CHA-EPC-CM

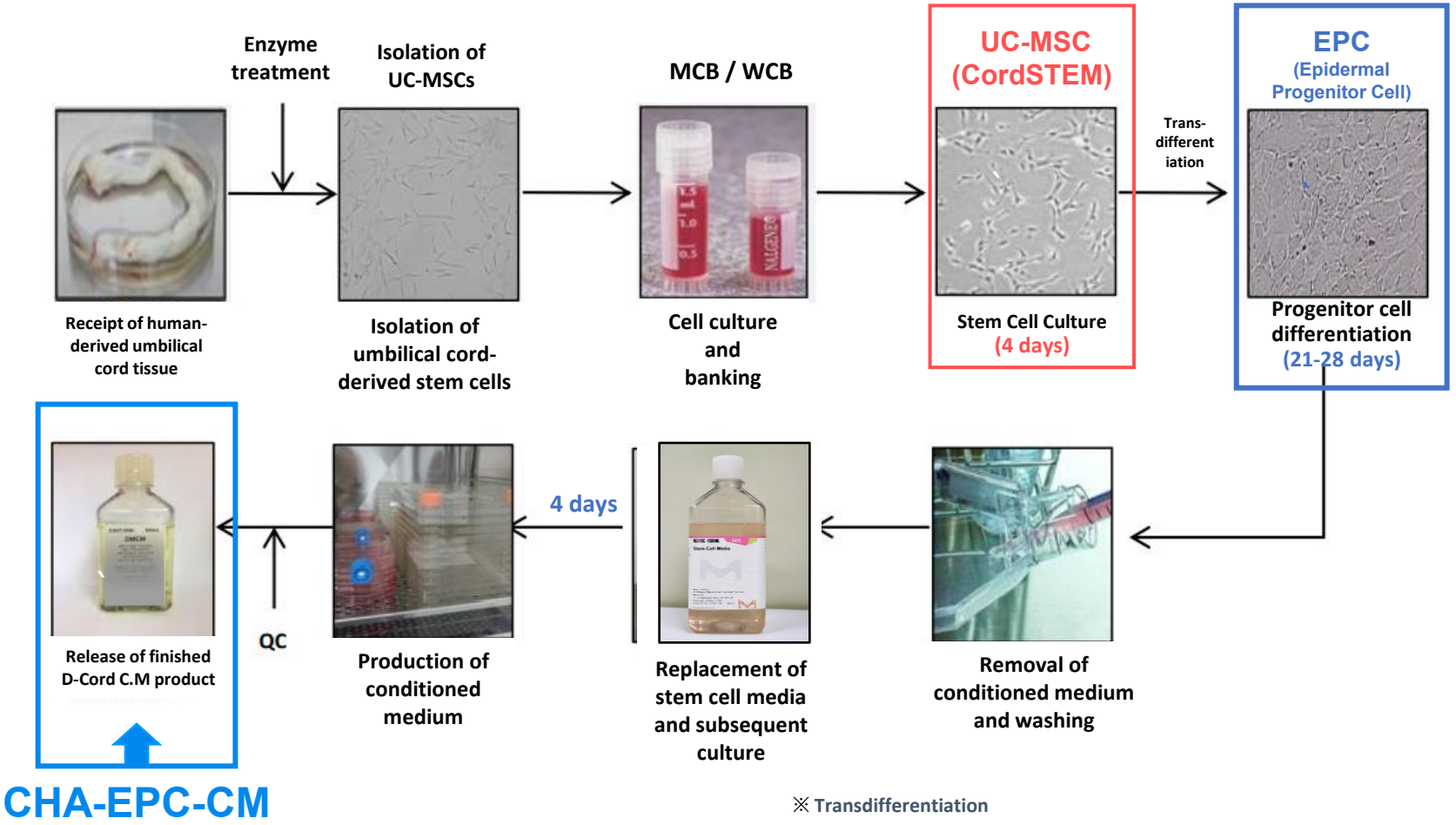
(Human Umbilical Mesenchymal Stem Cell Conditioned Media)

CHA Meditech

1. EPC-CM: Epidermal Progenitor Cell-Conditioned Medium

EPC-CM : Manufacturing Process (30–36 Day Process)

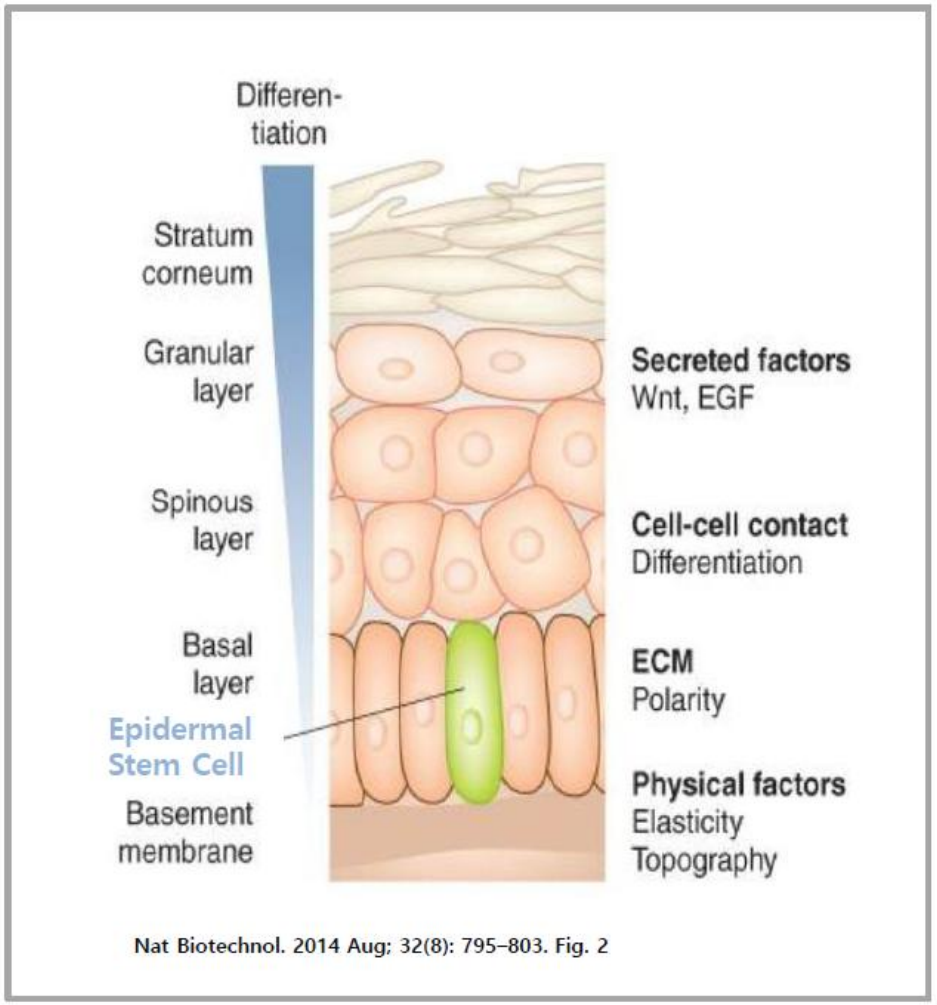
- Stem cell-derived epidermal progenitor cell-conditioned medium and its manufacturing method (Patent 10-2100608)
- Composition for preventing or improving skin aging comprising skin stem cell-conditioned medium and its use (Patent 10-1830062, Patent 10-2178778)



※ **Transdifferentiation**
The conversion of a cell type present in a tissue or organ into a cell type of another tissue or organ, without passing through a pluripotent state.

2. Why UC-MSC-derived EPC?

Challenges in the Industrialization of Epidermal Stem Cells, Which Are Crucial for Maintaining Skin Homeostasis

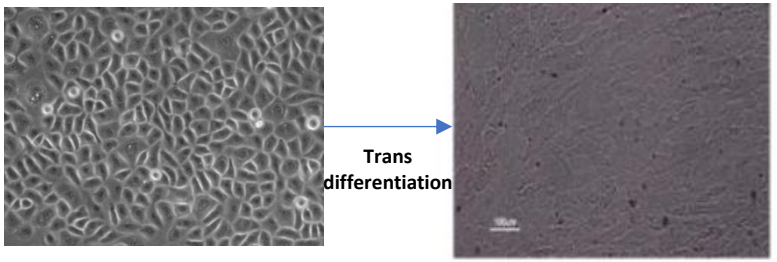


Nat Biotechnol. 2014 Aug; 32(8): 795–803. Fig. 2

Like other tissues, epidermal stem cells exist within the cutaneous epidermis.

Epidermal stem cells reside in the basal layer of the epidermis and play a critical role in maintaining healthy skin and driving the skin turnover process through the formation of keratinocytes.

However, due to their inherent tendency and rapid capacity to differentiate into keratinocytes, epidermal stem cells are highly difficult to maintain in vitro, making their direct industrial application currently unfeasible.



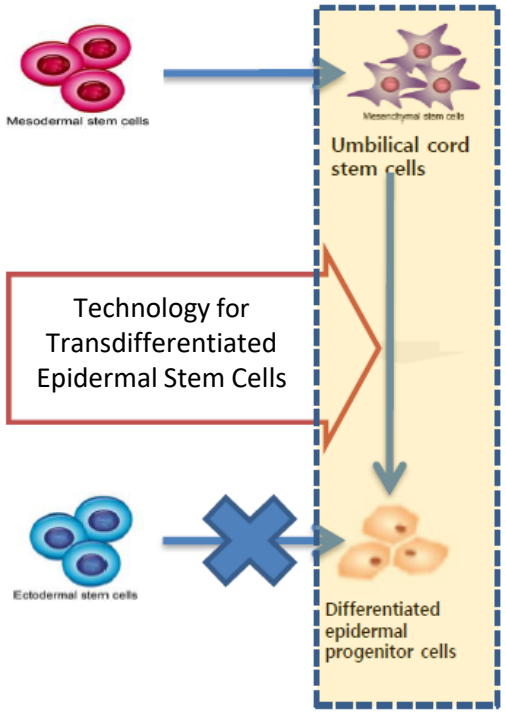
(Proliferating EPC) **(Differentiated keratinocytes)**

3. What's new in CHA-EPC?

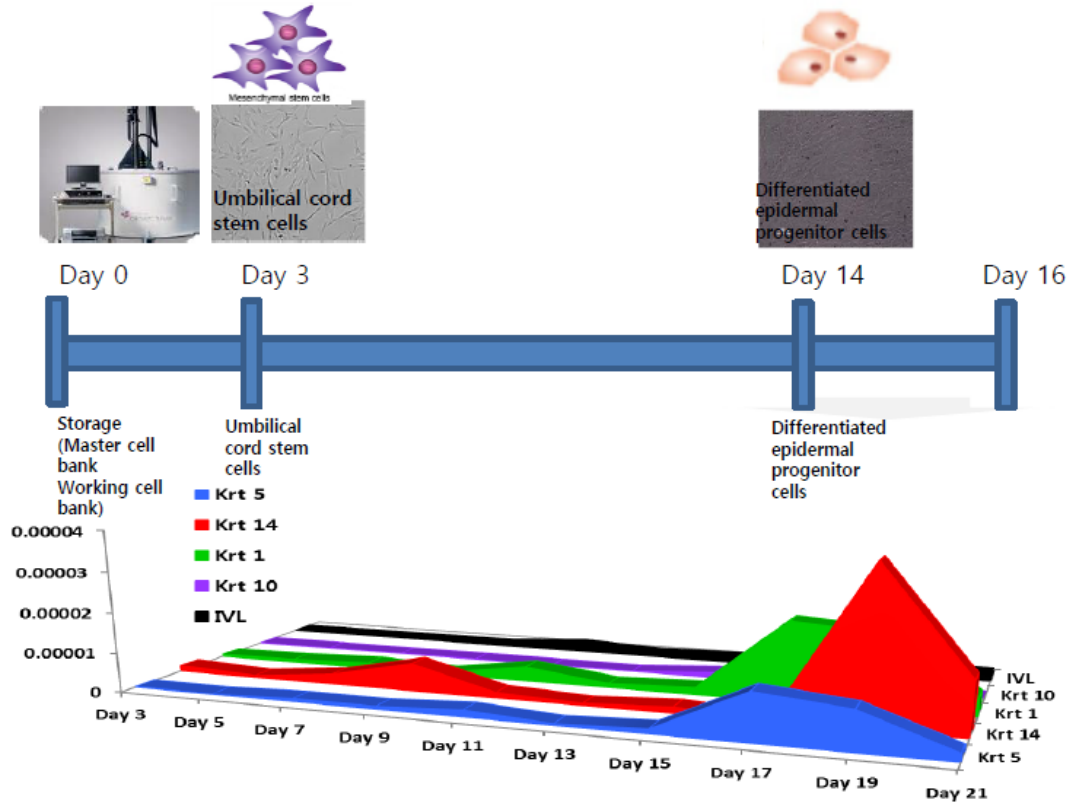
CHA Bio Group Achieves World's First Successful Transdifferentiation of Epidermal Stem Cells

Successfully cultured and differentiated epidermal stem cells—previously deemed impossible to industrialize—from umbilical cord-derived mesenchymal stem cells (UC-MSCs) for the first time in the world.

Differentiation Concept Map



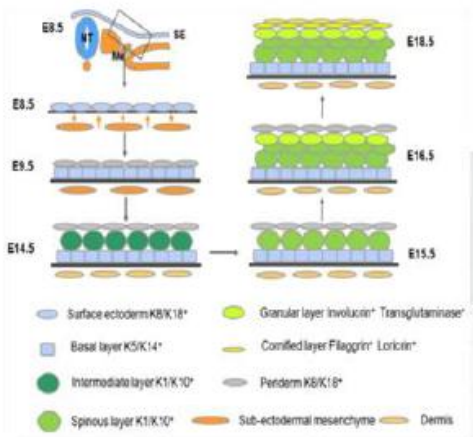
World's First Patented Technology for Transdifferentiated Epidermal Stem Cells

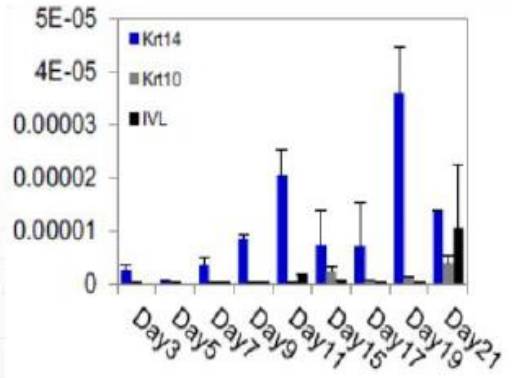


4. Characterization of EPC

Differentiation of Epidermal Progenitor Cells : Characterization

▶ Characterization Followed by the Induction of Epidermal Progenitor Cell Differentiation



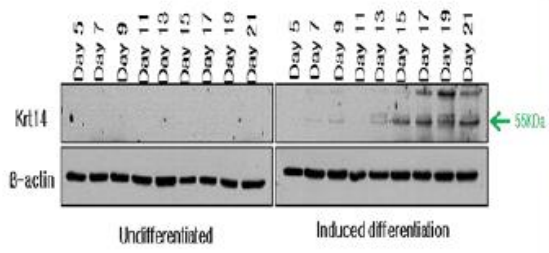


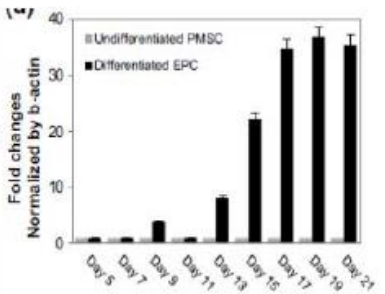
Rationale

· Marker Expression Varies by Stage of Skin Tissue Cell Differentiation
· Krt8/18→Krt5/14→Krt1/10

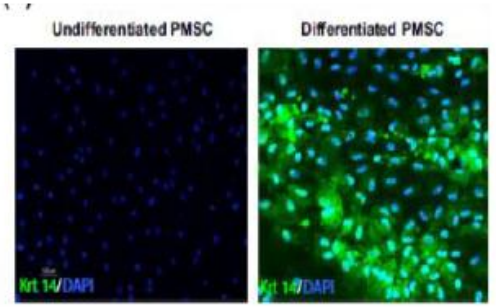
Evaluation Items

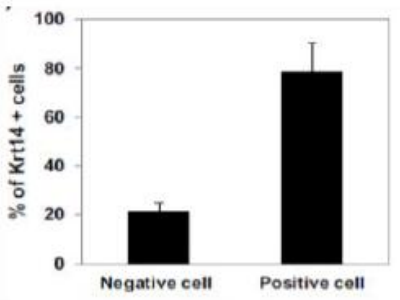
· Assessment of Cellular Characteristics by Differentiation
· Keratinocyte : Low Secretion Rate of Active Ingredients
→ Keratinocyte progenitor cell Differentiation





Comparison of Krt14 Expression by Differentiation Stage (Quantification of Protein Expression: WB)





Comparison of Krt14 Expression Levels at Day 21 of Differentiation (Immunostaining)

Dermatol Ther (Heidelb). 2018 Jun;8(2):229-244.

5. Reliability and safety of CHA-EPC-CM:

※ Notice by the Korean MFDS: Safety Standards for Human Cell and Tissue Conditioned Media (Regulations on Cosmetic Safety Standards, etc.)

Highly Reliable and Safe Ingredient Production Technology Regulated under Strict National Standards

▶ Secure production of raw materials powered by CHA Meditech's comprehensive, end-to-end solutions spanning from raw material manufacturing to pharmaceutical therapeutics.

▶ Strict management of manufacturing processes and quality control across a total of 26 parameters, including the 17 items mandated by the MFDS.


[별표 3]

인체 세포·조직

1. 용어의 정의

가. "인체 세포·조직 배양액"은 조직을 제거하고 남은 액을

나. "공여자"란 배양액에 사용되

다. "공여자 적격성검사"란 공여 해당 공여자가 세포배양액에 격성이 있는지를 판정하는

라. "윈도우 피리어드(window 항원 항체 유전자 등을 검출

마. "청정등급"이란 부유입자 및 수준 이하로 유지되도록 관리

2. 일반사항

가. 누구든지 세포나 조직을 주고

나. 누구든지 공여자에 관한 정 조직을 사용하였다는 내용의

다. 인체 세포조직 배양액을 제 한 위생상의 관리가 가능한

라. 세포·조직을 채취하는 의료 수행에 필요한 문서화된 절 존하여야 한다.

마. 화장품 제조판매업자는 세포 대하여 안전하고 품질이 균 리 감독을 철저히 하여야 한

3. 공여자의 적격성검사

가. 공여자는 건강한 성인으로서 한다.

- B형간염바이러스(HBV), 인체T림프영양성바이러스 (CMV), 엡스타인-바 바이

- 건염성 해면상뇌증 및 견
- 매독트레포네마, 클라미디
- 패혈증 및 패혈증으로 의
- 세포조직의 영향을 미칠

나. 의료기관에서는 원도우 피리 에 필요한 기준서를 작성하,

4. 세포·조직의 채취 및 검사

가. 세포·조직을 채취하는 장소 한다.

나. 보관되었던 세포·조직의 균 법을 사용해야 하며, 그와

다. 세포 또는 조직에 대한 품질 의 내용을 포함한 세포·조직

(1) 채취한 의료기관 명칭
(2) 채취 연월일
(3) 공여자 식별 번호
(4) 공여자의 적격성 평가 결
(5) 동의서
(6) 세포 또는 조직의 종류,

5. 배양시설 및 환경의 관리

가. 인체 세포조직 배양액을 제 구역에 설치하여야 한다.

나. 제조 시설 및 기구는 정기적 록 배치되어야 한다.

다. 제조공정 중 오염을 방지하 고 이에 따라야 한다.

6. 인체 세포조직 배양액의 제조

가. 인체 세포조직 배양액을 제 는 제거하는 처리를 하여야

나. 배양액 제조에 사용하는 세 정보를 확인할 수 있도록 서를 작성 보존하여야 한다.

(1) 채취(보관을 포함한다)
(2) 채취 연월일
(3) 검사 등의 결과
(4) 세포 또는 조직의 처리
(5) 공여자 식별 번호
(6) 사람에게 감염성 및 결과

다. 배지, 첨가성분, 시약 등 규격을 설정한 인체 세 안전성이 확보된 물질 야 한다.

라. 제조기록서는 다음의 사

(1) 제조번호, 제조연월일,
(2) 사용한 원료의 목록,
(3) 사용된 배지의 조성,
(4) 각 단계별 처리 및 취

마. 채취한 세포 및 조직을 에 따라 균일한 품질을 었던 세포 및 조직에 대 여 적절한 보정시험을

바. 인체 세포조직 배양액 서를 포함한 표준지침서

7. 인체 세포조직 배양액의

가. 인체 세포조직 배양액의 보존하여야 한다.

(1) 단회투여독성시험자료
(2) 반복투여독성시험자료
(3) 1차피부자극시험자료
(4) 안점막자극 또는 기타
(5) 피부감작성시험자료
(6) 광독성 및 광감작성 자료를 제출하는 경우
(7) 인체 세포조직 배양액
(8) 유전독성시험자료
(9) 인체첨포시험자료

나. 안전성시험자료는 「비임상 자료이어야 한다. 다만, 실시하여야 하며, 관련분 이상 해당시험에 경력을

다. 안전성시험자료는 인체 가위원회의(독성전문가 평가결과를 기록 보존하여 평가 결과에 따라 기타 토록 권고할 수 있다.

8. 인체 세포조직 배양액의 시

가. 인체 세포조직 배양액의 포조직 배양액 품질관리

(1) 성상
(2) 무균시험
(3) 마이코플라스마 부정시
(4) 외래성 바이러스 부정시
(5) 확인시험
(6) 순도시험

- 기원 세포 및 조직 부재
- '항생제', '혈청' 등 [별표 에 해당 원료를 사용한

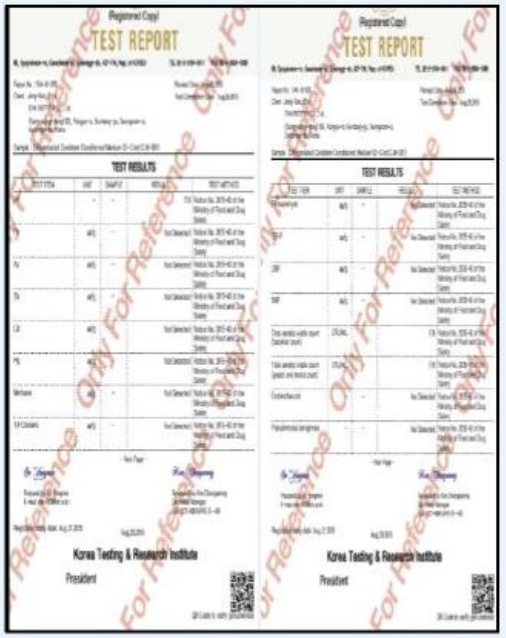
나. 품질관리에 필요한 각 항 되어야 한다.

다. 인체 세포조직 배양액의 그 시험성적서를 보존하여

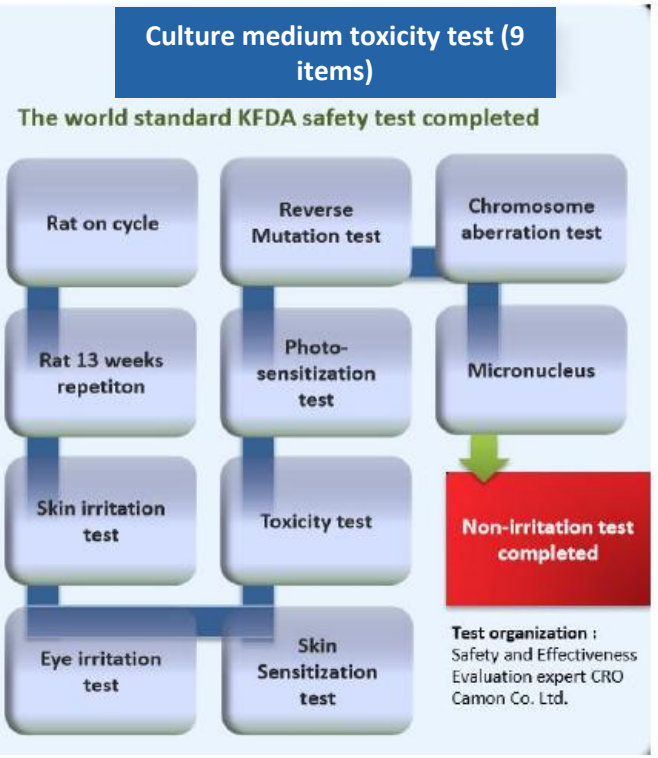
9. 기록보존

화장품 제조판매업자는 이 안 류를 받아 완제품의 제조연월

17 items notified by the Ministry of Food and Drug Safety



Culture medium toxicity test (9 items)



6. CHA-EPC-CM: Intellectual Property(IP)

Epidermal Stem Cell Culture Medium (EPC-CM) Technology Validation Data

Paper

Dermatol Ther **(Heidelb) 2018 8(2):229-244**

Dermatol Ther (Heidelb)
https://doi.org/10.1007/s13555-018-0229-2

ORIGINAL RESEARCH

Anti-aging Properties of Conditioned Media of Epidermal Progenitor Cells Derived from Mesenchymal Stem Cells

Su Ji Sohn · Ji Min Yu · Eun Young Lee · You Jin Nam · Jinwan Kim · Sukho Kang · Dong Hyun Kim · Aeri Kim · Sangjin Kang

Received: December 26, 2017
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ABSTRACT

Introduction: Reduced number and activities of epidermal stem cells are related to the features of photoaged skin. It was reported that conditioned media from various stem cell cultures are capable of improving the signs of cutaneous aging. This work was performed to establish epidermal progenitor cells derived from mesenchymal stem cells, and to evaluate the anti-aging efficacy of its conditioned media. ***Methods***: Epidermal progenitor cell culture was established by differentiation from mesenchymal stem cells, and its conditioned medium (EPC-CM) was prepared. Normal human dermal fibroblasts were exposed to hydrogen peroxide and the protective effects of EPC-CM were investigated, monitoring intracellular reactive oxygen species (ROS), cellular defense enzymes, collagen biosynthesis, and mitogen-associated protein kinase (MAPK) signaling. Anti-aging efficacy of cosmetic essence (5% EPC-CM) was evaluated by a clinical test with 25 Korean women aged between 29 and 69. ***Results***: Hydrogen peroxide hindered proliferation of fibroblasts and increased the levels of intracellular ROS. Pretreatment of EPC-CM

Enhanced content To view enhanced content for this article go to https://doi.org/10.6084/m9.figshare.5882878.

Su Ji Sohn and Ji Min Yu contributed equally to this study.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13555-018-0229-2) contains supplementary material, which is available to authorized users.

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Published online: 02 March 2018

Patent

10-1830062 / 10-2178778

특허증
CERTIFICATE OF PATENT

특허 제 10-1830062 호
Patent Number

출원번호 제 10-2017-0083862 호
Application Number
출원일 2017년 06월 30일
Filing Date
등록일 2018년 02월 12일
Registration Date

발명의 명칭 Title of the Invention
피부줄기세포 배양액을 포함하는 피부 노화 예방 또는 개선용 조성물 및 이의 용도

특허권자 Patentee
등록사항란에 기재

발명자 Inventor
등록사항란에 기재

위의 발명은 「특허법」에 따라 특허등록원부에 등록되었음을 증명합니다.
This is to certify that, in accordance with the Patent Act, a patent for the invention has been registered at the Korean Intellectual Property Office.

2018년 02월 12일

특허청
Korean Intellectual Property Office

특허청장
COMMISSIONER,
KOREAN INTELLECTUAL PROPERTY OFFICE

Listed in the International Dictionary of Cosmetic Ingredients

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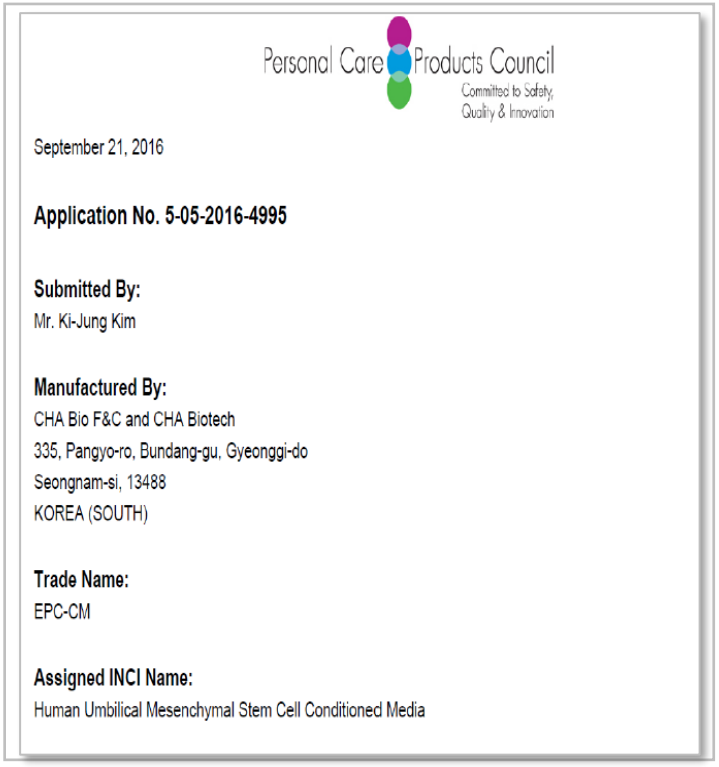


Personal Care Products Council
Committed to Safety, Quality & Innovation

September 21, 2016

Application No. 5-05-2016-4995

Submitted By:
Mr. Ki-Jung Kim

Manufactured By:
CHA Bio F&C and CHA Biotech
335, Pangyo-ro, Bundang-gu, Gyeonggi-do
Seongnam-si, 13488
KOREA (SOUTH)

Trade Name:
EPC-CM

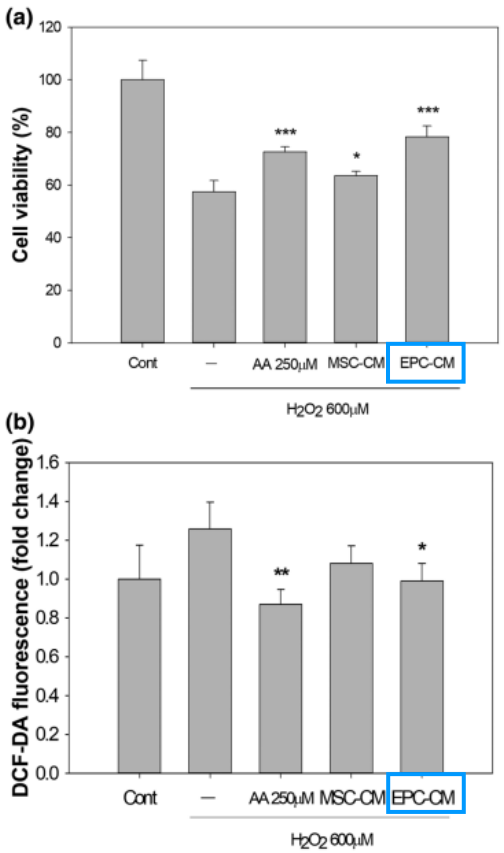
Assigned INCI Name:
Human Umbilical Mesenchymal Stem Cell Conditioned Media

7. CHA-EPC-CM Efficacy: Skin Cells and Artificial Skin

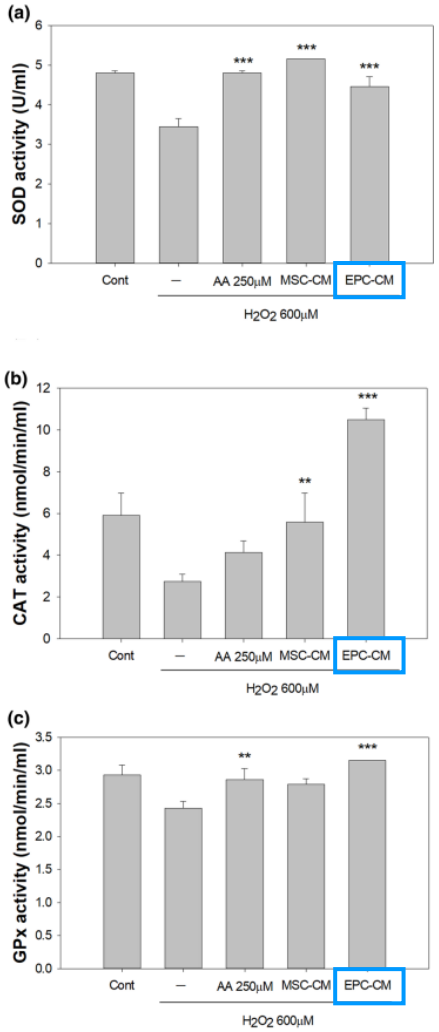
■ UNDER OXIDATIVE STRESS CONDITION

(skin) cell survival ↑, antioxidant ↑ , collagen production ↑, epidermal basal layer proliferation marker ↑, melanin inhibition ↓

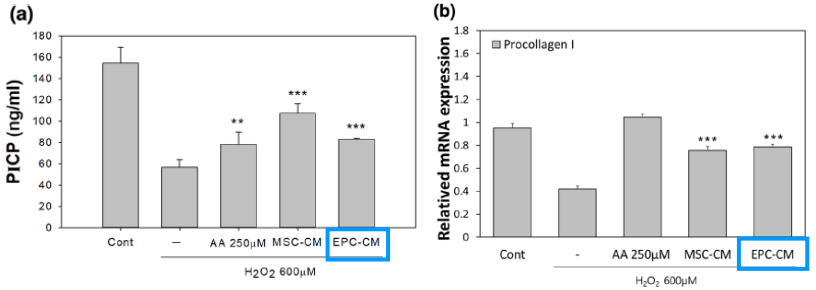
Increased Cell Viability (Fibroblasts)



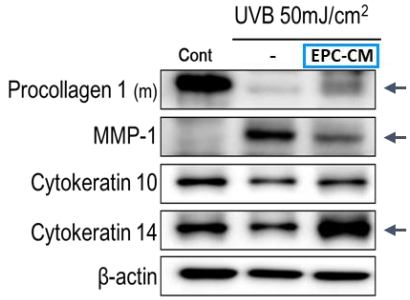
Increased Antioxidant Enzyme Activity (Fibroblasts)

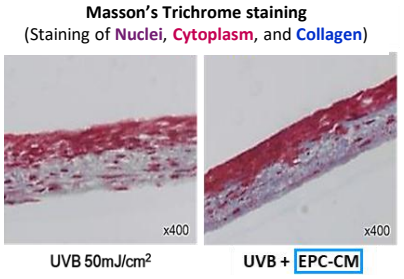


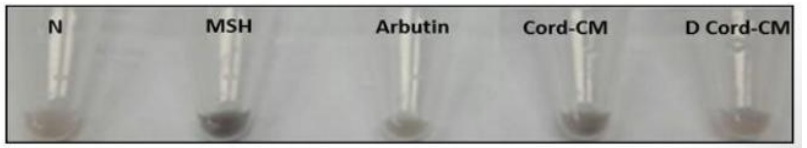
Increased Collagen Synthesis (Fibroblasts)



Under UVB-irradiated conditions: Increased collagen production, inhibition of collagenase, and upregulation of keratinocyte proliferation markers (artificial skin); Inhibition of melanogenesis (melanocytes)







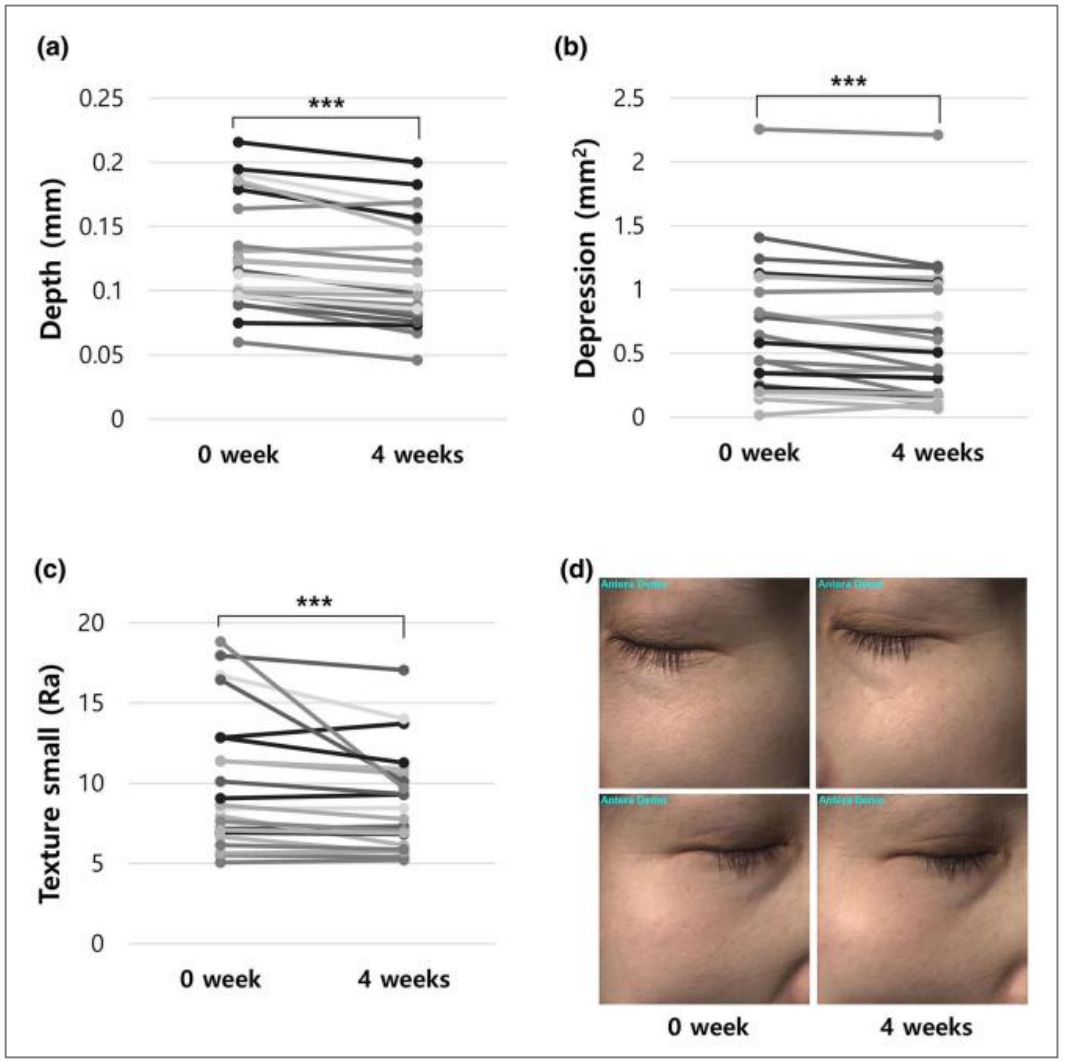
Stem Cell-Derived Epidermal Progenitor Cell-Conditioned Medium: Melanin Content

Dermatol Ther (Heidelb). 2018 Jun;8(2):229-244.
(Patent 10-1830062, Patent 10-2178778)

8. Efficacy of CHA-EPC-CM: Clinical Studies

Clinical Effects of EPC-CM: Reduction of fine lines, improvement in wrinkles, lifting of sagging skin, and enhancement of skin texture and tone

5% Essence
Twice per day



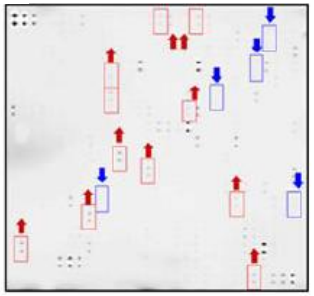
*Dermatol Ther (*Heidelb). 2018 Jun;8(2):229-244.

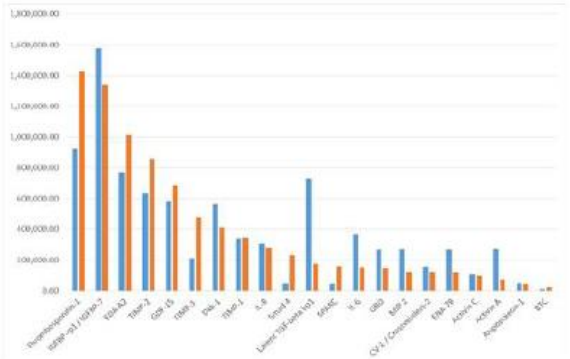
9. CHA-EPC-CM Analysis : Contains 59 types of epidermal stem cell-specific growth factors and 313 types of beneficial proteins

Analysis of Undifferentiated vs. Differentiated Conditioned Media

Undifferentiated Cell-Derived CM

Differentiated Cell-Derived CM



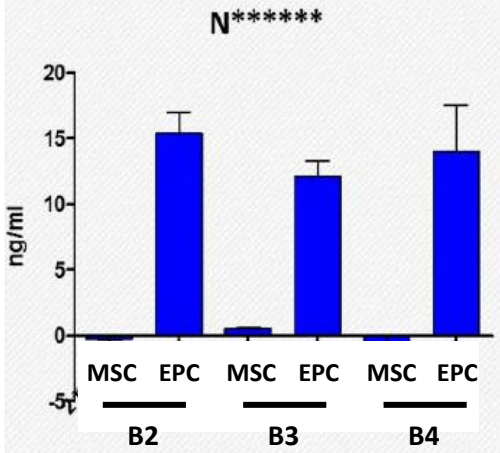


: Downregulated Cytokines (vs. Undifferentiated)

: Upregulated Cytokines (vs. Undifferentiated)

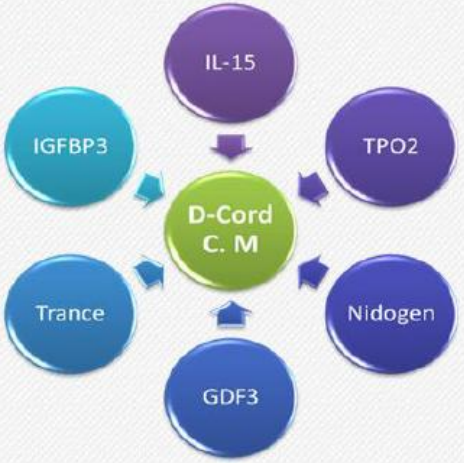
Highly Expressed Cytokines in Differentiated C.M.

Analysis of Secretory Factors Highly Expressed in Differentiated Conditioned Medium



IL-**	TH**	M**	G**	FS**	TR**	TM**	IG**
19 (pg/ml)	63.65 (pg/ml)	15180 (pg/ml)	108.2 (pg/ml)	–	29 (pg/ml)	–	<1 (pg/ml)

T**1	T**2	IL-*	IL-**	IL-***	M***-1	M**	M###	T***I	I***	G****
96,508 (pg/ml)	145,408 (pg/ml)	21.44 (pg/ml)	3,776 (pg/ml)	3,430(pg/ml)	2,508 (pg/ml)	2,112 (pg/ml)	1,092 (pg/ml)	1,651 (pg/ml)	36,226 (pg/ml)	28,656 (pg/ml)



10. CHA-EPC-CM Marker Protein: Nidogen-1

Top 24 Proteins with Higher Expression Intensity in EPC-CM Compared to MSC-CM
(RayBio® L-Series Human Antibody Array **507** Membrane Kit)

Table 2 List of highly upregulated cytokines on differentiation in EPCs

Name	Full name	Signal intensity
TSP	Thrombospondin	5,886,805
IGFBP-rp1/IGFBP-7	Insulin	3,970,222
TIMP2	Tissue inhibitor of metalloproteinase-2	3,950,540
EDA-A2	Ectodysplasin A2	3,938,694
XEDAR	Edar and X-linked Eda-A2 receptor	1,572,912
Angiopoietin-1	Angiopoietin-1	1,167,638
SPARC	Secreted protein acidic and rich in cysteine	1,020,366
GDF-15	Growth differentiation factor 15	1,009,469
sFRP-4	Secreted frizzled-related protein 4	999,123
GRO	Grow regulated oncogen	963,242
MIP2	Macrophage inflammatory protein 2	865,819
TIMP-1	Tissue inhibitor of metalloproteinases 1	785,939
Latent TGF-beta bp1	Latent TGF-beta binding protein 1	554,608
CV-2/crossveinless-2	Crossveinless-2	475,676
IL-6	Interleukin 6	441,917
TMEFF1/tomoregulin-1	Transmembrane protein with EGF-like and two follistatin-like domains 1	430,143
Nidogen-1	Nidogen-1	413,625
Smad 4	Mothers against decapentapiegic homolog 4	391,810
Activin C	Actin C	228,189
IGFBP-3	Insulin-like growth factor-binding protein 3	182,444
Thrombospondin-2	Thrombospondin-2	112,674
TRANCE	Tumor necrosis factor-related activation-induced cytokine	100,065
Activin A	Activin A	76,102
IL-15 R alpha	Interleukin-15 receptor alpha	51,270

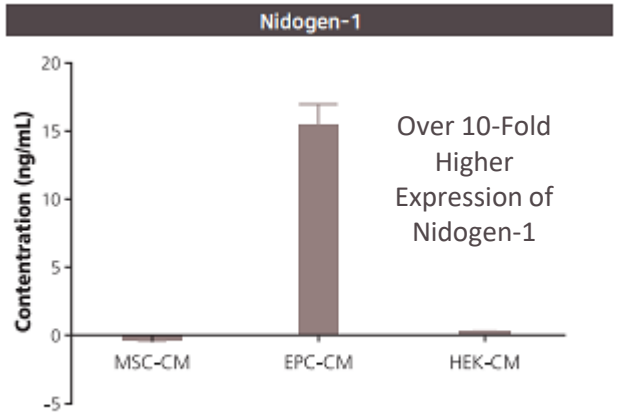
Dermatol Ther (Heidelb). 2018 Jun;8(2):229-244.

Quantification of Protein Expression Levels in EPC-CM
(Protein-specific ELISA kit)

No.	Cytokine	Concentration (pg/mL)	Signal Intensity
1	Thrombospondin	63.65	5,886,805
2	**Nidogen-1**	15,180	413,625
3	TRANCE	29	100,065
4	IL-15R alpha	19	51,270

(ELISA)

Comparison of Nidogen-1 Expression Across Different Stem Cell Types



※ MSC-CM : Mesenchymal stem cell - conditional media
※ HEK-CM : Human epidermal keratinocyte - conditional media

Composition for Preventing or Improving Skin Aging Comprising Epidermal Stem Cell-Conditioned Medium and Use Thereof (Patent No. 10-1830062, Patent No. 10-2178778)

11. CHA-EPC-CM: Catalog of Raw Material

STEM CELL MEDIUM
줄기세포배양액

Product Name	CHA-EPC-CM (Epidermal progenitor cell-conditioned medium)
INCI Name	Human Umbilical Mesenchymal Stem Cell Conditioned Media
Identification	Total proteins (≥ 300 μg/mL); Nidogen-1 (≥ 1 ng/mL)
Appearance	Very light yellow ~ light pink transparent liquid
Purity	≤ 10 cells/mL
Endotoxin	< 1 EU/mL
Use dosage	0.01 ~ 1.0%
Patent & Paper	Patent No.: 10-1830062 / 10-2178778 / Dermatol Ther (Heidelb). 2018 Jun:8(2):229-244
Result	

CHA-EPC-CM

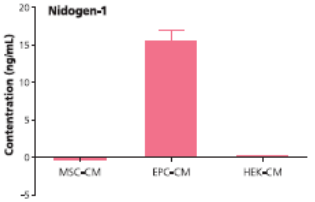
Antioxidant, Anti-aging, Anti-wrinkle
항산화, 항노화, 주름 개선

CHA-EPC-CM can be used as a functional material for antioxidant, anti-aging, and anti-wrinkle effects
표피줄기세포 배양액의 항산화, 항노화, 주름개선 소재로의 활용

The conditioned medium of epidermal progenitor cells, which was derived from umbilical cord mesenchymal stem cells, specifically contains nidogen-1 protein, a key component of the basement membrane. CHA-EPC-CM exhibits strong antioxidant effects in addition to inducing collagen production in human dermal fibroblasts. In a clinical study using a essence containing 5% CHA-EPC-CM, wrinkle, depression and texture was significantly improved.

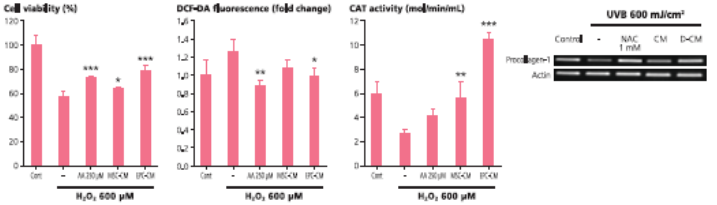
인텟줄 중간엽줄기세포에서 유래한 표피줄기세포 배양액에는 기저막 핵심 구성요소인 니도겐 단백질이 특이적으로 함유되어 있어 진피층 섬유아세포 대상 콜라겐 생성 유도 외에 강력한 항산화 효과를 제공

1. Active component in EPC-CM



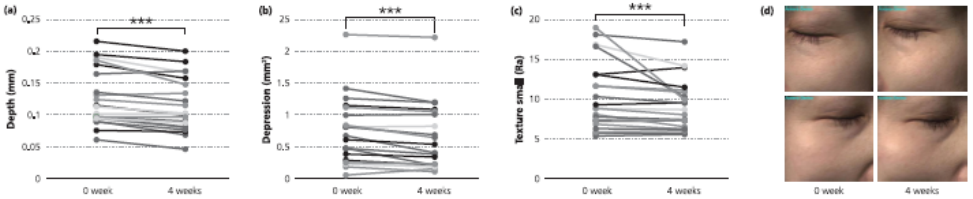
CHA-EPC-CM contains 10 times more nidogen-1 protein (15.2 ng/mL) than the conditioned media (CM) of undifferentiated mesenchymal stem cells or human primary keratinocytes.

2. Effect on skin cells and reconstructed skin model under oxidative stress conditions



Under oxidative stress conditions (H2O2), CHA-EPC-CM treatment better restored cell viability (left, top), reduced reactive oxygen species levels (right, top), and increased the activity of antioxidant enzyme CAT (left, bottom) compared to MSC-CM in human dermal fibroblasts. In reconstructed skin model, EPC-CM (D-CM) treatment restored procollagen protein levels reduced by UVB irradiation better than MSC-CM (CM) (right, bottom).

3. Clinical results



A cosmetic essence containing 5% CHA-EPC-CM was applied to the face of female volunteers (n = 25) twice a day for 4 weeks. As results, wrinkle, depression, and texture were improved significantly during clinical test period. *** p < 0.001 versus control.

- E.O.D -