CONTACT: GSI Creos Corporation TEL.: 06-6944-2621 E-MAIL:mail: cosme@gsi.co.jp

Next-generation CICA ingredient

- Purified using patented technology and prepared a high concentration Exosome
- Standardized number of Exosome (10 million / ml)
- Better performance than ordinary CICA

Patented ingredient

100% plant origin

Derived from
Centella asiatica

Number of Exosome
10 million/ml

GSI Creos

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CONTENTS

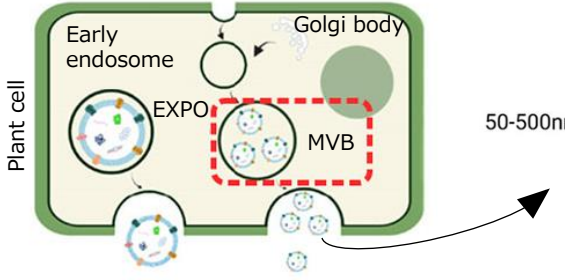
CICA EXOSOME™

What is plant exosomes?

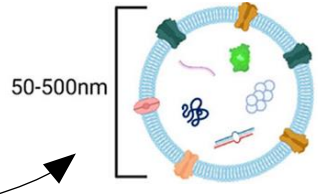
Exosomes are secreted into extracellular fluids and are found in most body fluids, including blood, urine, saliva, breast milk, and cerebrospinal fluid. A number of biologically active molecules including proteins, mRNA, miRNA, DNA and phospholipids are found in exosomes.

Plant cells also secret **PELNs : P**lant-derived **E**xosome **L**ike **N**anoparticle**s.** It has been under investigation for many years.

『Feature of plant exosomes』



Biosynthetic and secretion



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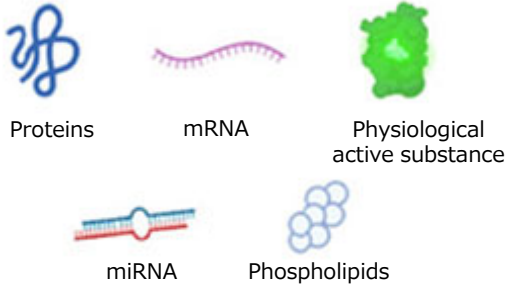


Included component

Application of plant exosomes to cosmetics

『Safety』

『Stability』

『Efficacy』

When we take in plants as food or apply plant-derived cosmetics, we are ingesting plant exosomes into our body on a daily basis. **However so far, there are no reports that irritation and toxicity are suspected to be due to plant exosomes.**

Plant exosome is made up of phospholipid bilayer that is **physically stabilized**. It is similar in structure to a human cell. So, **it has a high affinity and penetrativeness to skin.**

Plant exosome has **safety, stability and efficacy** that are required for cosmetic ingredient recently. **It can be a game changer** that is an alternative to nanocarrier such as polymer or carbon nanoparticle.

Manufacturing method of CICA EXOSOME™

CICA EXOSOME™ is purified from C. asiatica using **a patented technology.**

The technology enables **to prepare high concentration exosome without any physical damage on the structure.**

『Manufacturing process by our technology』

Selection of raw materials

Ultra-high-pressure treatment

Separation

Fraction

About Centella asiatica

Centella asiatica thatis used for CICA EXOSOME™ is **one of the most famous traditional herbs**, and used for medical care and beauty care from 5,000 years ago. It has **a reliability and experience based on long history.**

In recent years, products containing Centella asiatica ingredients under the name **CICA*** have been rapidly increasing. **It is a perfect plant as a source of plant exosomes** in terms of popularity, reliability, and effectiveness.

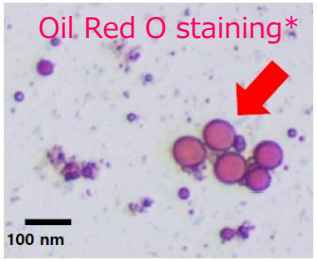
Centella asiatICA

CICA EXOSOME™

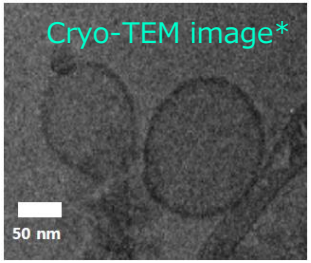
Analysis of C. asiatica exosomes

In order to investigate plant exosomes in CICA EXOSOME™, we conducted photography using two methods and nanosite analysis that allows us to measure the number of exosome particles.

As a result of the analysis, the average size of exosomes obtained from Centella asiatica is **174.6 nm**. It was found that the number of exosomes **can be adjusted in the range of 100,000 to 100 million/ml**. From the viewpoint of effectiveness, we have standardized the amount of exosomes at **10 million cells/ml**.



*Method for staining phospholipids red



*Method of irradiating electron beams under liquid nitrogen cooling

Efficacy evaluation by in-vitro test

In order to investigate the effectiveness of CICA EXOSOME™ on the skin and the optimal exosome concentration, we added this product adjusted to four different exosome concentrations to human epidermal keratinocytes and human dermal fibroblasts. We conducted a test to evaluate the anti-inflammatory, anti-wrinkle and cell renewal effect.

For comparison, commercially available Centella asiatica extract (1% to 10% concentration) and single active ingredients according to the evaluation items were used.

Test① : Evaluation of the inhibitory effect on NF-kB, a transcription factor involved in skin inflammation.　(Positive control : Allantoin 0.01%)

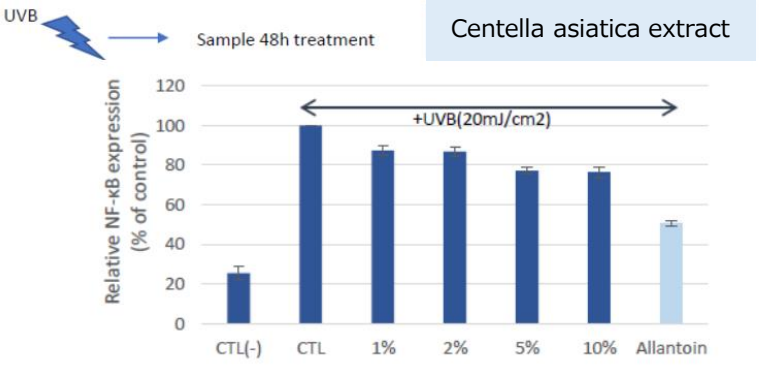
Test② : Evaluation of the influence on collagen and collagenase involved in anti-wrinkle effects.　(Positive control : TGF-β1 20nM)

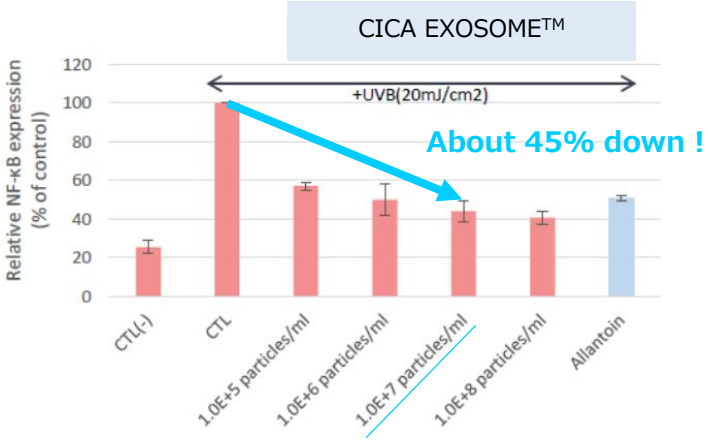
Test③ : Evaluation of cell renewal effect (wound healing effect)
(Positive control : Madecassoside 0.1%)

CICA EXOSOME™

Test① : Anti-inflammatory effect

Anti-inflammatory effect is one of the typical efficacy expected from Centella asiatica and CICA cosmetics. To investigate the anti-inflammatory effects of CICA EXOSOMETM, we evaluated its inhibitory effect on NF-kB, a transcription factor involved in skin inflammation.



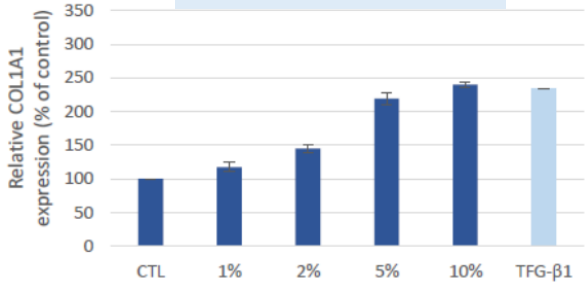


As a result of the test, CICA EXOSOME™ inhibited the expression of NF-kB by approximately 45% when the number of exosomes was 1.0E+7 (1.0 x 10 to the 7th power = 10 million)/ml. **It showed better results than allantoin and positive control, centella asiatica extract.**

Test② : Anti-wrinkle effect (Collagen synthesis)

Anti-wrinkle effect is one of the typical effects expected from Centella asiatica and CICA cosmetics. To investigate the anti-wrinkle effect of CICA EXOSOME™, we evaluated the expression level of COL1A1, a gene involved in type I collagen production.

Centella asiatica extract



CICA EXOSOME™

As a result of the test, CICA EXOSOME™ increases the expression level of genes related to collagen production by approximately 300% when the number of exosomes is 1.0E+7 (1.0 x 10 to the 7th power = 10 million)/ml.

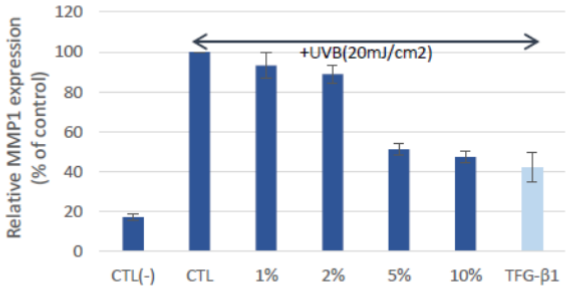
It showed better results than TGF-β1 and positive control.

Test② : Anti-wrinkle effect (Collagenase inhibition)

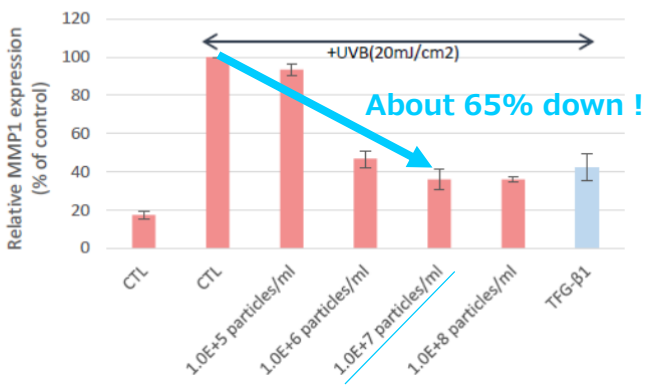
Anti-wrinkle effect is one of the typical effects expected from Centella asiatica and CICA cosmetics. To investigate the anti-wrinkle effect of CICA EXOSOMETM, we evaluated its effect on the expression level of collagenase MMP-1, which degrades collagen and is involved in wrinkle formation.

UVB → Sample 48h treatment

Centella asiatica extract



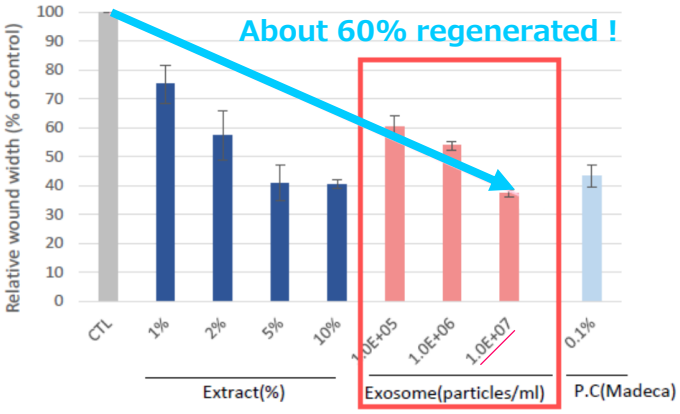
CICA EXOSOME™

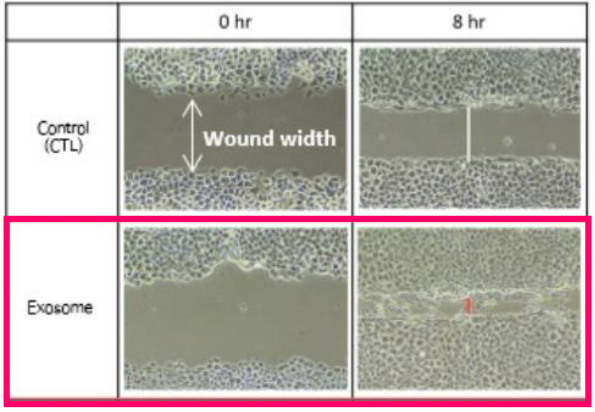


As a result of the test, CICA EXOSOMETM inhibited the expression of MMP-1 by approximately 65% when the number of exosomes was 1.0E+7 (1.0 x 10 to the 7th power = 10 million)/ml. **It showed better results than TGF-β1 and positive control, centella asiatica extract.**

Test③ : Cell renewal effect (wound healing)

Cell renewal effect is one of the typical effects expected from Centella asiatica and CICA cosmetics. In order to investigate the cell renewal effect of CICA EXOSOMETM, we performed a scratch test in which cultured human keratinocytes were scratched and we could see the repair of the scratches (width) .





As a result of the test, with CICA EXOSOMETM (exosome number 1.0E+07 = 10 million pieces/ml) approximately 60% of the width of the wound was repaired in 8 hours compared to the untreated case. **It showed almost same cell renewal effects as madecassoside and positive controls, such as Centella asiatica extract.**

Summery of in-vitro test

As a result of some tests using human epidermal keratinocytes, CICA EXOSOMETM showed excellent performance of anti-inflammatory, anti-wrinkle and cell renewal that are expected from Centella asiatica and CICA cosmetics. It was more effective than common Centella asiatica extract.

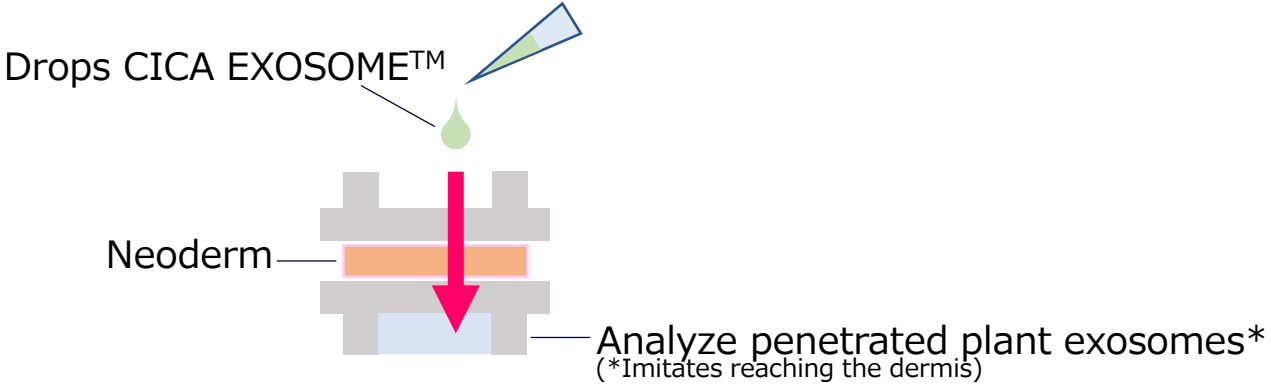
And as a result of reviewing the difference in effectiveness due to the difference in exosome concentration in CICA EXOSOME™, we found that there was no significant difference in effectiveness between exosome concentrations of 10 million/ml and 100 million/ml. Based on this result, **the product standard for CICA EXOSOME™ was set at a exosome concentration of 10 million/ml.**

- ◆Anti-inflammatory effect : **Inhibits about 45%** of the transcription factor NF-kB involved in inflammation.
- ◆Anti-wrinkle effect : **Promotes about 300%** of expression of collagen production-related gene COL1A1.

  Inhibits about 65% of the expression level of collagen degrading enzyme MMP-1.
- ◆Cell renewal effect : **Promotes about 60%** of wound healing performance in 8 hours compared to untreated cases.

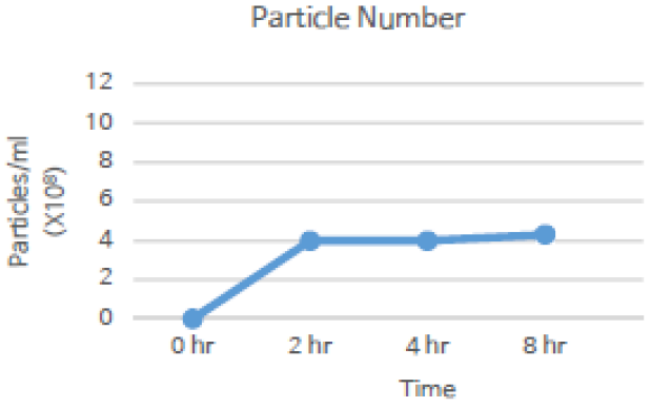
Skin penetration efficacy

Plant exosomes are composed of the same phospholipid bilayer as skin cells, so they have a high affinity for the skin, and have excellent skin penetration. In order to investigate the skin penetration of CICA EXOSOMETM, we conducted a test using a cultured skin model membrane (Neoderm) that imitates the epidermis, and measured the penetrated amount of plant exosomes using nanoparticle tracking analysis (NTA) and miRNA detection.



Evaluation for skin penetration① : Nanoparticle tracking analysis (NTA)

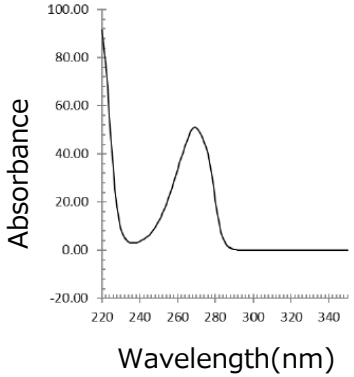
CICA EXOSOME™ was dropped onto the surface layer of an artificial skin model membrane (Neoderm), and the number of plant exosomes that penetrated the membrane was measured using nanoparticle tracking analysis (NTA) after 2, 4, and 8 hours.



As a result of the test, we found that approximately 10% of the plant exosomes in CICA EXOSOME™ penetrated the artificial skin model membrane in a short period of 2 hours.

Evaluation for skin penetration② : miRNA detection

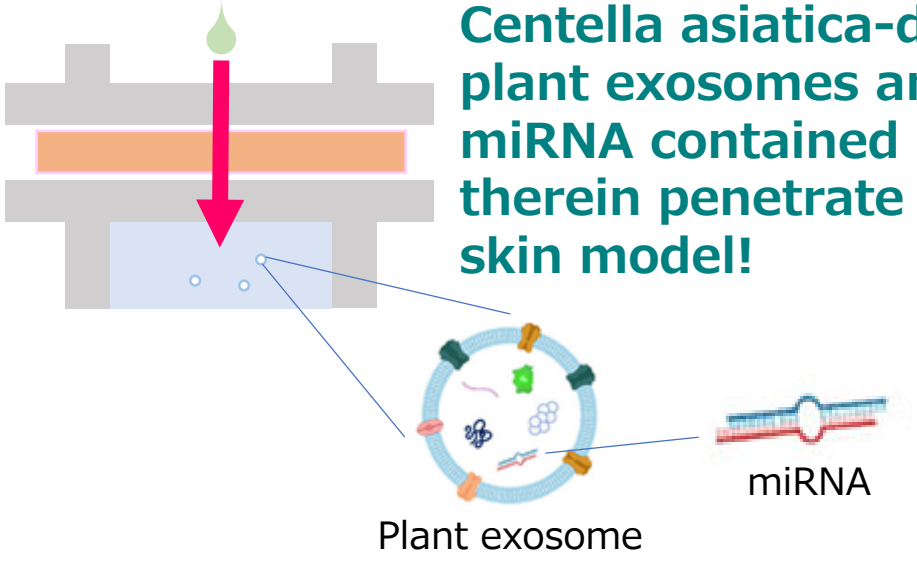
miRNA (microRNA) is one of the main components contained in plant exosomes, and it is involved in post-transcriptional expression regulation of genes in humans and plants. We dropped CICA EXOSOME™ onto the surface layer of a cultured skin model membrane (Neoderm) and investigated whether miRNA could be detected by absorbance from the solution that penetrated the membrane.



As a result of the test, miRNA, which is the main active substance of exosomes, was detected, and we found that exosomes can penetrate the skin and allow their contents to penetrate.

Summary of skin penetration

A penetration test using a cultured skin model membrane showed that the plant exosomes contained in CICA EXOSOME™ can penetrate the skin. Furthermore, as a result of miRNA detection using absorbance, we found that plant exosomes can penetrate the skin while containing miRNA.



Trademark and patent

◆Trademark

Applying for trademark in Japan

CICA EXSOSOME

◆Patent

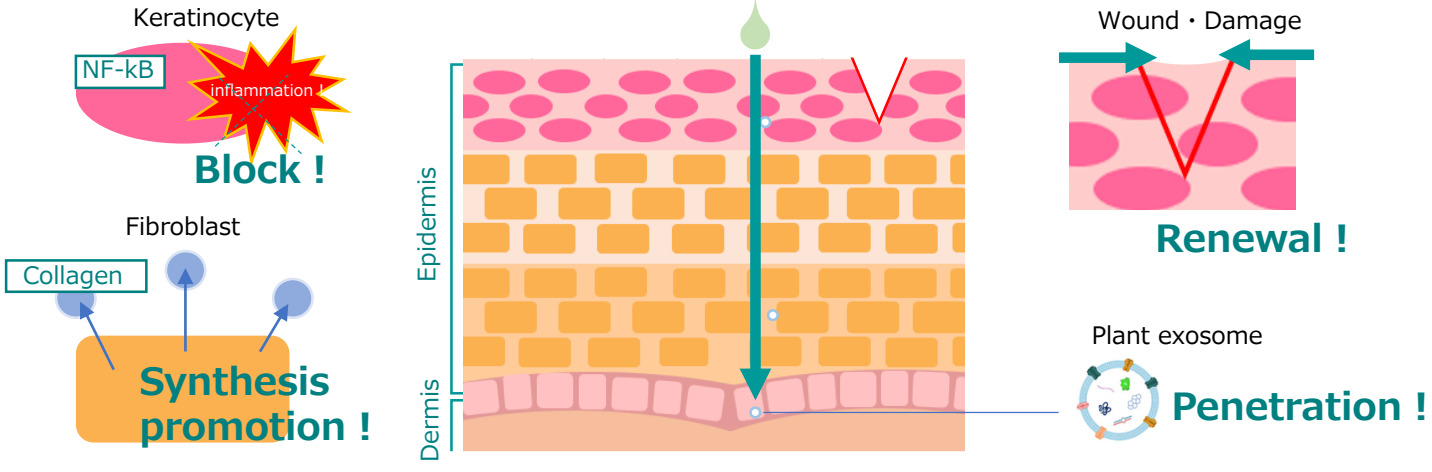
Patented in Korea

Method for isolation and purification of Centella asiatica exosome and composition containing the same

CICA EXOSOME™

Summery of CICA EXOSOME™

- ○High concentration of plant exosomes using patented technology.
- ○Standardized over 10 million plant exosomes per ml.
- ○Anti-inflammatory, collagen production, and cell renewal effects that exceed those of regular Centella asiatica extract.
- ○Excellent skin penetration.
- ○100% natural derived. Applying for trademark.



Product summary

Product name	CICA EXOSOME™
INCI	Centella Asiatica Leaf Vesicles, Water, Glycerin, Pentylene Glycol
Identification	Over 1.0x10^7 particles/mL (plant exosomes: over10 million pieces/ml)
Appearance	Colorless to light yellowish colored liquid
Solubility	Water soluble
pH	6.00～8.00
Safety data*	Cytotoxicity: negative (MTT assay) Dermal irritation: negative (Patch test)
Recommend Use level	1-10%

- Tested using another grade product with a similar composition instead of this product.
- For NMPA comply version, please contact local GSI office