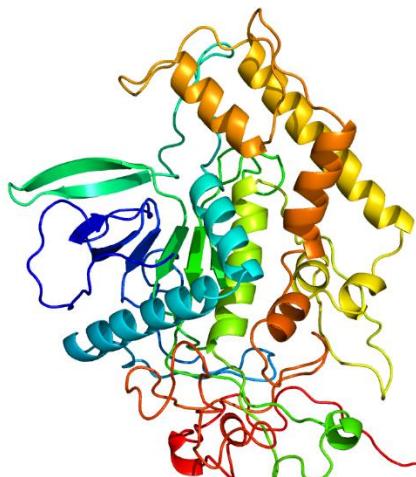


Skin Transmissive Botulinum-derivative Polypeptide

Technical description

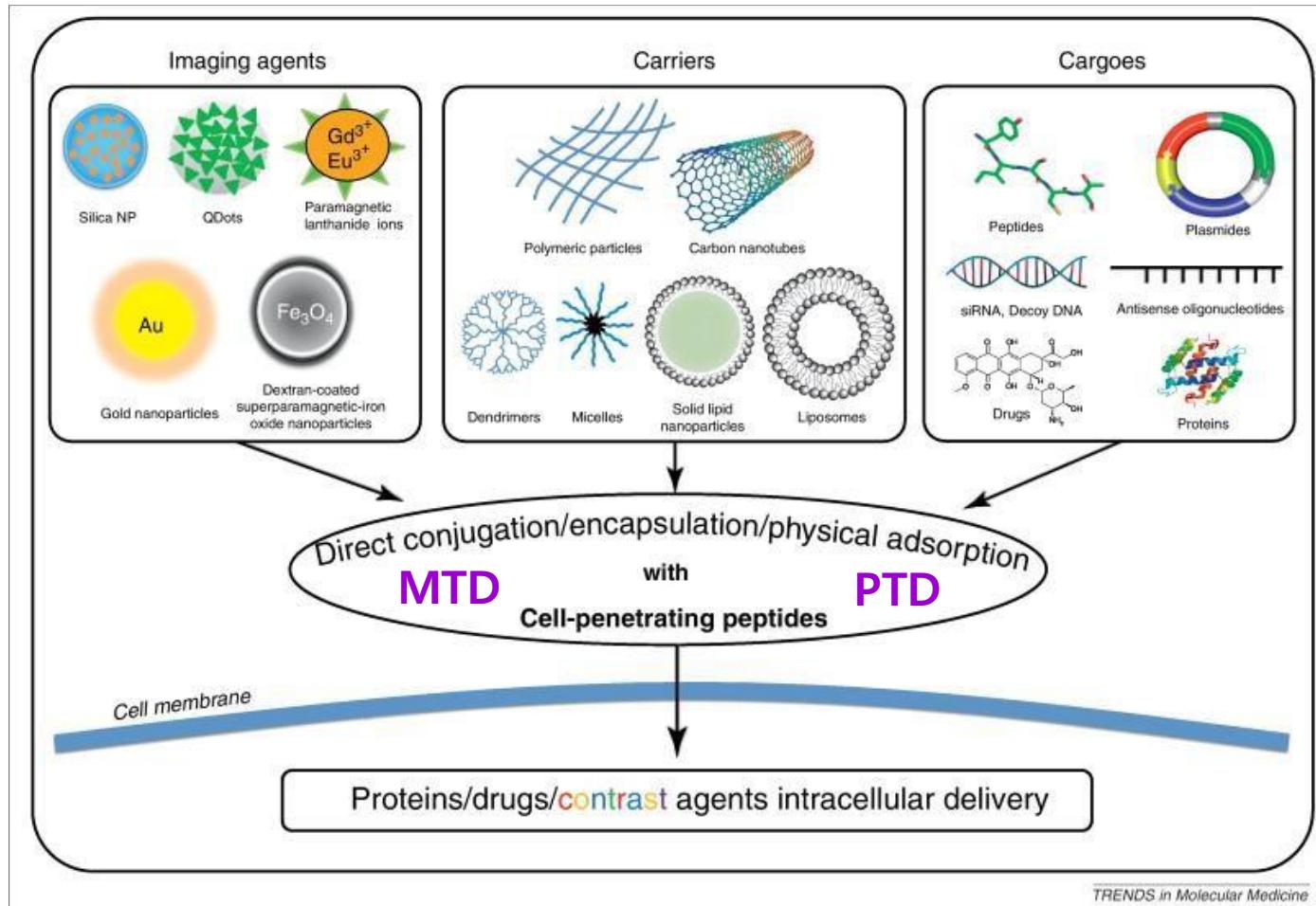
2019.05.13



About MTD compare to PTD _ Needs for Intracellular Transduction

2

Cell Permeable Peptide (CPP)



Protein Transduction Domain (PTD)

1. PTD history

- Tat(trans-activating transcriptional activator) was discovered from HIV1 (1988) as first PTD
- Other PTDs were identified.
- Amphiphilic PTDs were subsequently found

2. 1st generation PTD

Tat	YG RKKRRQRRR (11mer)	1988
Penetratin	Q R I K WFQNRRM KWKK (15mer)	1994
Buforin II	T RSSRAGLQFPV GRV HRLLRK (21mer)	2000
SynB1	RGGRLSYSRRRF STST GR (18mer)	2004
CTP-512	YGRRARRRRRR (11mer)	2006

3. 2nd generation PTD

Transportan	GWTLNSAGYLLGKINLKALAALAKKIL (27mer)	1996
PEP-1	Ac- KETWWETWWTEWSQP KKKRKV -cya(21mer)	2001
MAP	KLALKLALKAAALKLA (15mer)	1999

Macromolecule Transduction Domain (MTD)

1. MTD history

- MTS(Macromolecule Translocation Sequence) was discovered from Kaposi fibroblast factor residues 129-144(1995) as first MTD
- kFGF4 : modified MTS sequence(reduce amino acid residue)
- 193 MTD were developed by Dr. D-W. Jo (ProCell)

2. MTS(MTD origin)

MTS	AAVALLPAVLLALLAP(16mer)	1995
kFGF4	AAVLLPVLLAAP(12mer)	1999

3. ProCell MTD (1st Generation)

MTD-008	AVLAPVVAV(9mer)	2005
MTD-077	AVLLLILAV(8mer)	2005
MTD-141	ALAVIVLVLL(11mer)	2005
MTD-181	AVLLLPAAA(9mer)	2005

- Hydrophobic amino acids & central proline or not
- Proteinase resistant and Low immunogenicity

About MTD compare to PTD _ Cell Penetration Mechanism

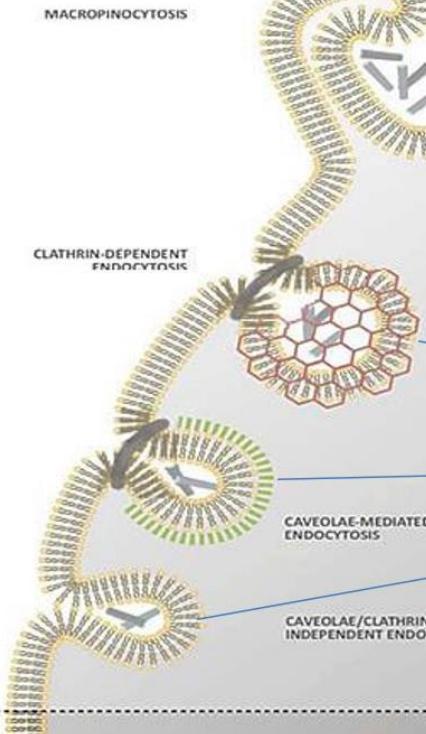
5

Cell Penetration Mechanism (PTD & MTD)

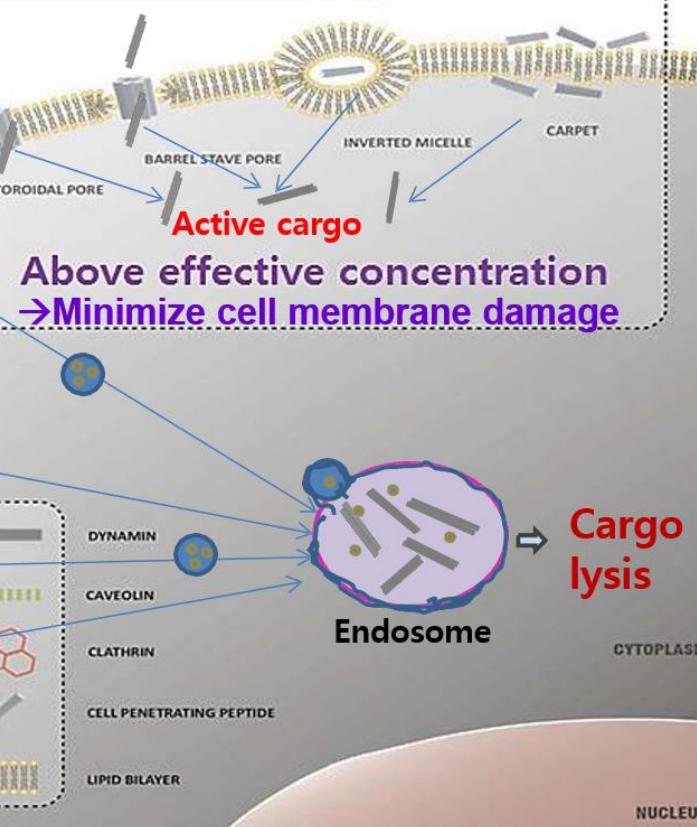
PTD :

Endocytosis

Endocytosis



Direct Translocation



MTD :

Direct
Membrane
Translocation

About MTD compare to PTD

- ✓ Currently, most cosmetic companies are using PTD (Protein Transduction Peptide) technology
 - ✓ BPMed Cosmetic is using advanced MTD (Macromolecule transduction domain) technology

	PTD	MTD
Definition	Protein Transduction Peptide	Macromolecule Transduction Domain
Penetration	<ul style="list-style-type: none"> - The penetration of effective ingredient to the dermis layer is very low and not meaningful. - It needs very high concentration for efficacy in cells. - There is no successful case with PTD yet. 	<ul style="list-style-type: none"> - The penetration of effective ingredient to the dermis layer is significant. - Moreover, actively permeates target cells. (Nerve cell, for our Botulinum Ingredient)

Example of PTD technology linked to Botulinum

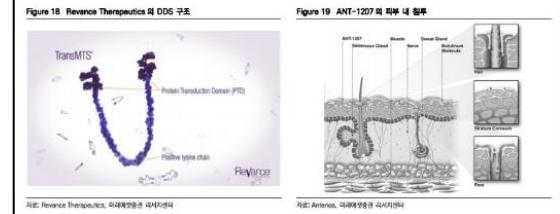
REVANCE THERAPEUTICS (NASDAQ)

Announced **Failure** of Topical Botulinum Toxin Development (JAN. 2016.)

They linked PTD technology to there Botulinum Toxin.



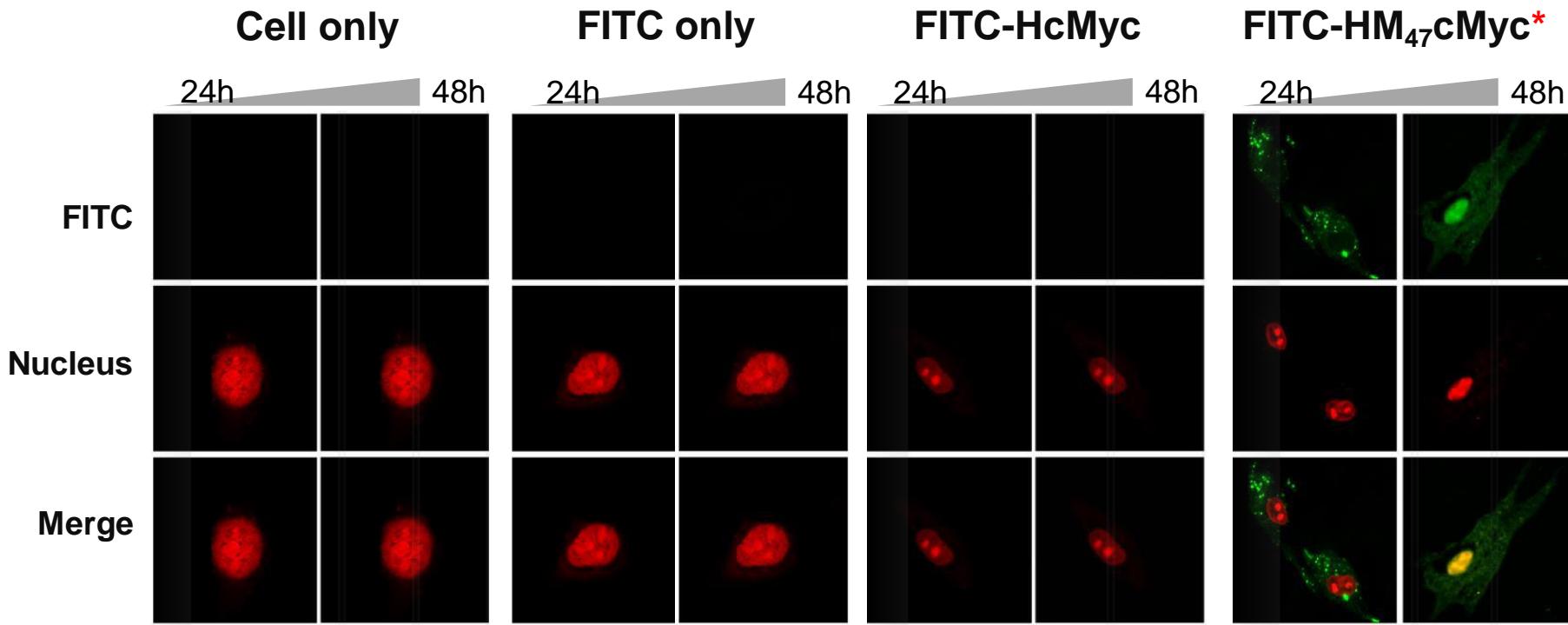
보툴리눔 속신 사건은 항후 구조 및 재활의 다양화가 예상된다. 페리ックス & 이센은 액상형 보툴리눔 독신을 개발하고 있다. 악성생물은 주로 미세 세균과 악성 세균으로만 인식되는 바이러스를 줄여주며, 청정한 환경도 주입할 수 있는 경향이 있다. DOS 기술을 이용하여 바이러스를 보툴리눔 독신을 주입하는 혼 태입법은 보툴리눔 독신을 개발하고 있다. Allergen은 지난 17일 파피와 미세 생물 세균을 개발하고 있다.는 Anterior을 출시했다. Allergen은 미세생물의 결합을 위한 NC3D 기관과 ANT-1207 파이로리아의 관계에서 대체로 9년 전에 암모스를 출시했지만, 기계화 및 상용화에 따른 미세생물은 기초 연구 자체를 예상된다. Anterior은 더불어 Revance Therapeutics도 PTD를 통한 보툴리눔 독신을 개발하고 있다.



- Cell permeable protein conjugation mode

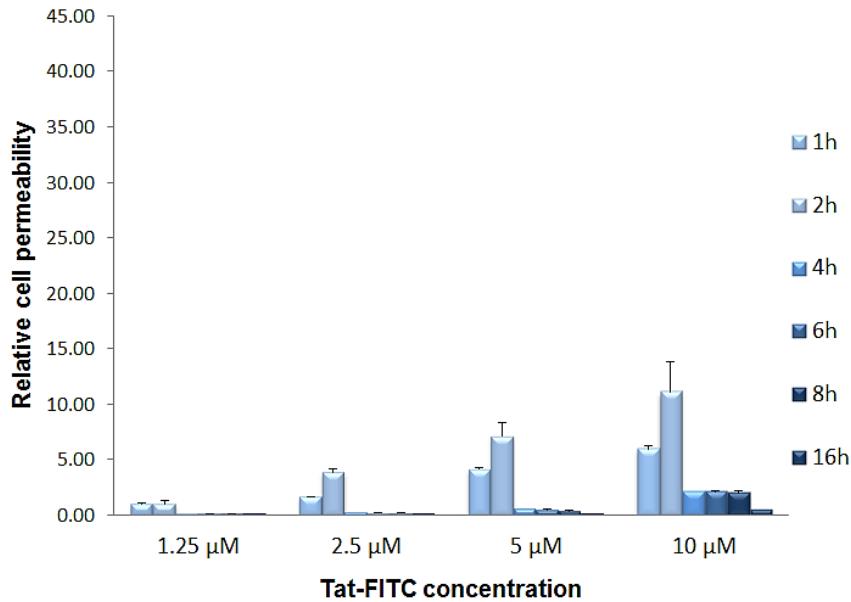


* Nucleus Localizing Sequence : homing peptide

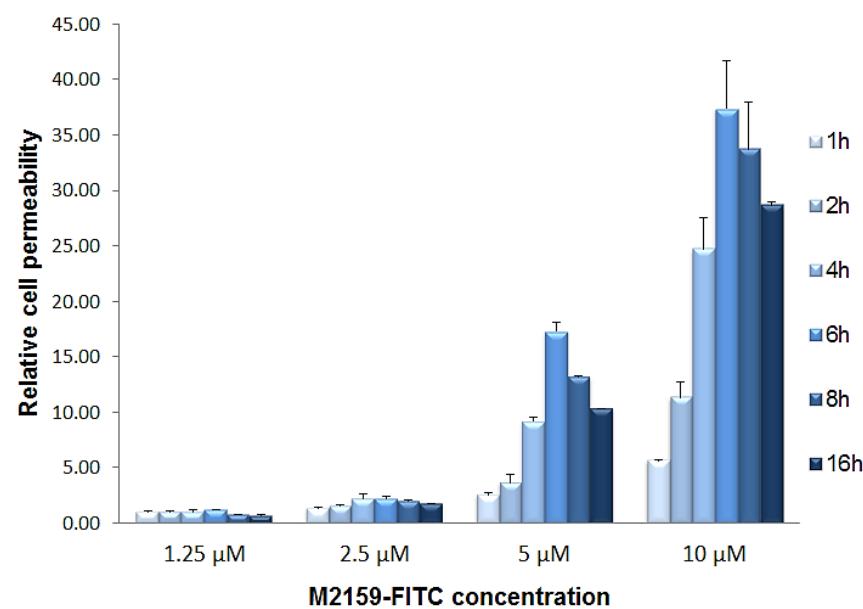


- MTD is compatible with other homing sequence, but PTD's not.

● PTD: TAT-FITC



● MTD: MTD-FITC

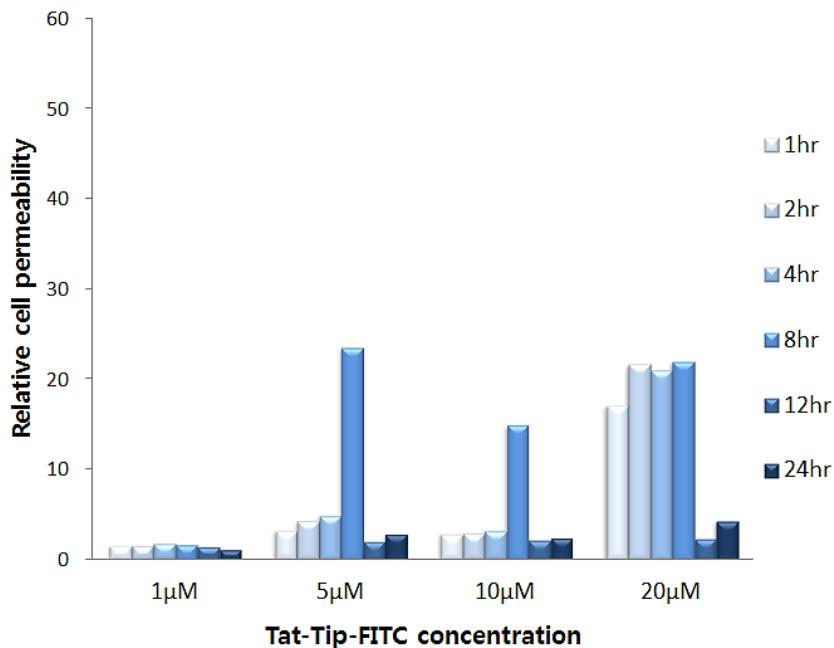


- HCT116 cell, FACS analysis
- MTD permeability to colon cancer cells increased in a concentration-dependent manner.
- MTD permeability to colon cancer cells increased in a time-dependent manner

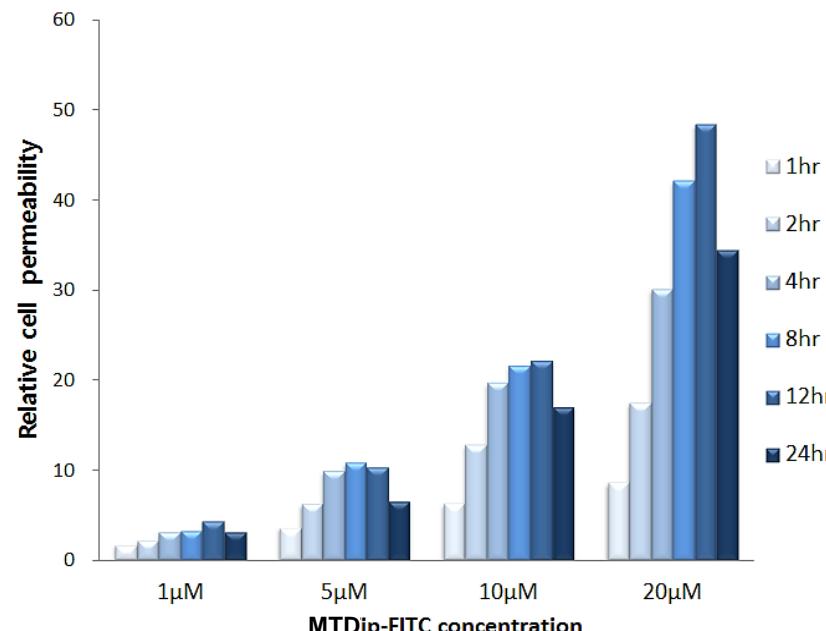
About MTD compare to PTD _ Cell permeability of PTD & MTD-TIP-FITC

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● PTD: TAT-TIP-FITC



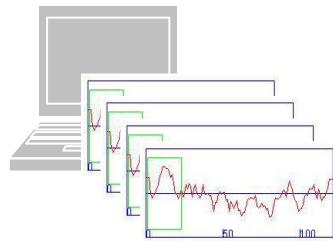
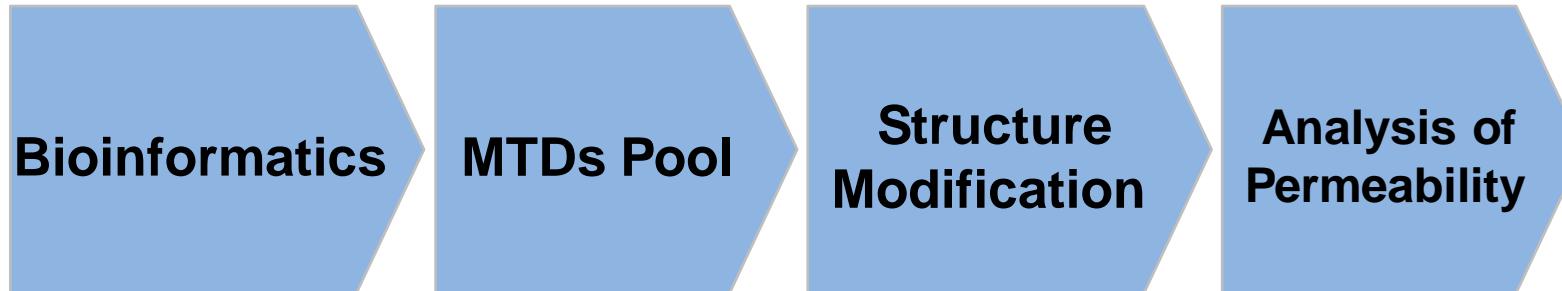
● MTD: MTD-TIP-FITC



- HCT116 cell, FACS analysis
- TAT- TIP*- FITC : TAT- PPLSMPPRFMDYWEGLNENG- FITC
- MTD- TIP- FITC : MTD- PPLSMPPRFMDYWEGLNENG- FITC
- TIP : Thioredoxin Insert Protein Peptide
(reference peptide, 19 amino acids)

Concentration & treatment time dependency is very important,

1. 1st Generation



- 7~15 aas
- Alpha helix
- hydrophobic
- Eliminate Proteinase cleavage sequence
- Proline position
- GFP-MTD fusion protein
- Cell permeability analysis

2. 2nd Generation

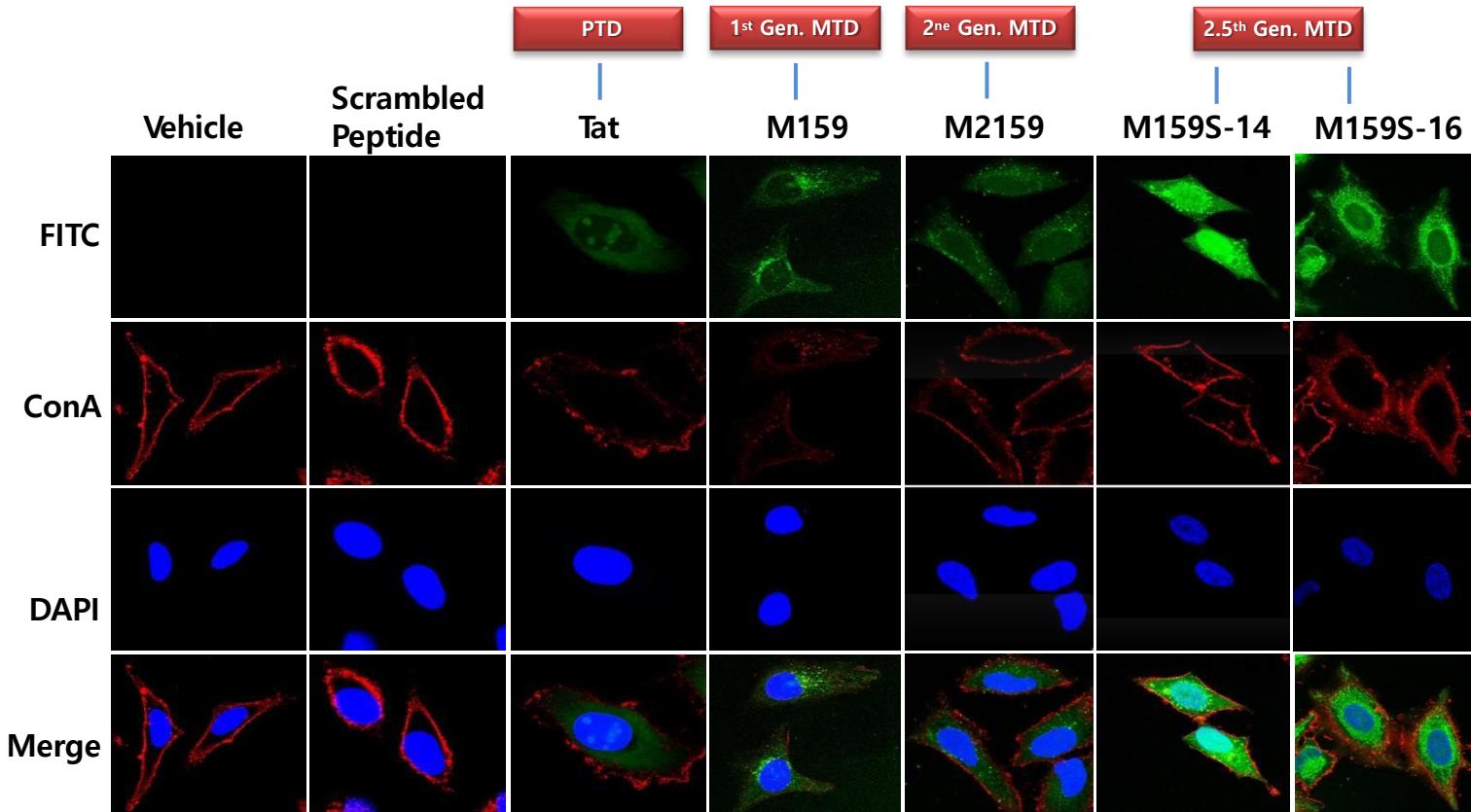
- Amino acid substitution
- Enhance hydrophilicity
- Improve cargo compatibility
- Easy biological expression



- Cargo delivery potential
 - Improve more than 10~30 folds
- Improve cargo delivery speed
- Improve cargo compatibility

MTD
Library

- Confocal Microscopy with FITC-labeled CPP(PTD & MTD)



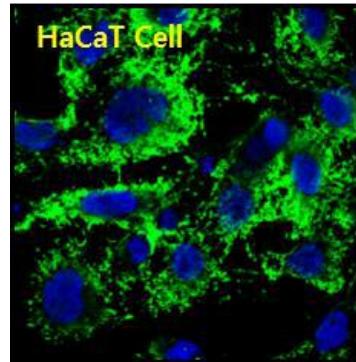
HeLa cell, 2.5uM, 6hr treatment, X400

Gain value : high(Tat/M159), low 2 & 2.5 Gen. MTD

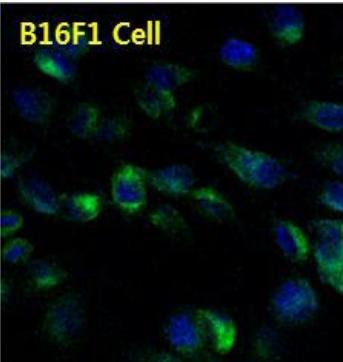
Example of MTD _ Development of skin barrier penetrating MTD

12

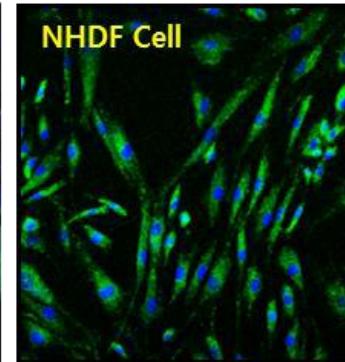
1. 3 types of skin cell permeability of FITC labeled MTD



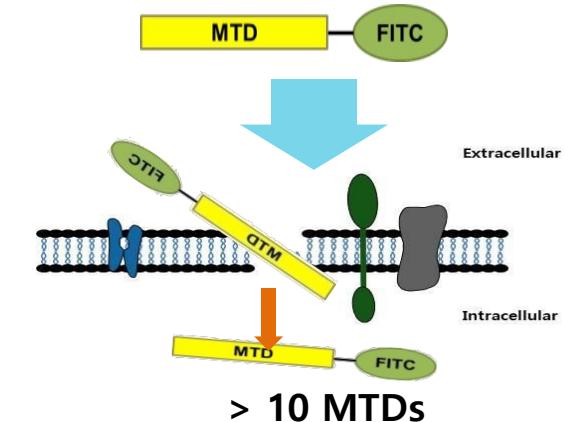
Keratinocyte



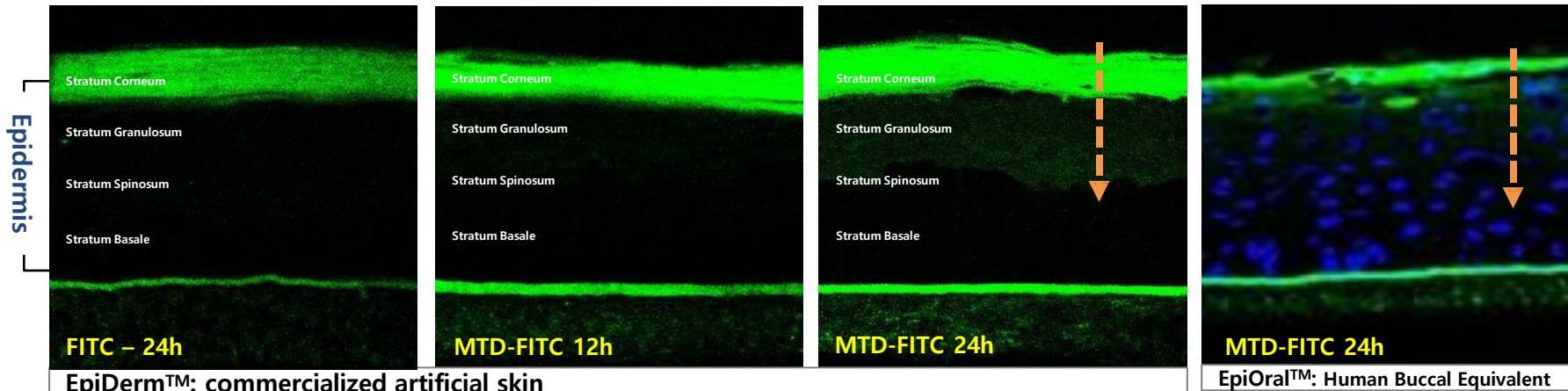
Melanocyte



Fibroblast Cells

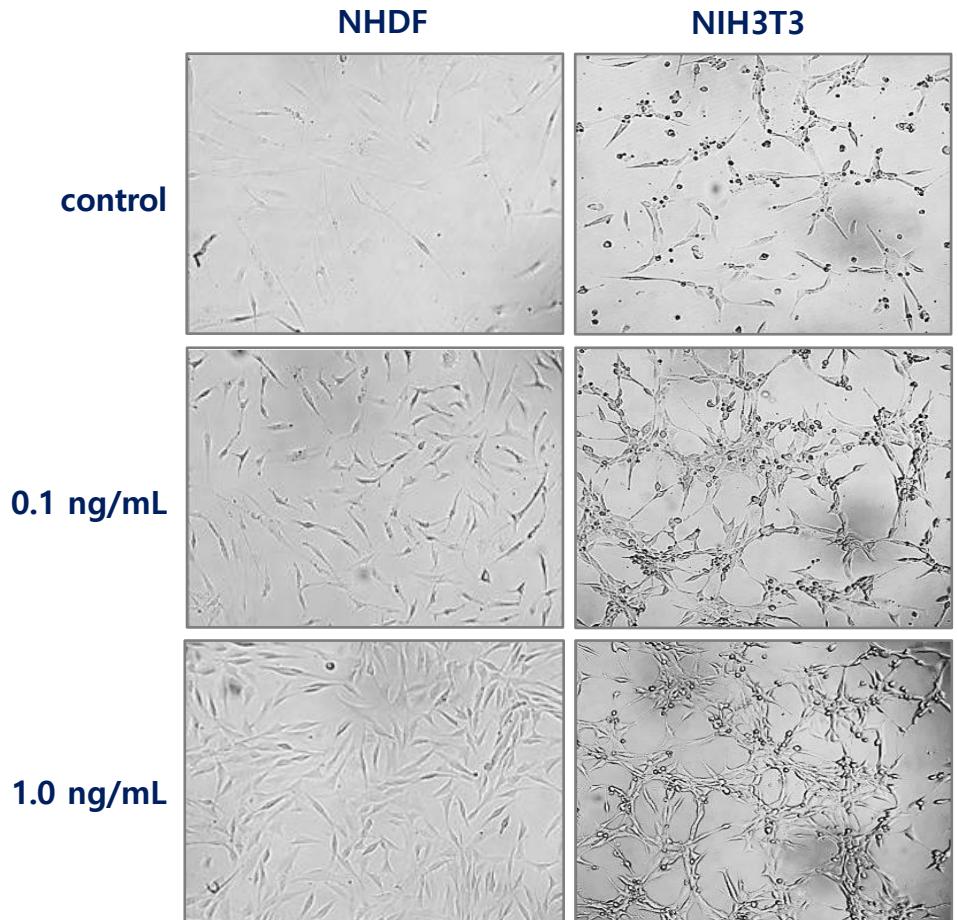
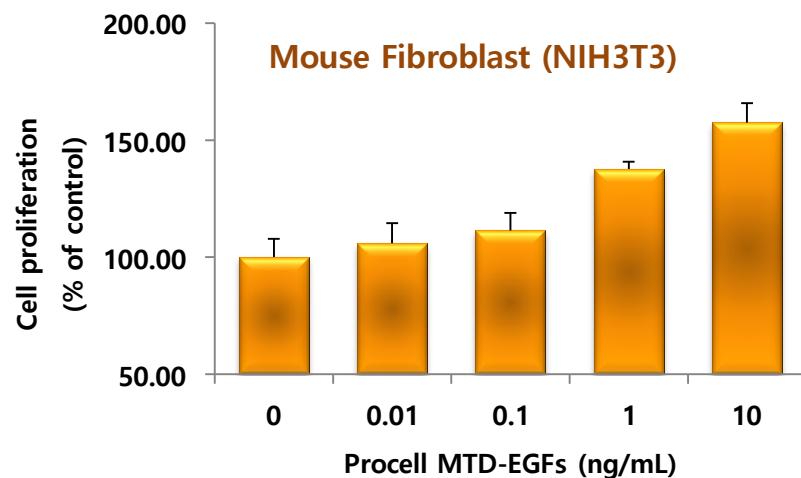
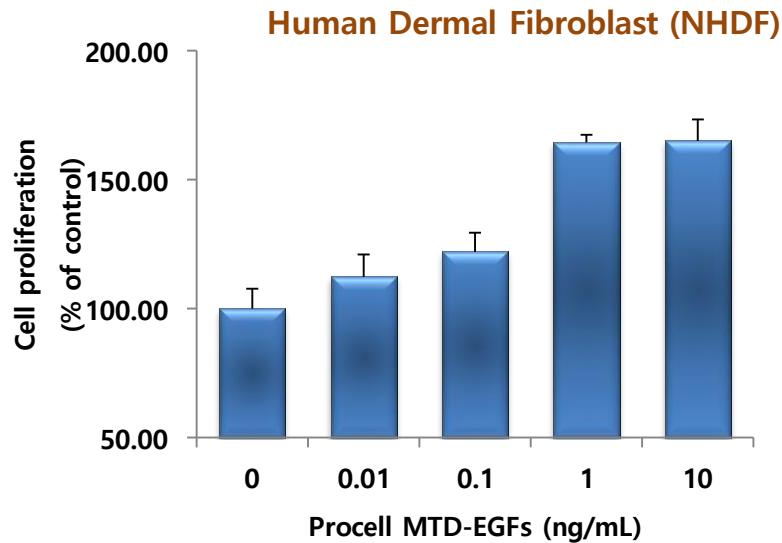


2. Artificial skin penetration of FITC labeled MTD



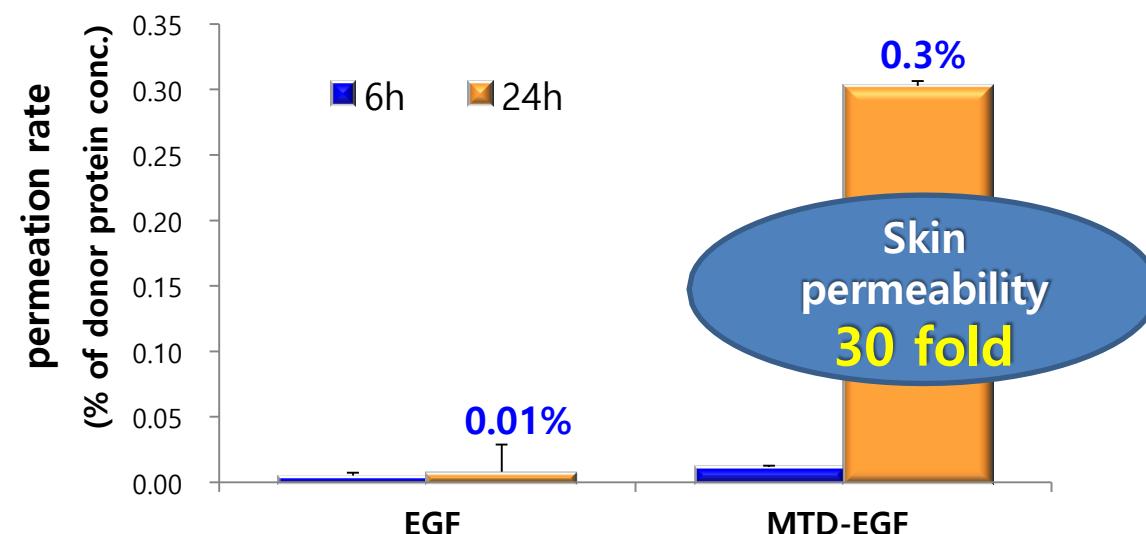
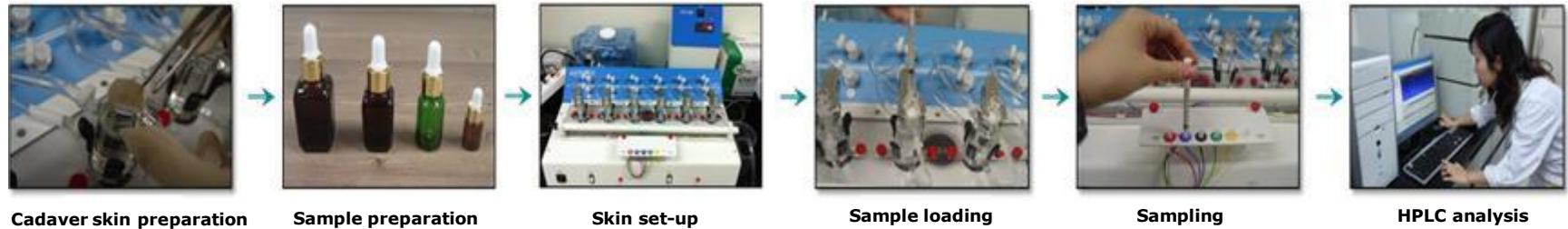
Example of MTD _ Fibroblast proliferation effect of MTD-EGF

13



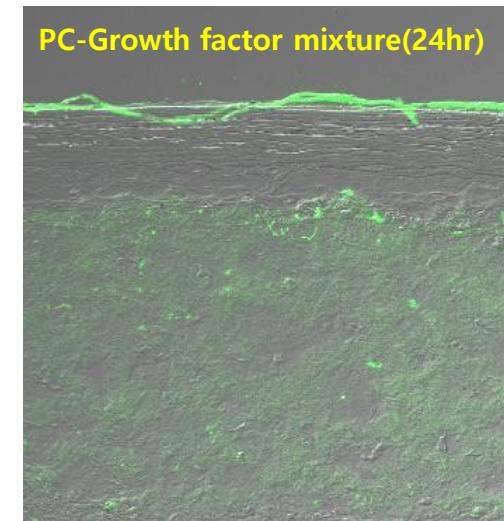
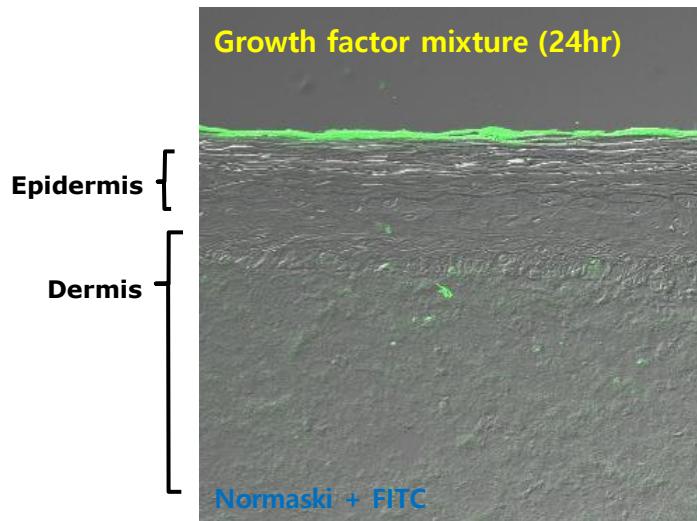
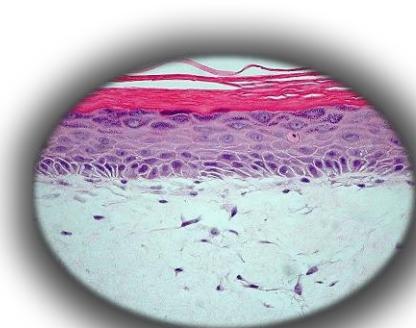
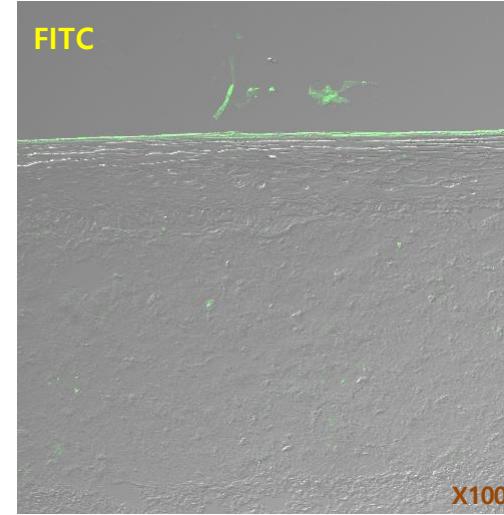
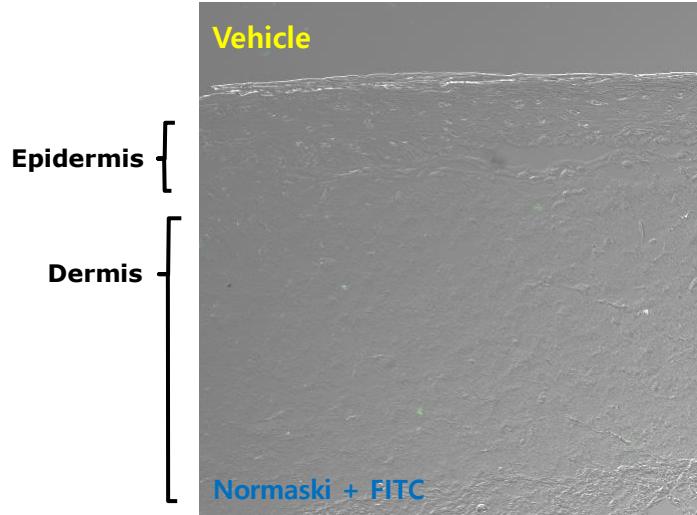
Skin fibroblast Cell proliferation was increased after
72 hr treatment of MTD-EGF

- *In vitro* Cadaver skin penetration assay



Skin permeability of MTD-conjugated EGF(PC-EGF) is 30 times more than that of EGF after 24 hour treatment on cadaver skin.

- Tissue permeability of FITC labeled GF mixture conjugated with MTD or not



EGF/PC-EGF

bFGF/PC-bFGF

VEGF/PC-VEGF

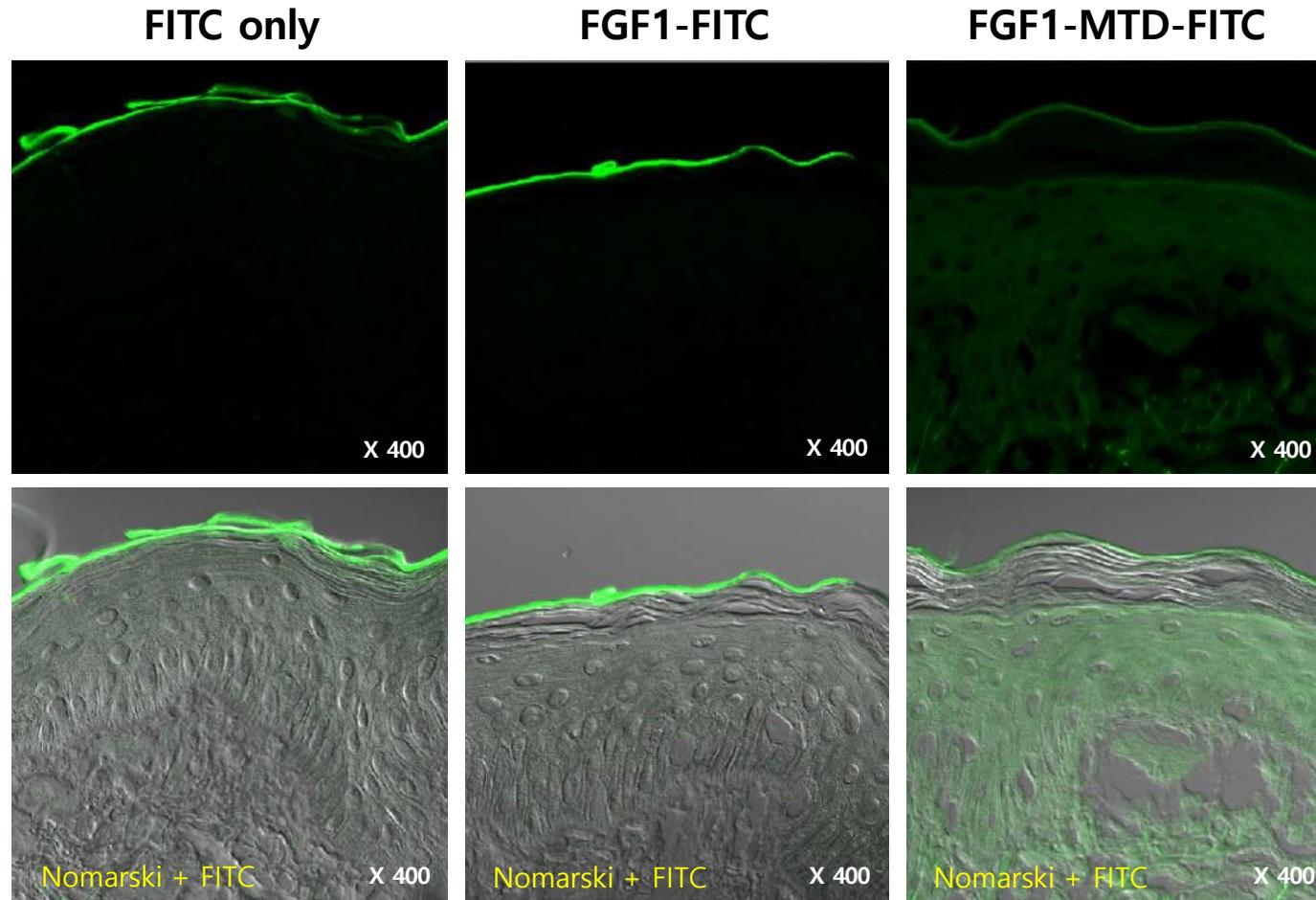
Treatment conc.

5ug/cm² each GF

ZEISS LSM 700

Example of MTD _ Porcine skin penetration of FGF1 & FGF1-MTD

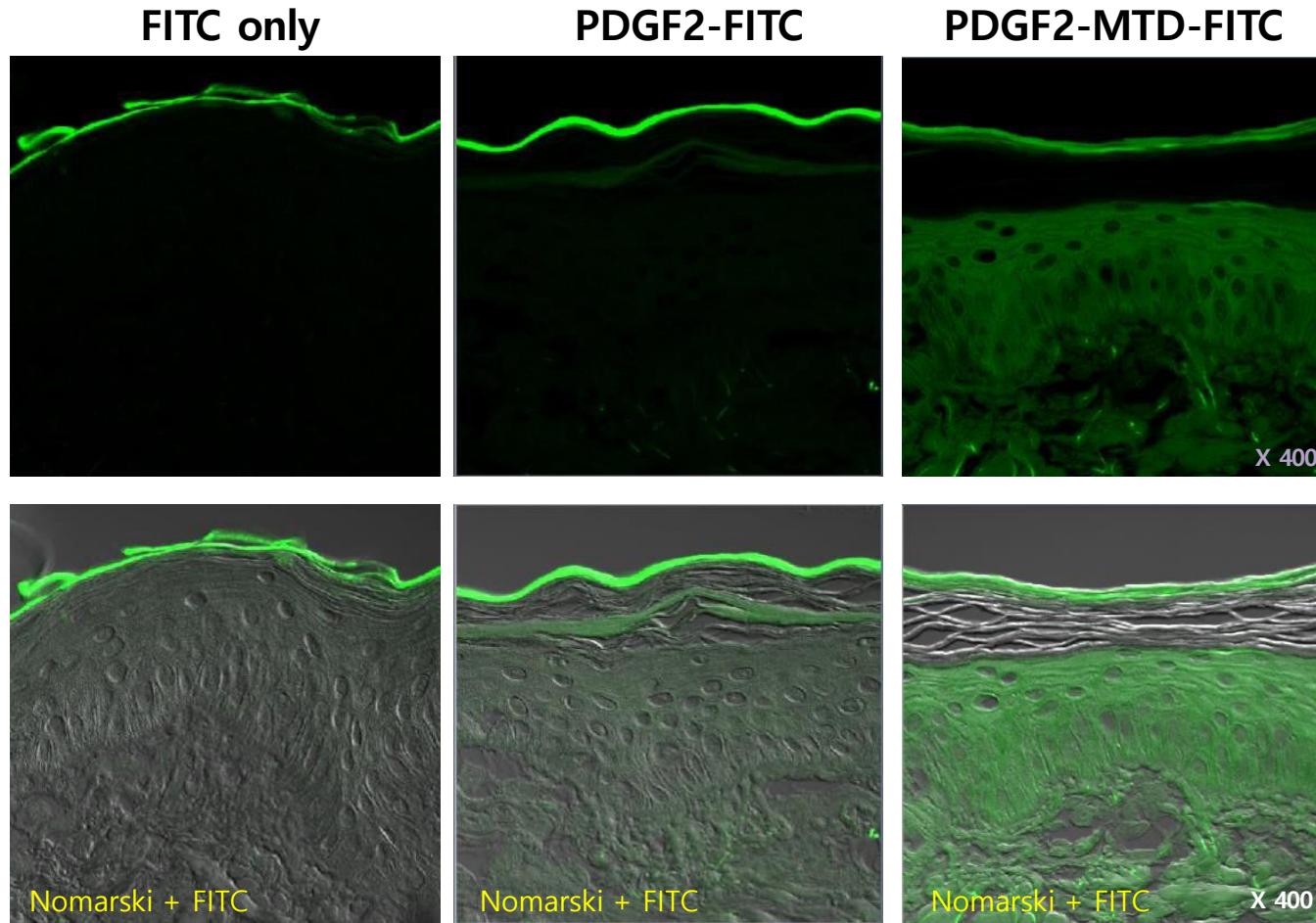
16



**10 μ g of each FGF1 and FGF1-MTD in 50 μ L of PBS(pH 7.0)
for 24 hours at 37°C on 1cm² porcine(mini pig) skin**

Example of MTD _ Porcine skin penetration of PDGF2 & PDGF2-FITC

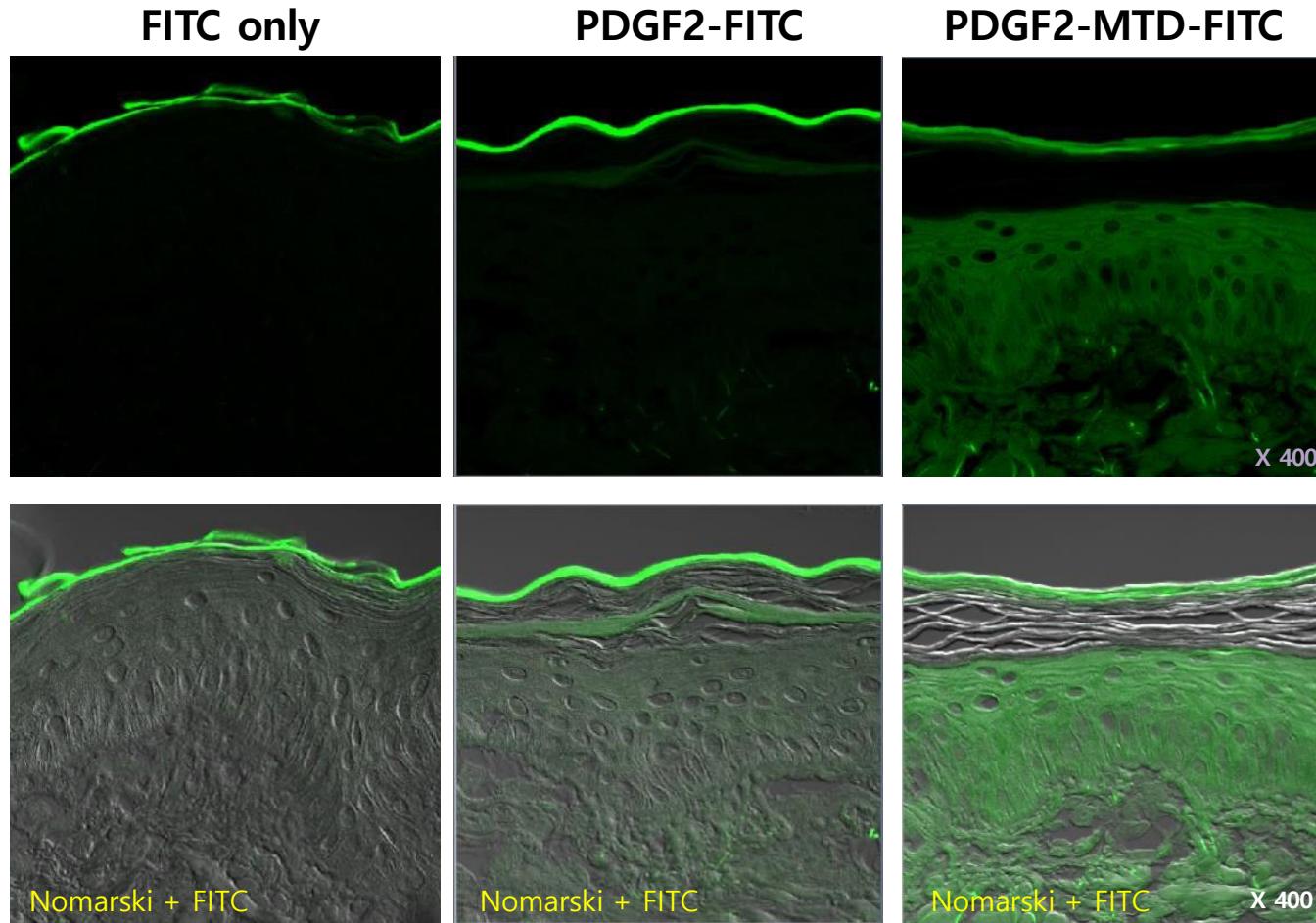
17



10 μ g of each FITC labeled PDGF2 and PDGF2-MTD in 50 μ L of PBS (pH 7.0) treatment for 24 hours at 37°C on 1cm² porcine(mini pig) skin

Example of MTD _ Porcine skin penetration of PDGF2 & PDGF2-FITC

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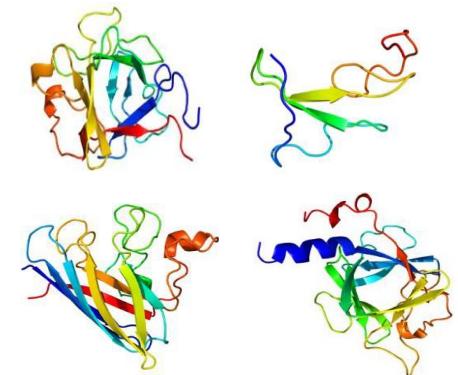
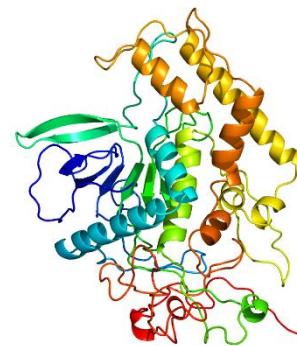


10 μ g of each FITC labeled PDGF2 and PDGF2-MTD in 50 μ L of PBS (pH 7.0) treatment for 24 hours at 37°C on 1cm² porcine(mini pig) skin

BPMed
COSMETIC



Product Solutions
What ever you want



World First
Botulinum Cosmetic Ingredient

"Methionyl r-Clostridium Botulinum
Polypeptide-1 Hexapeptide-40"

Innovative MTD linked
Peptides and Growth Factors

Most Advanced Cosmetic Formula
Which makes Real Efficacy

Appendix _ Facility

20

Facilities : FACS, Confocal microscope, HPLC(2 set), Fermentors, PCR etc.

Lab A



Lab B



Process Dev. Lab.



Imaging Room



Animal breeding Room



Cell cultivation room A



room B



Thank You