생체활성 각질층 지질

박 병 덕

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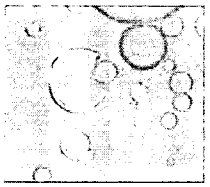
Multi-lamellar emulsion(MLE)

생체활성 각질층 지질



박 병 덕

NeoPharm Co., Ltd

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Multi-lamellar emulsion(MLE)

Contents

- Introduction
- Skin barrier recovery
- Immune-modulation
- New S1P 유사체
- 맺음말

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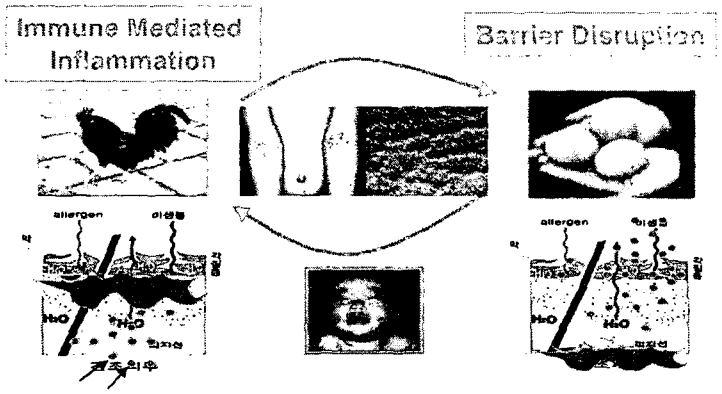
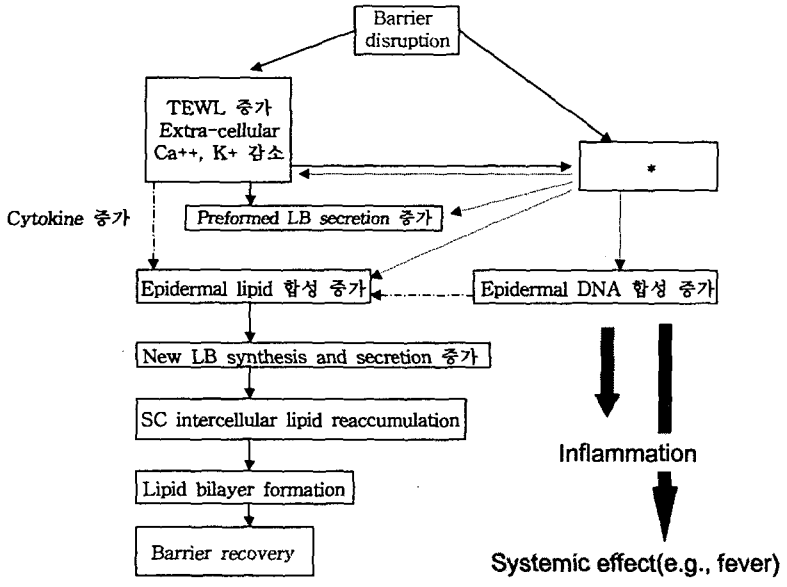
Inside-outside vs. outside-inside



Inside-Outside paradigm
Schellander F, Marks R 1973

Outside-Inside paradigm
Elias P M 2001



Key issues on skin homeostasis

- **Recovery of skin barrier function**
- **Immune-modulation**

Skin barrier recovery(1)

Composition

The ratio between intercellular lipid is very important for barrier recovery -> Physiologic lipid mixture (Man et al, 1995)

Multi-lamellar emulsion(MLE)

2. Skin barrier recovery

Skin Physiological lipid mixture and Skin barrier recovery

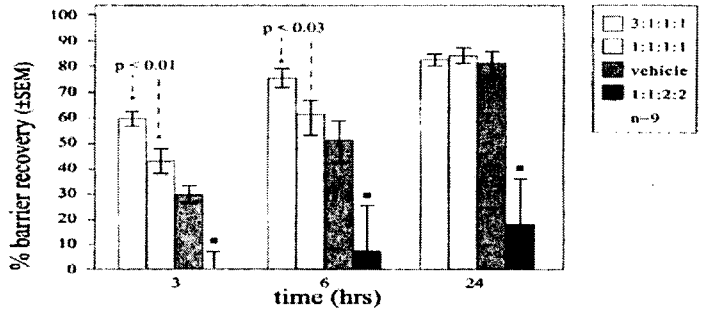


Figure 5: Optimized physiological lipids improve barrier recovery in aged mice.

Ref. From JID 106, 1096~1101, Mao-Qiang, M. et al.

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Multi-lamellar emulsion(MLE)

2. Skin barrier recovery

Logical barrier repair strategies recommended by Elias group

Clinical Indication	Repair Strategy
	Dressings
Healing wounds	Vapor permeable
Keloids	Vapor impermeable
	Nonphysiologic lipids (NPL)
Radiation dermatitis or sunburn	Petrolatum jelly or lanolin
Premature infants (aged <34 wk or >33 wk [add physiological lipids at optimal molecular ratio])	Petrolatum jelly or lanolin
Irritant contact dermatitis (some surfactants or retinoids)	Petrolatum jelly or lanolin
	Physiological lipids at optimal molecular ratio
Aging or photoaging	Cholesterol dominant
Atopic dermatitis (with added NPL)	Ceramide dominant
Diaper dermatitis (with added NPL) or psoriasis (with added NPL)	Free fatty acid dominant (any)
Irritant contact dermatitis (most causes)	Cholesterol, ceramide, or free fatty acid dominant (any)

Ref. From Archive Derm., 2001, P.M. Elias. et al.

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Skin barrier recovery(2)

Structure

1) Intercellular lipid lamellar phase has two periodicity of about 13nm and 6nm. 13nm phase is very important for the permeability barrier function.

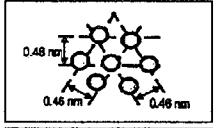
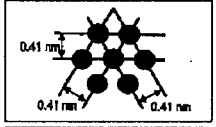
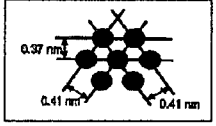
2) The coexistence of orthorhombic and and hexagonal chain packing lattice in isolated stratum corneum(Bouwstra et al, 2002).

3) Atopic dermatitis and lamellar ichthyosis show increased hexagonal lattice, along with deficiencies in long chain fatty acids and certain type of ceramides (Bouwstra et al, 1996).

4) Intercellular lipid is a single and coherent gel phase(Gopi and Norlén, 2002).

5) The coexistence of orthorhombic and and hexagonal chain packing lattice in isolated stratum corneum is due to the phase transition from hexagonal to a more orthorhombic chain packing(Gopi and Norlén, 2002).

Types of lamellar structure in SC

	Types	Lateral packing	Permeability barrier
Liquid Lamellar phase			Weak
Hexagonal Gel phase			Middle
Orthorhombic Crystalline phase			Strong

Multi-lamellar emulsion(MLE)

2. Skin barrier recovery

SC lipid types and composition

Lipid species	Description	Mean carbons Per alkyl chain	Mean double bonds per alkyl chain	□95mol%	Notes
Ceramide 1	Long chain base(sphingosine) Amide linked fatty acid(ω- OH) Ester linked fatty acid(non-OH)	18.7 29.9 18.4	0.8 0 0.8	C17-22 C26-32 C14-24	33mol% C18:1 59mol% C30:1 24mol% C18:2
Ceramide 2	Long chain base (sphingosine) Amide linked fatty acid (non-OH)	18.7 23.5	0.8 0	C17-22 C16-30	37mol% C18:1 54mol% C24-26
Ceramide 3	Long chain base(phytosphingosine) Amide linked fatty acid (non-OH)	20.2 23.4	0 0	C16-25 C16-28	61mol% C19-22 51mol% C24-26
Ceramide 4□5	Long chain base (sphingosine) Amide linked fatty acid(α-OH)	18.4 23.3	0.7 0	C16-22 C16-26	32mol% C18:1 70mol% C24-26
Ceramide 6 i	Long chain base(phytosphingosine) Amide linked fatty acid(α-OH) Ester linked fatty acid(α-OH)	19.5 23.1 20.4	0 0 0	C16-25 C16-28 C16-26	61mol% C18-20 69mol% C24-26 70mol% C16,24,26
Ceramide 6 ii	Long chain base(phytosphingosine) Amide linked fatty acid(α-OH)	20.2 23.9	0 0	C16-24 C16-26	48mol% C20-22 81mol% C24-26
Free fatty acids		21.3	0.1	C16-26	45mol% C22-24
Cholesterol esters		17.9	0.7	C16-18	69mol% C18:1
Cholesterol					

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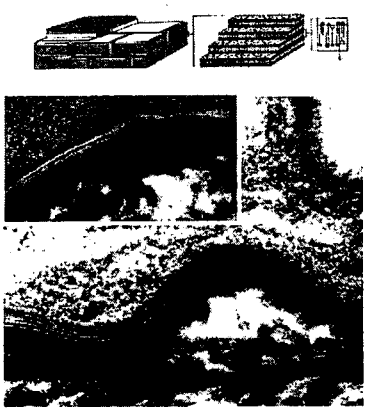
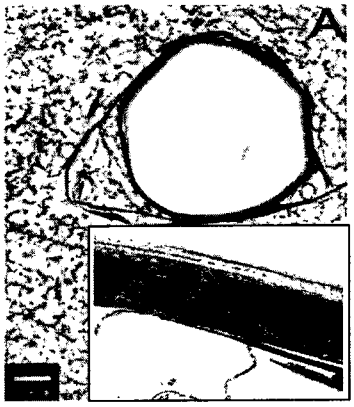
Multi-lamellar emulsion(MLE)

2. Skin barrier recovery

Structure similarity(1) -lamellar structure



Lamellar structure of skin



Lamellar structure of MLE

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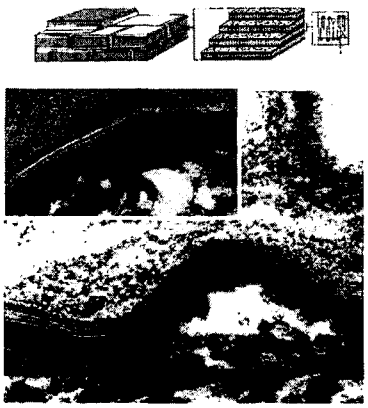
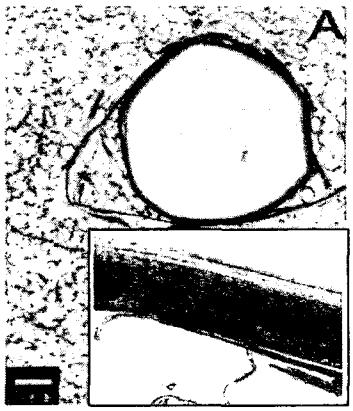
Multi-lamellar emulsion(MLE)

2. Skin barrier recovery

Structure similarity(1) -lamellar structure



Lamellar structure of skin



Lamellar structure of MLE

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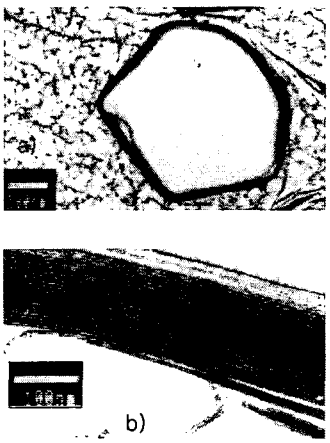
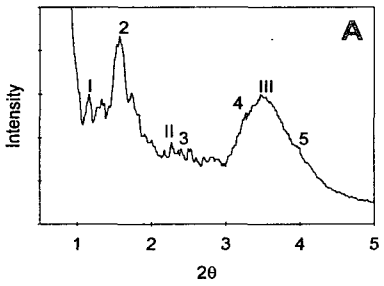
Multi-lamellar emulsion(MLE)

2. Skin barrier recovery

Structure similarity(2)-long repeated periodicity





The diffraction pattern of MLE at 30℃. (A) The peaks indicated by the Arabic numbers in the figure are attributed to the long periodicity lamellar phase of 11.5nm (second, third, fourth, and fifth order).

The relatively short distance of the MLE compared to that in native stratum corneum lipids might be due to the short fatty acid chain length of the pseudoceramide and free fatty acid used in MLE.

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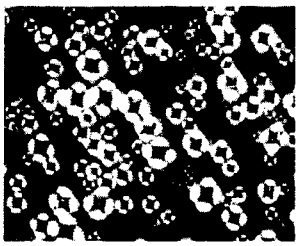
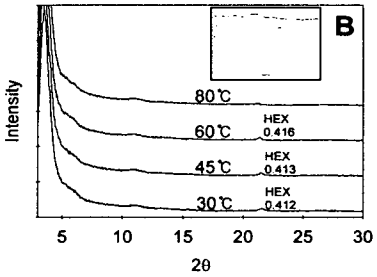
Multi-lamellar emulsion(MLE)

2. Skin barrier recovery

Structure similarity(3) - single hexagonal gel structure





The WAXD peak of MLE reveals a weak but distinct peak at approximately 0.411nm, which could be attributed to the hexagonal lateral packing only.
Although an increase of temperature could result in an increase of chain mobility of lipid components, an identical series of diffraction patterns were observed at elevated temperature up to 60℃. However, the slight increase of packing space was also seen with elevated temperature and no distinct diffraction pattern was observed at 80℃.

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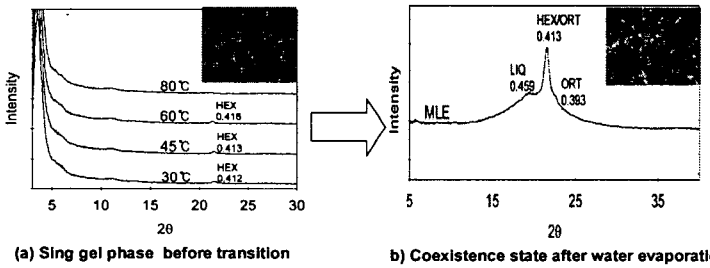
Multi-lamellar emulsion(MLE)

2. Skin barrier recovery

Structure similarity(4) - Phase transition and coexistence state of LIQ/HEX/ORT



(a) Sing gel phase before transition

b) Coexistence state after water evaporation

MLE itself is the single hexagonal phase, but, when MLE is applied on the skin, the water vaporized and resulted in phase transition. Figure (b) shows the phase transition of the MLE when it was dried. The shape of lamellar structure in the emulsion changed into broad laminated sheet shape. After incubation, MLE, a distinct peak at a spacing of 0.413nm and a weak peak at 0.393nm suggest the phase transition to orthorhombic and broad peaks at 0.459 nm suggest liquid lamellar, which is very similar with natural stratum corneum lipids.

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Multi-lamellar emulsion(MLE)

2. Skin barrier recovery

Characteristics of MLE structure

1) Intercellular lipid lamellar phase has two periodicity of about 13nm and 6nm. Long chain fatty acid and Cer I, IV are very important for long periodicity.

   : Long chain fatty acid 나 Cer I, IV를 사용하지 않아도 적절한 비율조절로 long periodicity 구조를 갖는 multiple emulsion을 얻을 수 있다.

2) Intercellular lipid is a single and coherent gel phase(Gopi and Norlén, 2002).

   : Multi-lamellar emulsion -> single coherent gel phase

3) The coexistence of orthorhombic and and hexagonal chain packing lattice in isolated stratum corneum is due to the phase transition from hexagonal to a more orthorhombic chain packing(Gopi and Norlén, 2002).

   : Single hexagonal gel phase -> Phase transition -> coexistence of orthorhombic and and hexagonal chain packing

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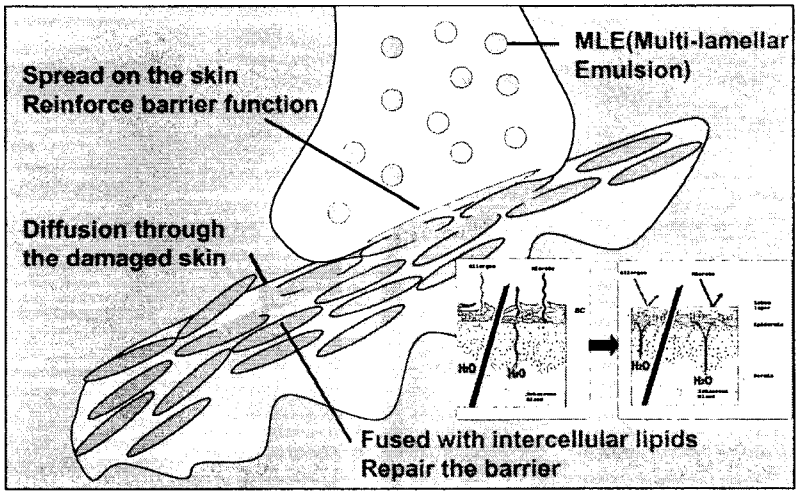
Multi-lamellar emulsion(MLE)

2. Skin barrier recovery

Barrier repair strategy by emulsion



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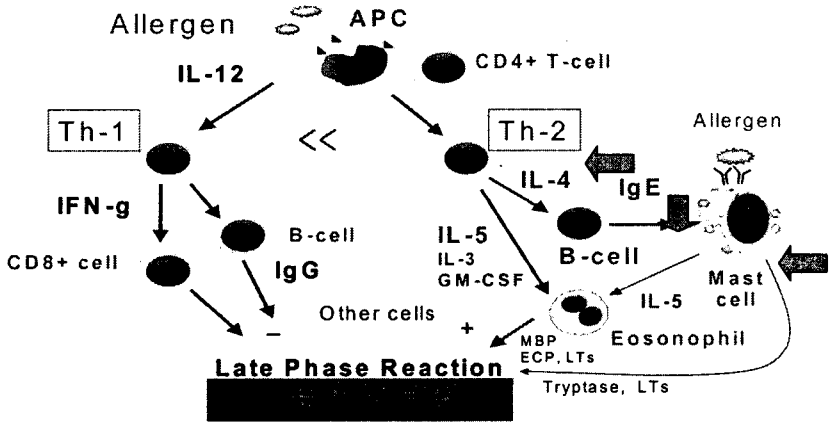
Multi-lamellar emulsion(MLE)

3. Immune-modulation

Immune disorder- atopy dermatitis



Th1 cell << Th2 cell -> 아토피 피부염

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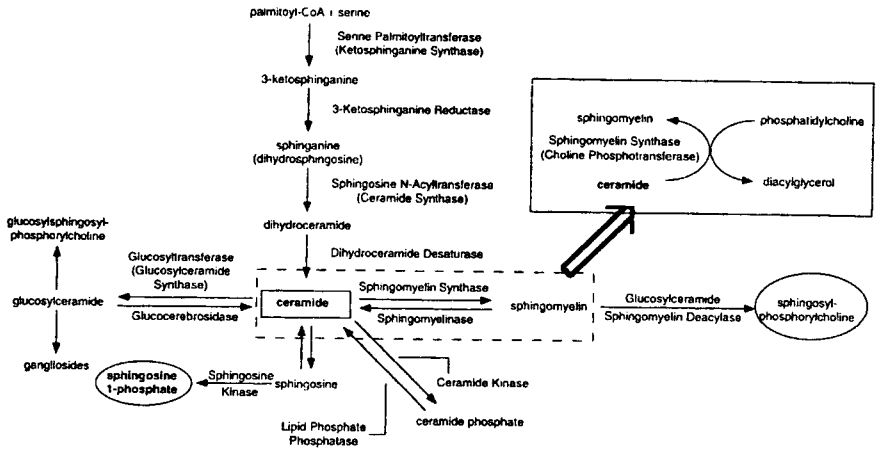
Multi-lamellar emulsion(MLE)

3. Immune-modulation

Sphingolipid metabolism



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Sphingosine-1-phosphate(S1P)

- **sphingosine kinase(SK)**활성화로 생성되는, 세라마이드 대사산물의 일종
- 세포증식, 분화, **survival, and motility**에 중요한 역할을 하는 **Bioactive** 물질
- 세포의 증식 및 콜라겐 합성을 촉진(주름방지효과)
- 세포의 이동과 분화촉진으로 상처회복 촉진
- **Ca2+ ion release**
- 면역세포**(T cell) trafficking**을 조절: 면역 조절능

Ref : Goetzl and An, 1998; Spiegel et al, 1998; Pyne and Pyne, 2000; Spiegel and Milstien, 2000, Yatomi et al, 1997; Ruwisch et al, 2001.

Lipid mediators in T cell-dependent immune responses

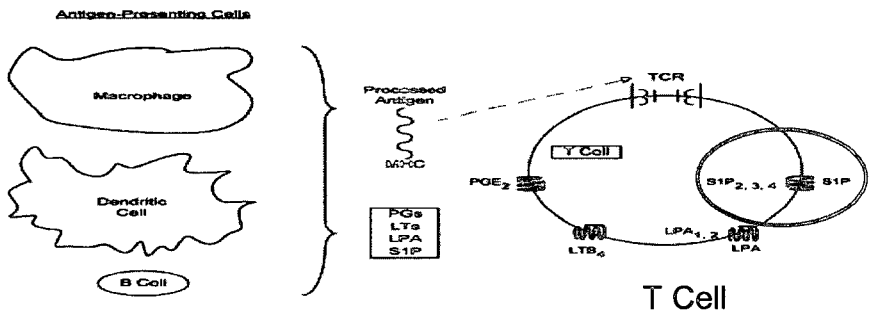

Fig. 1. Lipid mediators in T cell-dependent immune responses. Two shared functions of the different antigen-presenting cells (APCs) depicted (left) are: (a) antigen processing and presentation to T cell receptors (TCRs) in association with APC major histocompatibility complexes (MHCs), and (b) generation and secretion of diverse lipid mediators, including the eicosanoid subfamilies of prostaglandins (PGs) and leukotrienes (LTs), and the lysophospholipids (LPLs) lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P). **T cells (right) express G protein-coupled receptors (GPCRs) for PGE2, LTB4, LPA and S1P**. Huang et al, Lysophospholipid mediators of immunity and neoplasia Biochimica et Biophysica Acta (BBA), 2002

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3. Immune-modulation

S1P, SPC, S1P 유사체

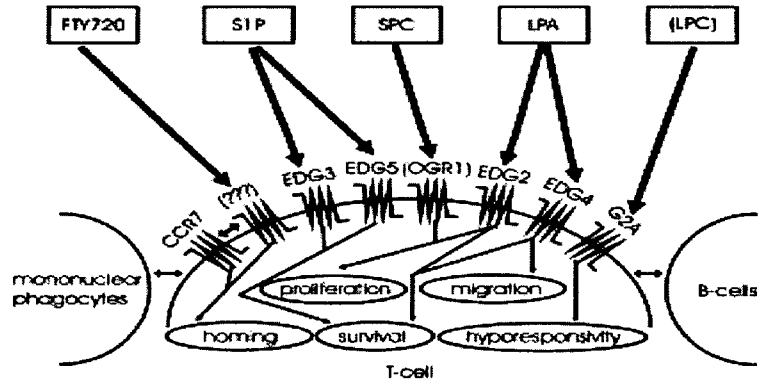

Fig. 1. Effects of lysophospholipids on T-cells. Various lysophospholipids bind to specific GPCRs inducing different cell physiological responses and affecting the ability of T-cells to interact with phagocytic monocytes or B-lymphocytes. The suggested involvement of molecules put in parentheses has not yet been shown.
Graler and Goetzl. LPA and their GPCR's in Inflam and Imm. BBA 2002

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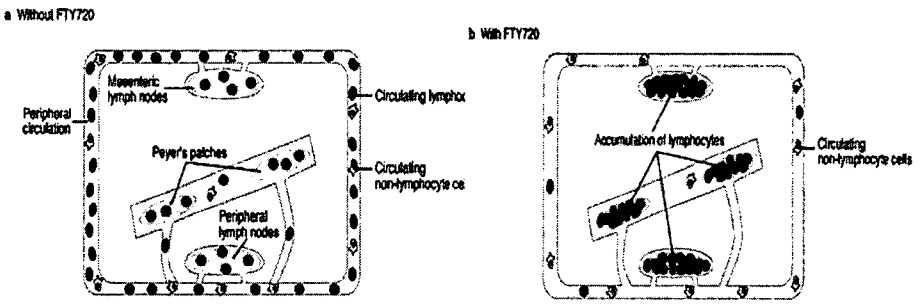
Multi-lamellar emulsion(MLE)

3. Immune-modulation

Immune disorder- atopy dermatitis



- 면역세포(T-cell)을 림프절로 모이게 하는 효과
- 면역력을 저하시키지 않으면서 면역조절기능
- 이상적인 면역조절제

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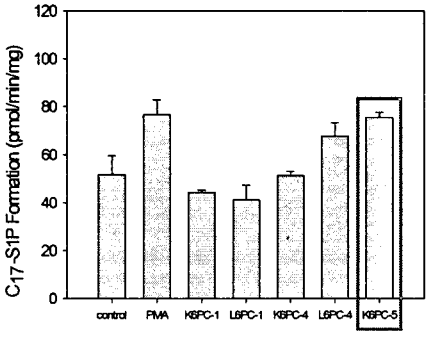
S1P 유사체의 합성 및 활성

- **sphingosine kinase(SK)활성화 확인**
- **Fibroblast 의 세포증식 효과 확인**
- **각질세포(HaCaT cell)의 분화 효과 확인**
- **Fibroblast 세포에서의 콜라겐 합성 촉진효과**
- **광 노화에 의한 주름 방지 효과 확인**

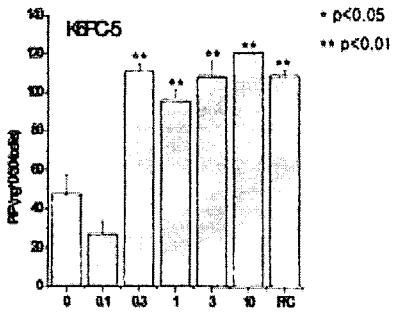
실험기관 : 충북대학교 약학대학, 생명공학연구원
관련연구과제 : Sphingosine kinase activation 물질의 합성 및 응용(충북대약대)
: 주름방지기능성소재개발(생명공학연구원)

SK 활성화능/콜라겐 합성능

Treatment PMA and Neopharm reagent in F_{9-12}cells



-SK 활성화능 확인
-실험: 충북대학교 약학대학(이용문교수)



-콜라겐 합성 촉진 확인
-실험:생명공학연구원(김환묵 박사)

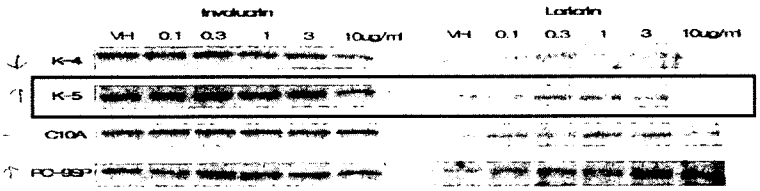
Multi-lamellar emulsion(MLE)

4. New S1P 유사체

Fibroblast증식/HaCaT 분화능

1) Fibroblast

	C10A	PC-DSP	K-1	K-4	K-5	Retinol
10 ug/ml	125.8±6.7	113.5±5.8	106.4±5.5	130.3±5.1	100.2±8.4	106.8±5.1
3	108.7±3.5	90.3±7.9	103.0±6.8	99.5±6.1	132.8±5.2	122.8±3.9
1	116.5±5.0	87.1±3.7	103.1±7.0	102.9±6.6	121.8±8.3	133.6±4.3
0.3	108.9±8.5	73.3±7.4	106.5±2.5	86.4±3.3	130.6±8.7	117.2±9.9
0.1	89.4±7.5	80.9±4.2	99.4±10.3	81.6±9.8	121.8±3.7	126.6±6.2



- Fibroblast에서의 증식/HaCaT cell에서 분화효과 확인
- 실험 :생명공학연구원(김환묵 박사팀)

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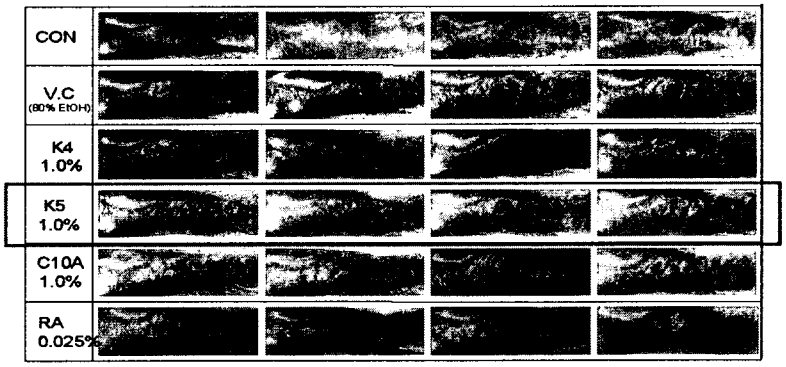
Multi-lamellar emulsion(MLE)

4. New S1P 유사체

In-vivo 광노화 억제효과



- 광 노화로 인한 주름방지 효과 확인
- 레티노익산(RA)에 비해 부작용 감소(생명공학연구원)

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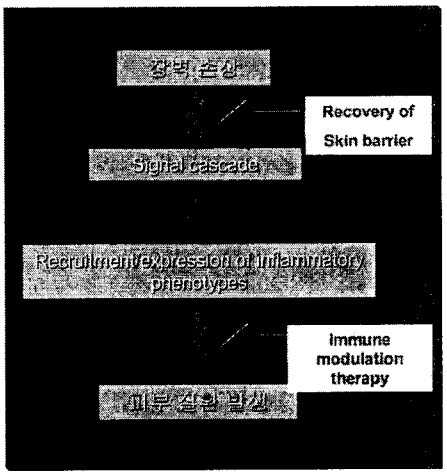
Conclusions



Recovery skin barrier function
- **Recovery of skin barrier**
- **Maintaining skin homeostasis**

Immune-modulator
- **T cell trafficking**
- **Imune-therapy**