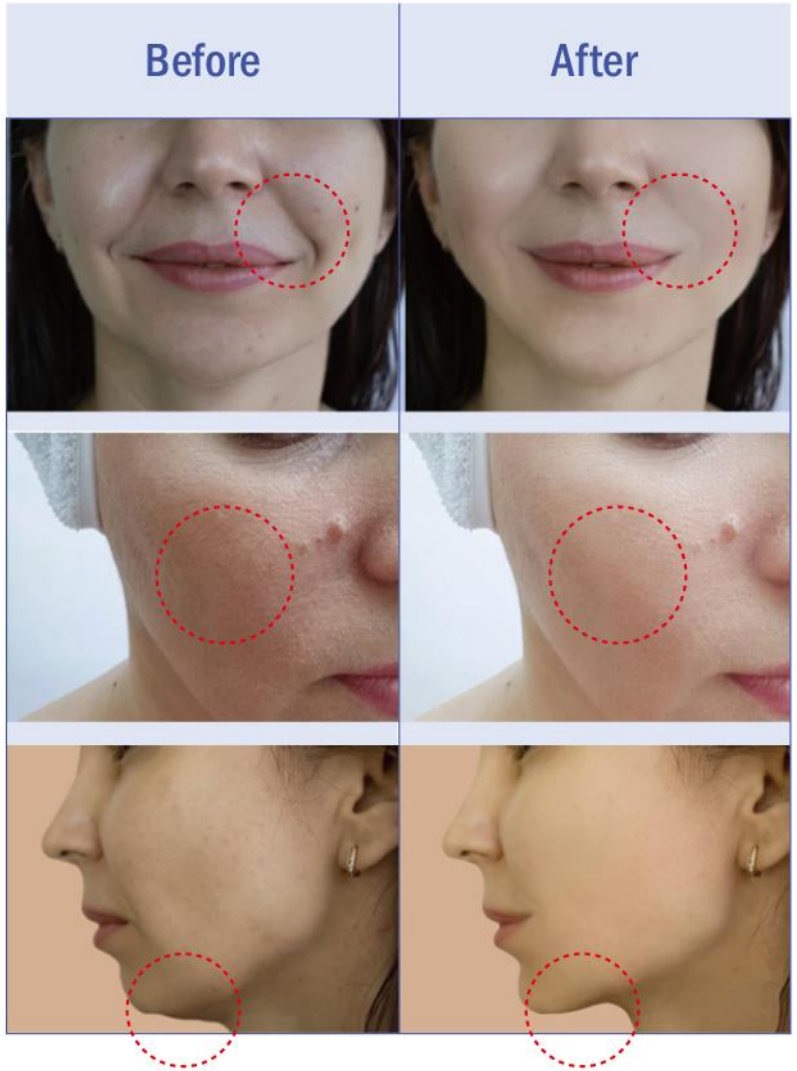
Before & After



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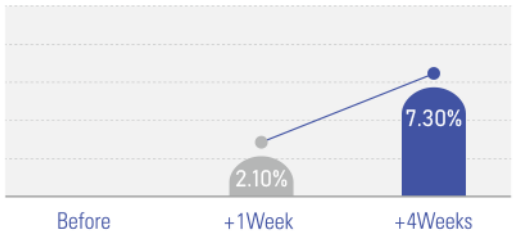
Rate of changes by testing item

Rate of changes by testing item(%)

Change rate (%)=[Analysis value after using the product-Analysis value before using the product/Analysis value before using the product] X 100

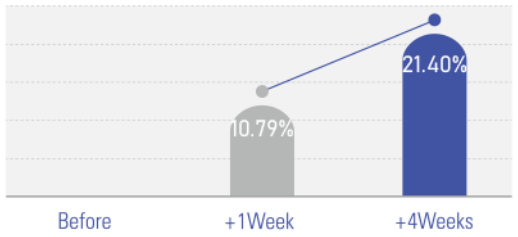
Reconstruction of damaged skin

7.30% improvement after 4 weeks



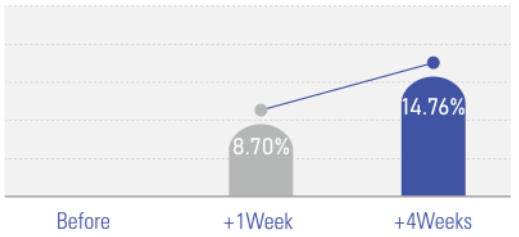
Improving skin moisturization

21.40% improvement after 4 weeks



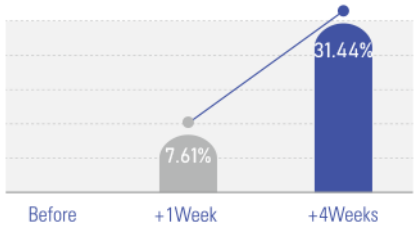
Melanin pigment improvement

14.76% improvement after 4 weeks



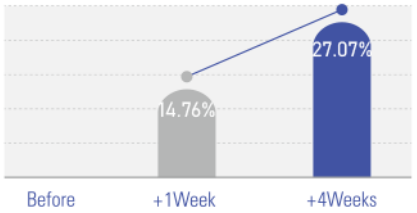
Anti-inflammation

31.44% improvement after 4 weeks



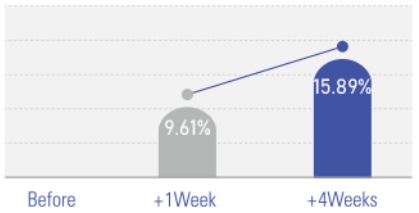
Increased skin elasticity

27.07% improvement after 4 weeks



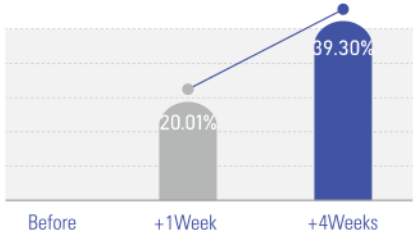
Anti-wrinkle

15.89% improvement after 4 weeks



Skin barrier density

39.30% improvement after 4 weeks



LACTO EXO COLLA
EXOSOME (LACTO, GINSENG, CICA, CAMELLIA)
PDRN + COLLAGEN

How to use

How to use Lacto Exo Colla serum

Only for topical application. Daily use at home.

1. Clean the skin.
2. Open the aluminum cap and remove the rubber stopper from both ampoules.
3. Pour the “PDRN Solvent Ampoule 2” into “EXOSOME Ampoule 1”.
4. Shake well to completely dissolve the freeze-dried exosome in Ampoule 1.
5. Apply the serum to the face and neck, allowing it to be absorbed.
6. Once absorbed, apply a moisturizing cream.

※ Rubber cones are enclosed in the box.

LACTO EXO COLLA

EXOSOME (LACTO, GINSENG, CICA, CAMELLIA)
PDRN + COLLAGEN

5th Generation Exosome Skin Booster

4 EXOSOMES + PDRN + Collagen & Peptides

LXC

LACTO EXO COLLA

EXOSOME (LACTO, GINSENG, CICA, CAMELLIA) + PDRN + COLLAGEN

5th Generation Exosome Skin Booster

4 EXOSOMES + PDRN + Collagen & Peptides

No1. Freeze-dried Ampoule

| 4 EXOSOMES |

Lactobacillus vesicles Vesicles
Panax Ginseng Callus Extracellular Vesicles
Centella Asiatica Callus Extracellular Vesicles
Camellia Japonica Callus Extracellular Vesicles

No2. Solvent Ampoule

| PDRN + Collagen & Peptides |

High-Purity PDRN
30,000 ppm
Atelocollagen, Hydrolyzed Collagen
8 Amino Acid Peptides