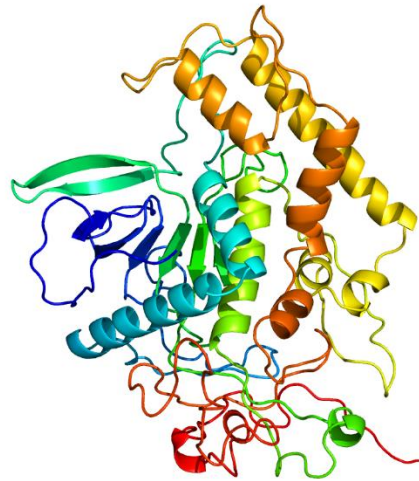


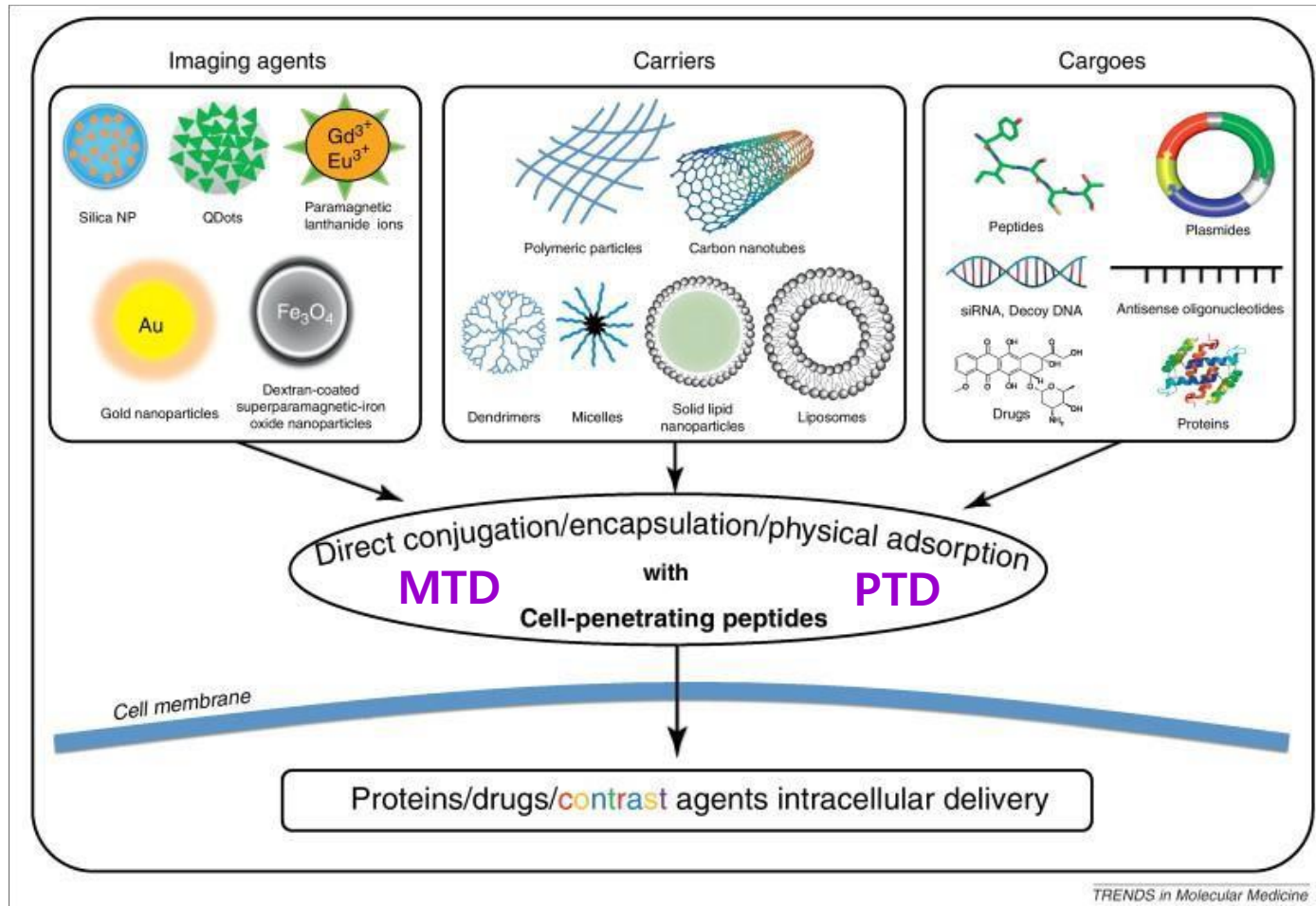
Skin Transmissive Botulinum-derivative Polypeptide

Technical description

2019.05.13



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.
- Ampiphilic PTDs were subsequently found

2. 1st generation PTD

Tat	YGRKKRRQRRR (11mer)	1988
Penetratin	QR IK WFQNRRMK WKK (15mer)	1994
Buforin II	TR SSR AGLQFPV GRVH LL RK (21mer)	2000
SynB1	RGG RLS YSRRR F STSTGR (18mer)	2004
CTP-512	YGRRA RRRRR (11mer)	2006

3. 2nd generation PTD

Transportan	GWTLNSAGYLLGKINLKALAALAKKIL(27mer)	1996
PEP-1	Ac- KET WW ET WW TE WSQP KKK R KV - cya(21mer)	2001
MAP	KLAL KL AL KA AL KL A (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

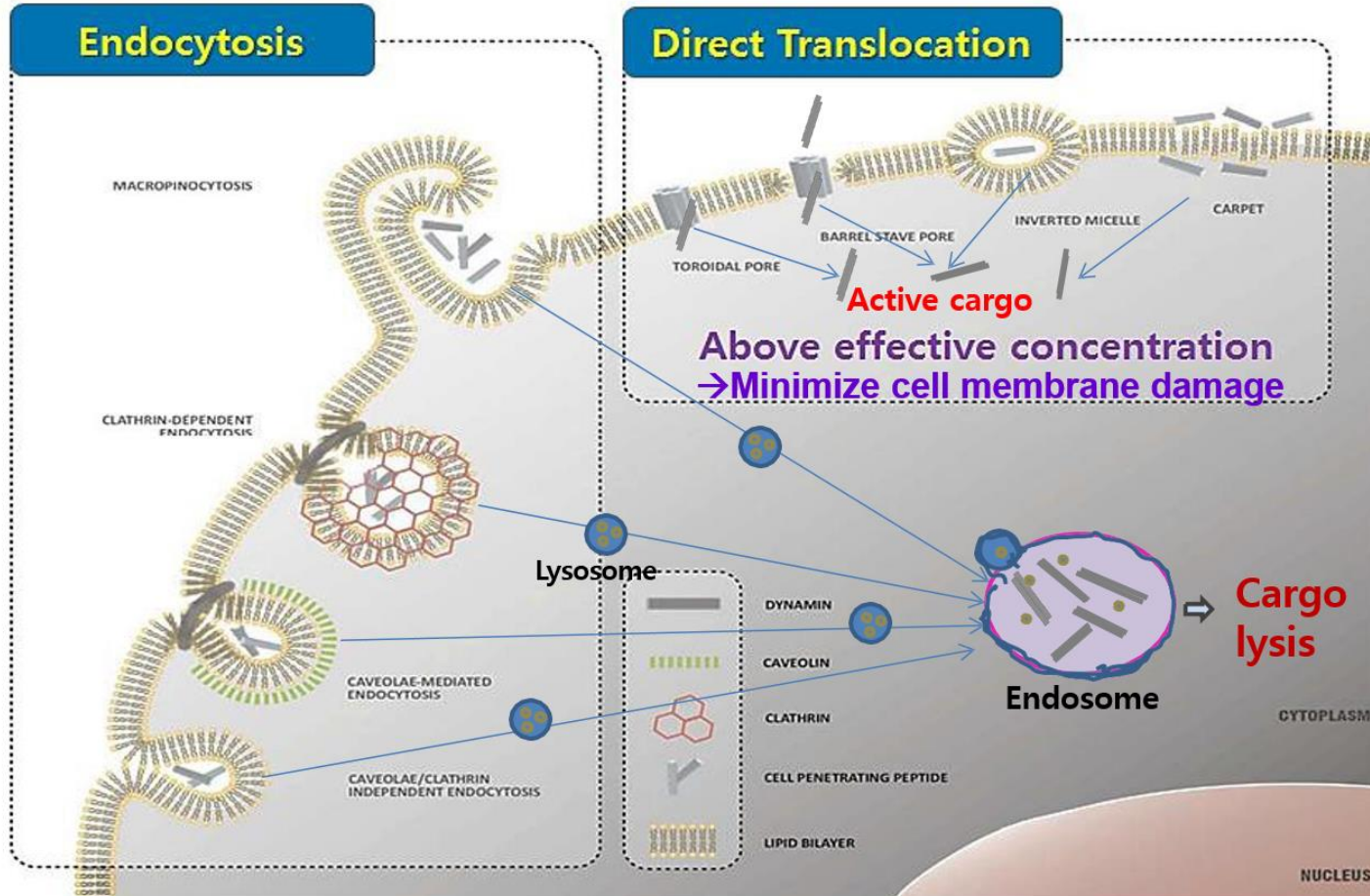
3. ProCell MTD (1st Generation)

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

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

Cell Penetration Mechanism (PTD & MTD)

PTD :
Endocytosis



MTD :
Direct Membrane Translocation

Pharmaceuticals 2010, V3, 961-993

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)

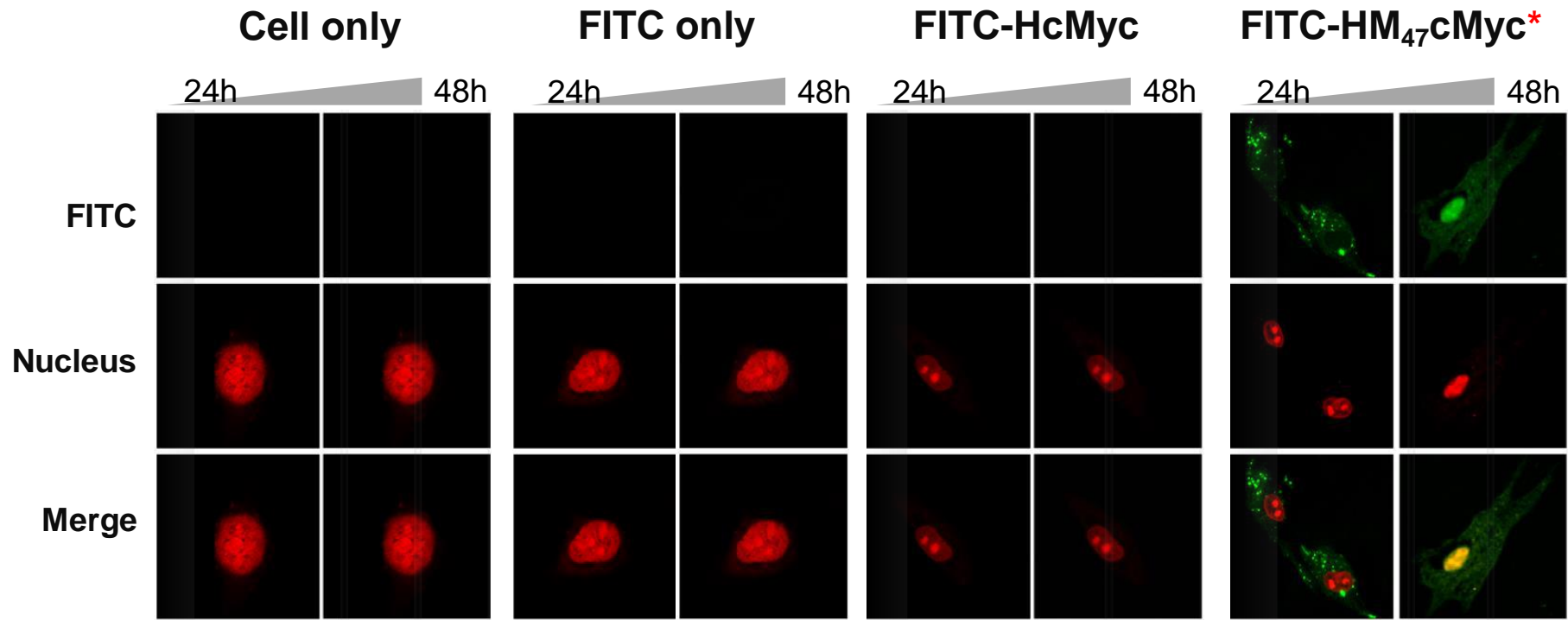
Example of PTD technology linked to Botulinum

REVANCE THERAPEUTICS (NASDAQ)
 Announced **Failure** of Topical Botulinum Toxin
 Development (JAN. 2016.)
 They linked PTD technology to there
 Botulinum Toxin.

- Cell permeable protein conjugation mode

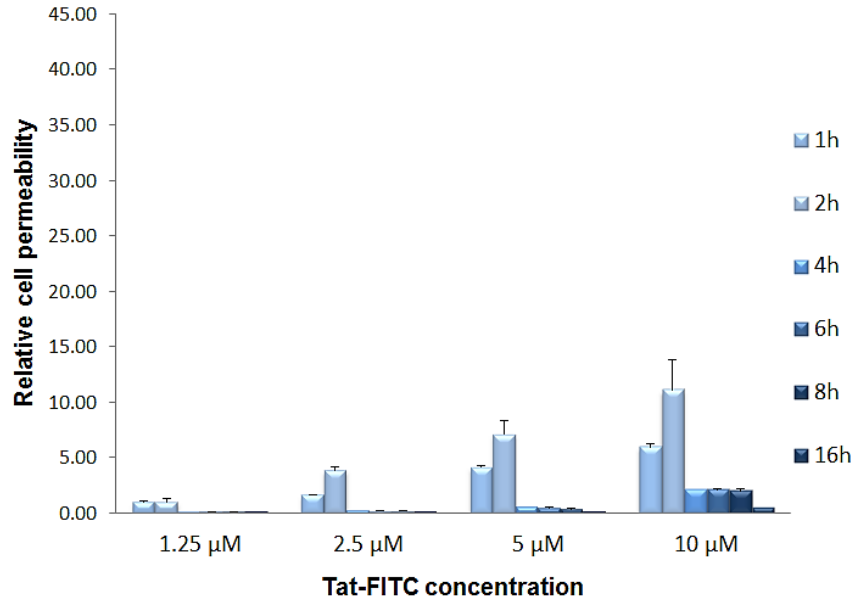


* Nucleus Localizing Sequence : homing peptide

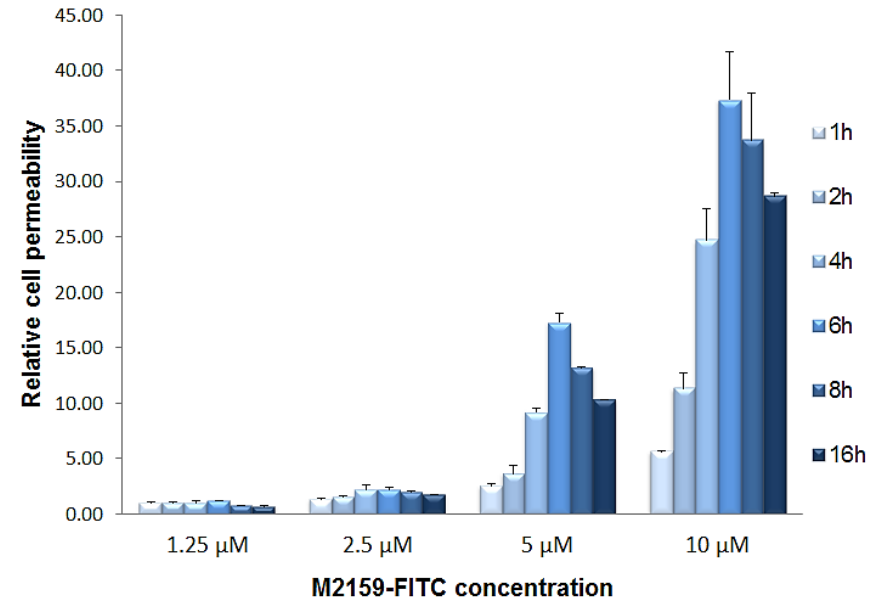


➤ MTD is compatible with other homing sequence, but PTD's not.

● PTD: TAT-FITC

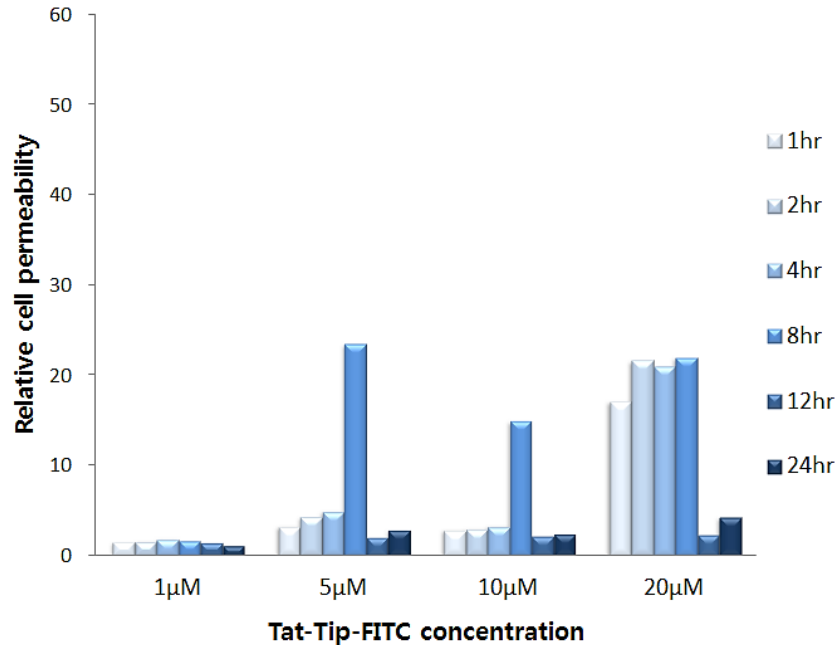


● MTD: MTD-FITC

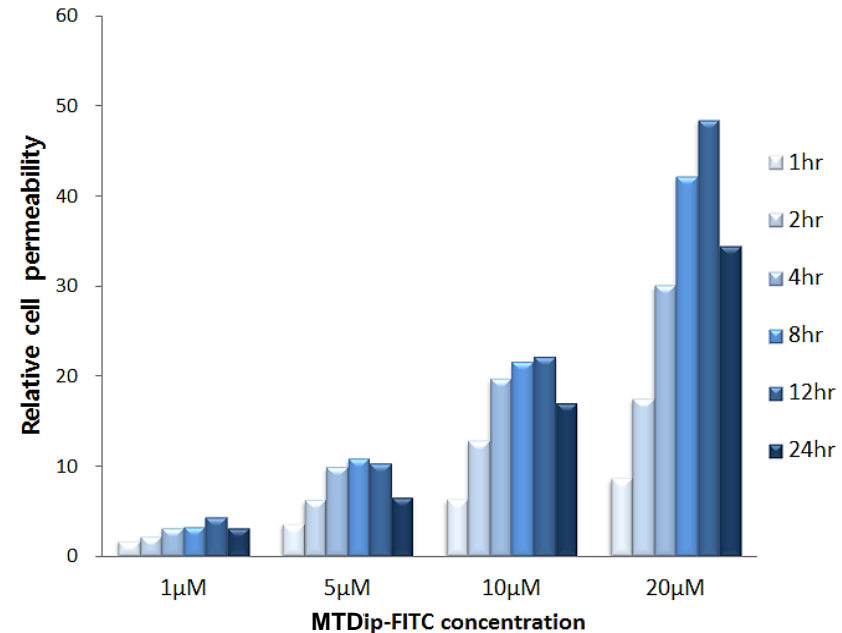


- HCT116 cell, FACS analysis
- MTD permeability to colon cancer cells increased in a concentration-dependent manner.
- MTD permeability to colon cancer cells increased in a time-dependent manner

● PTD: TAT-TIP-FITC



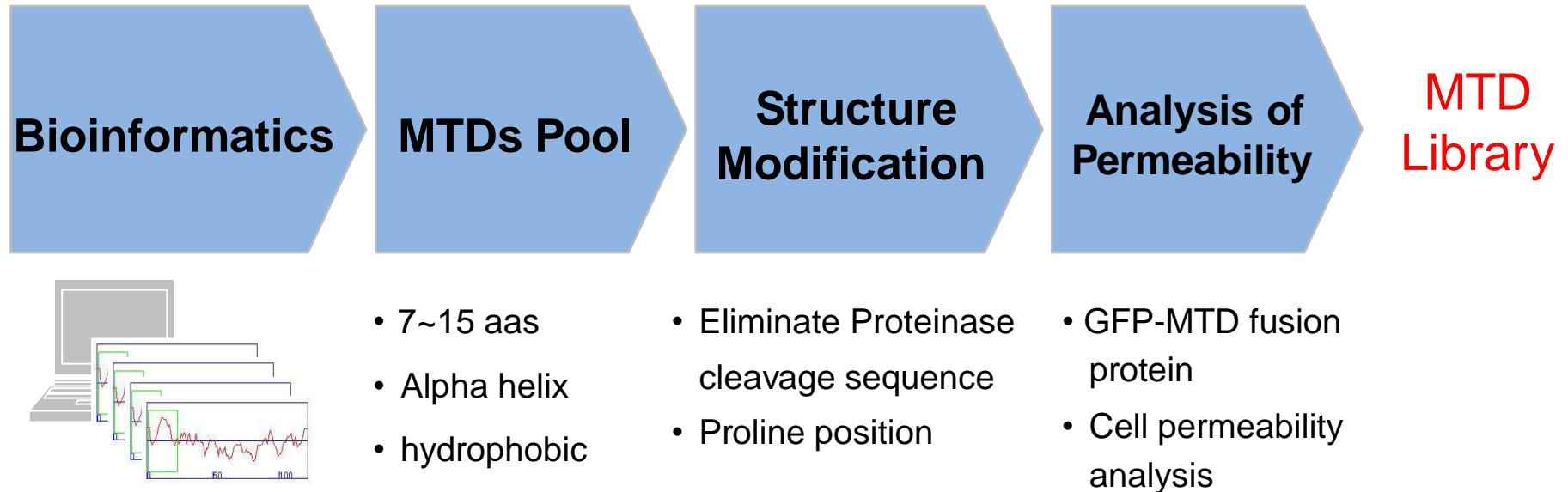
● MTD: MTD-TIP-FITC



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

Concentration & treatment time dependency is very impotent,

1. 1st Generation



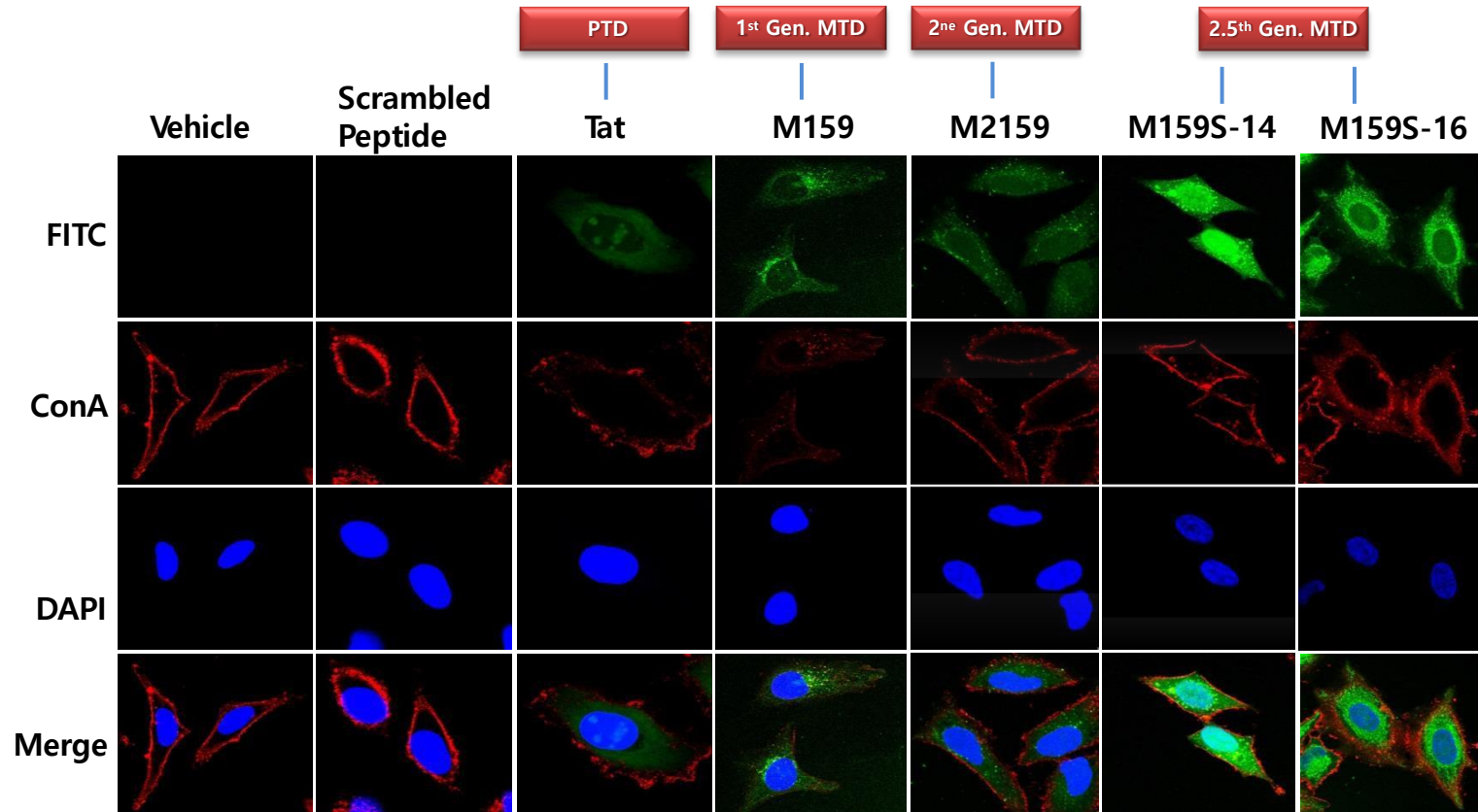
2. 2nd Generation

- 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

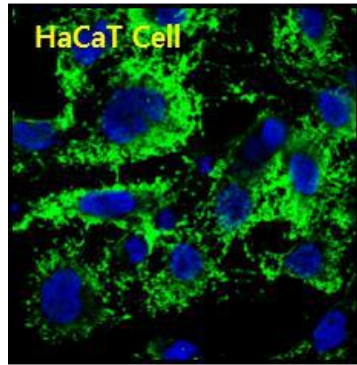
- Confocal Microscopy with FITC-labeled CPP(PTD & MTD)



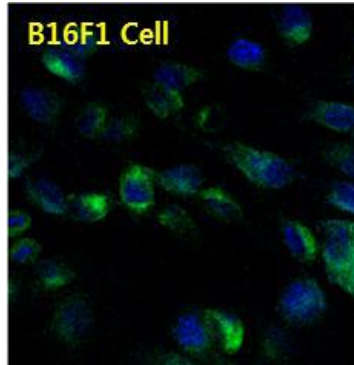
HeLa cell, 2.5uM, 6hr treatment, X400

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

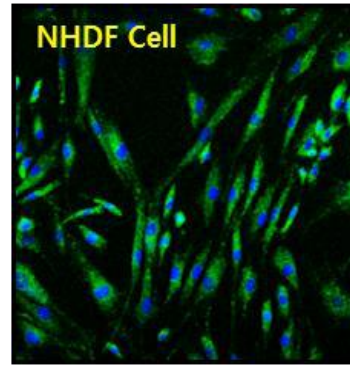
1. 3 types of skin cell permeability of FITC labeled MTD



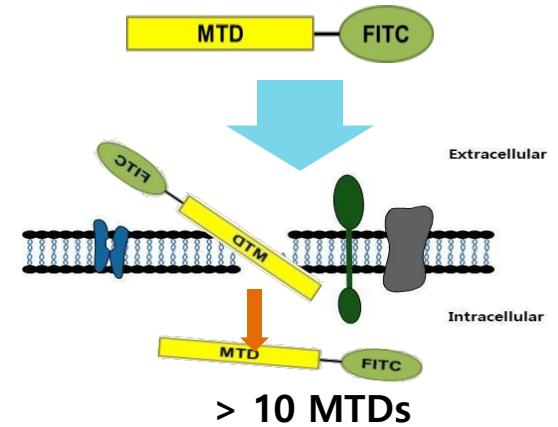
Keratinocyte



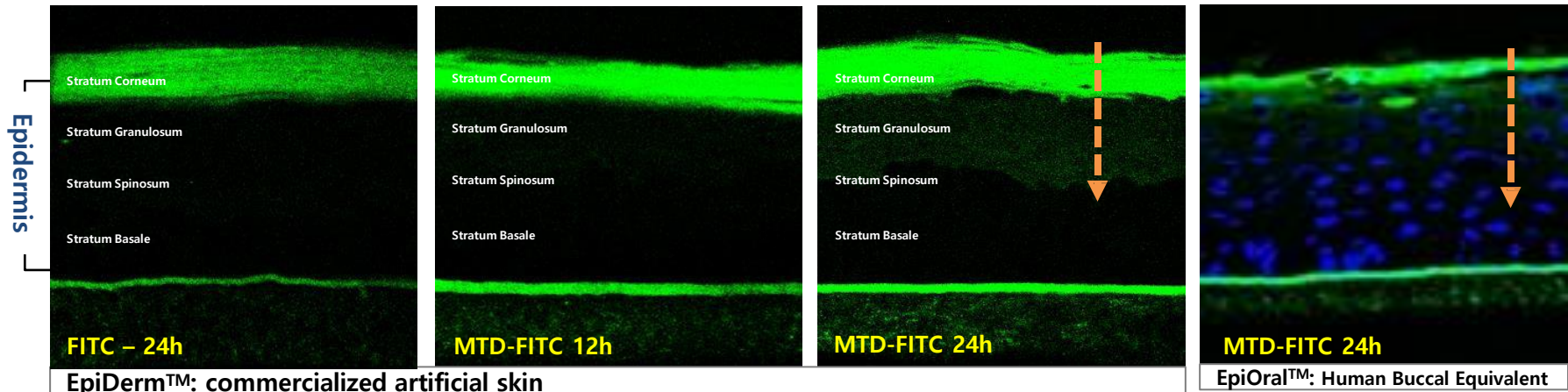
Melanocyte

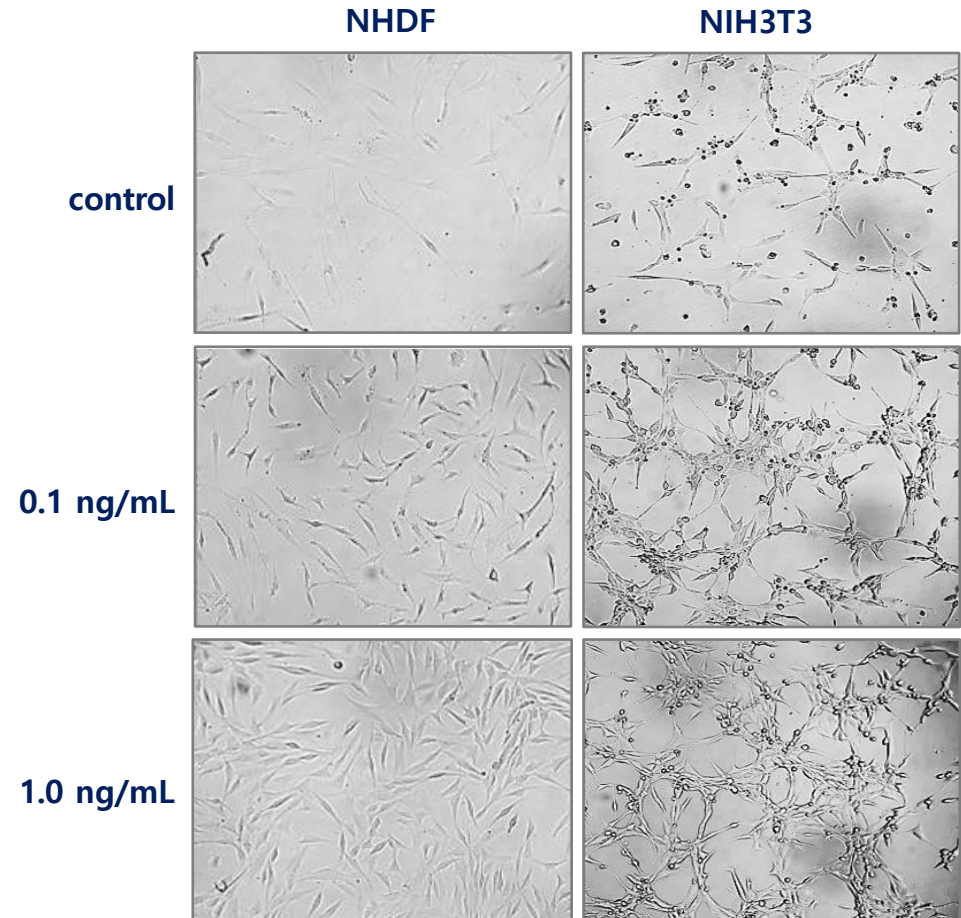
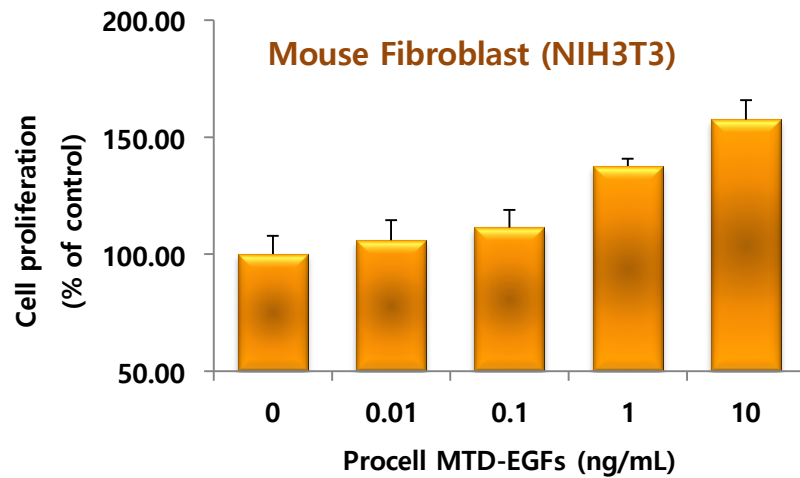
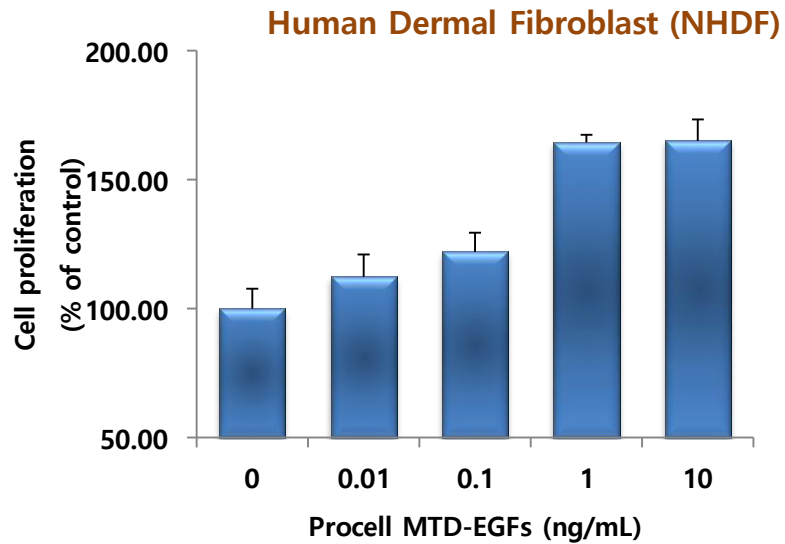


Fibroblast Cells



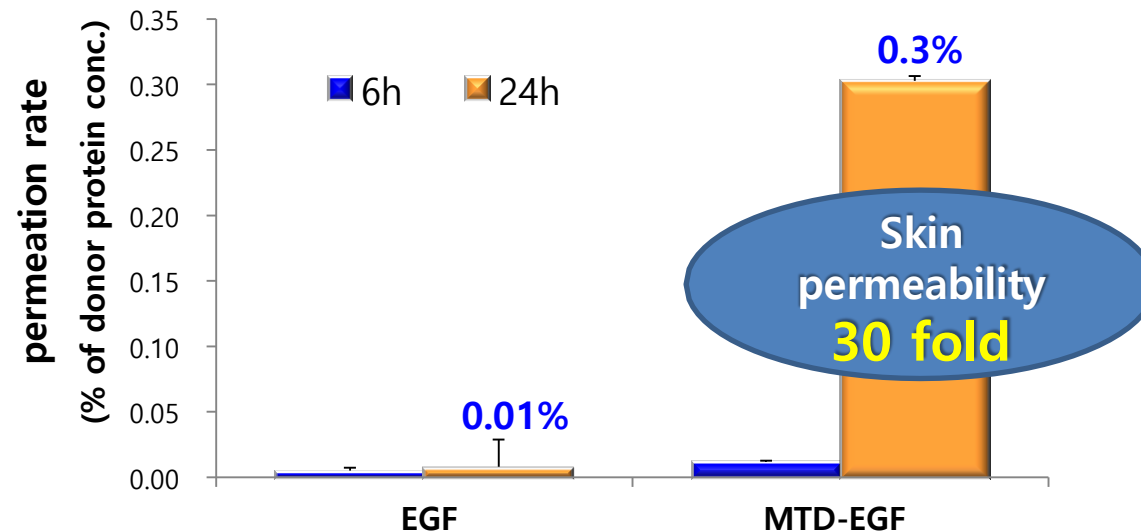
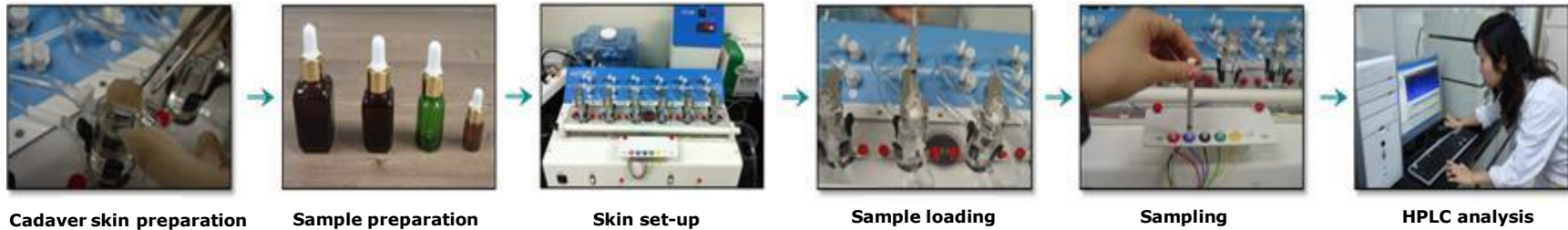
2. Artificial skin penetration of FITC labeled MTD





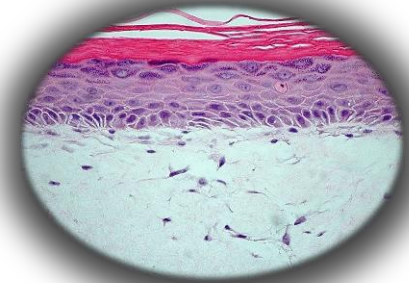
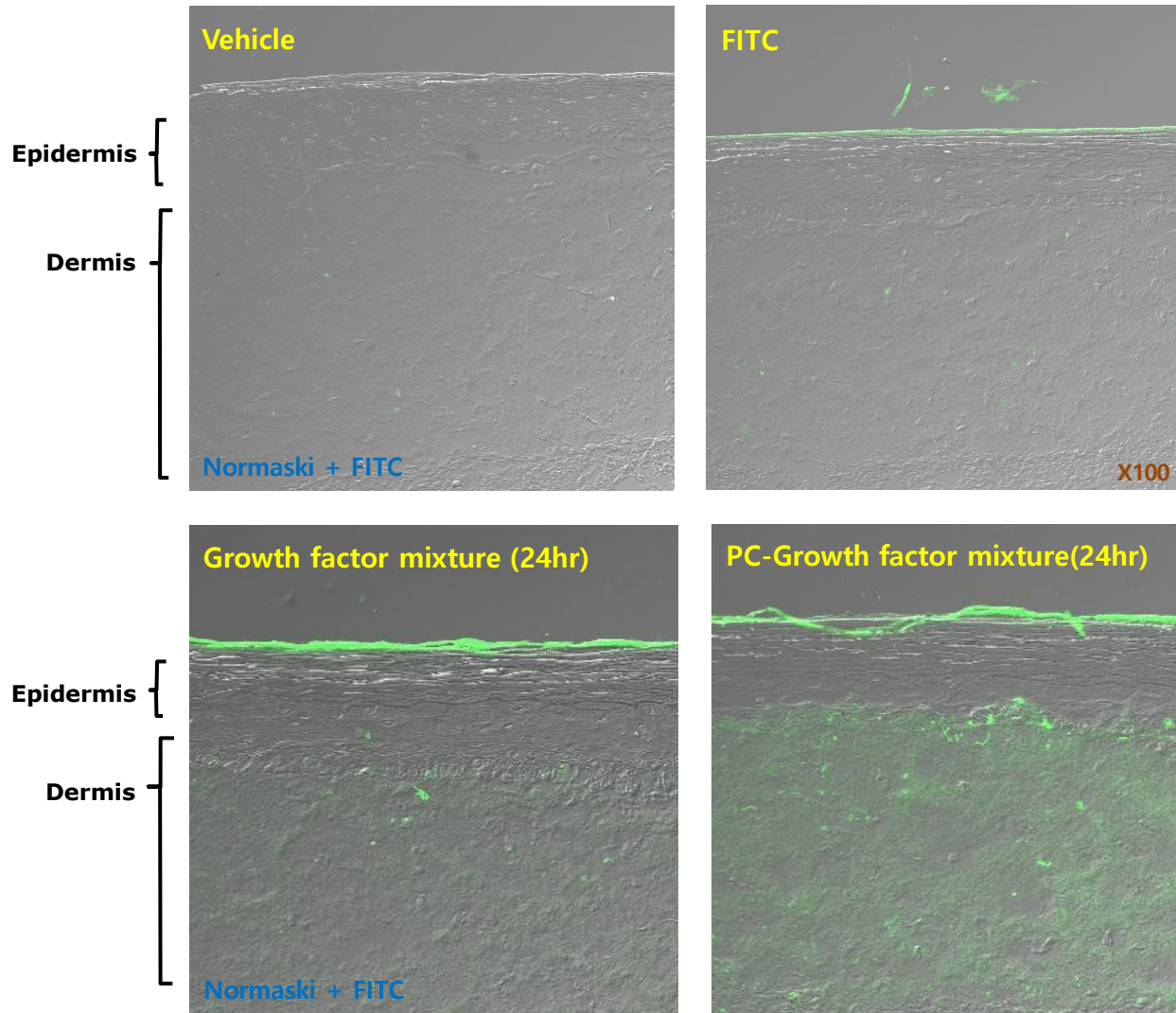
Skin fibroblast Cell proliferation was increased after 72 hr treatment of MTD-EGF

● *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



EpiDermFT Tissue Model

EGF/PC-EGF

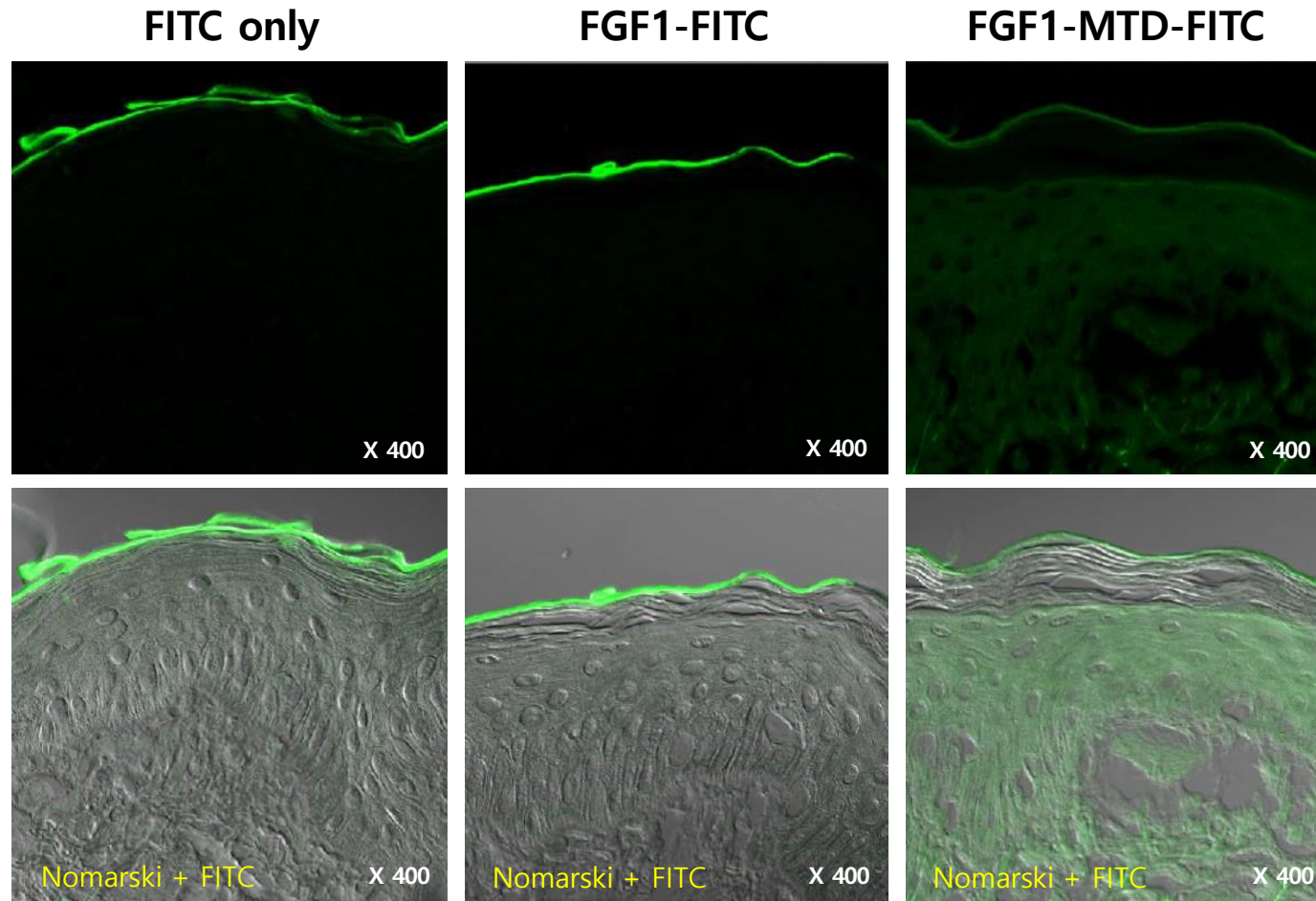
bFGF/PC-bFGF

VEGF/PC-VEGF

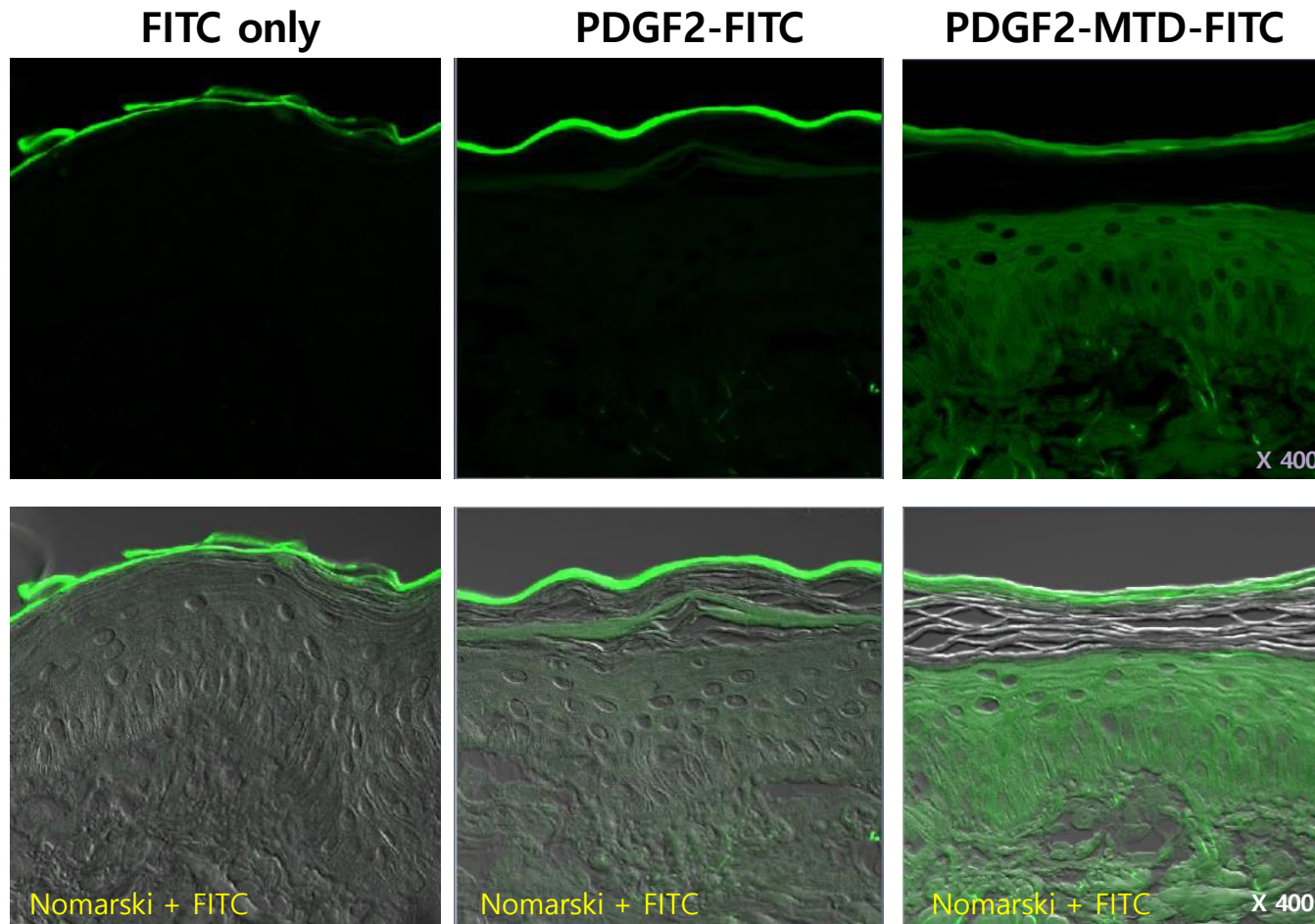
Treatment conc.

5ug/cm² each GF

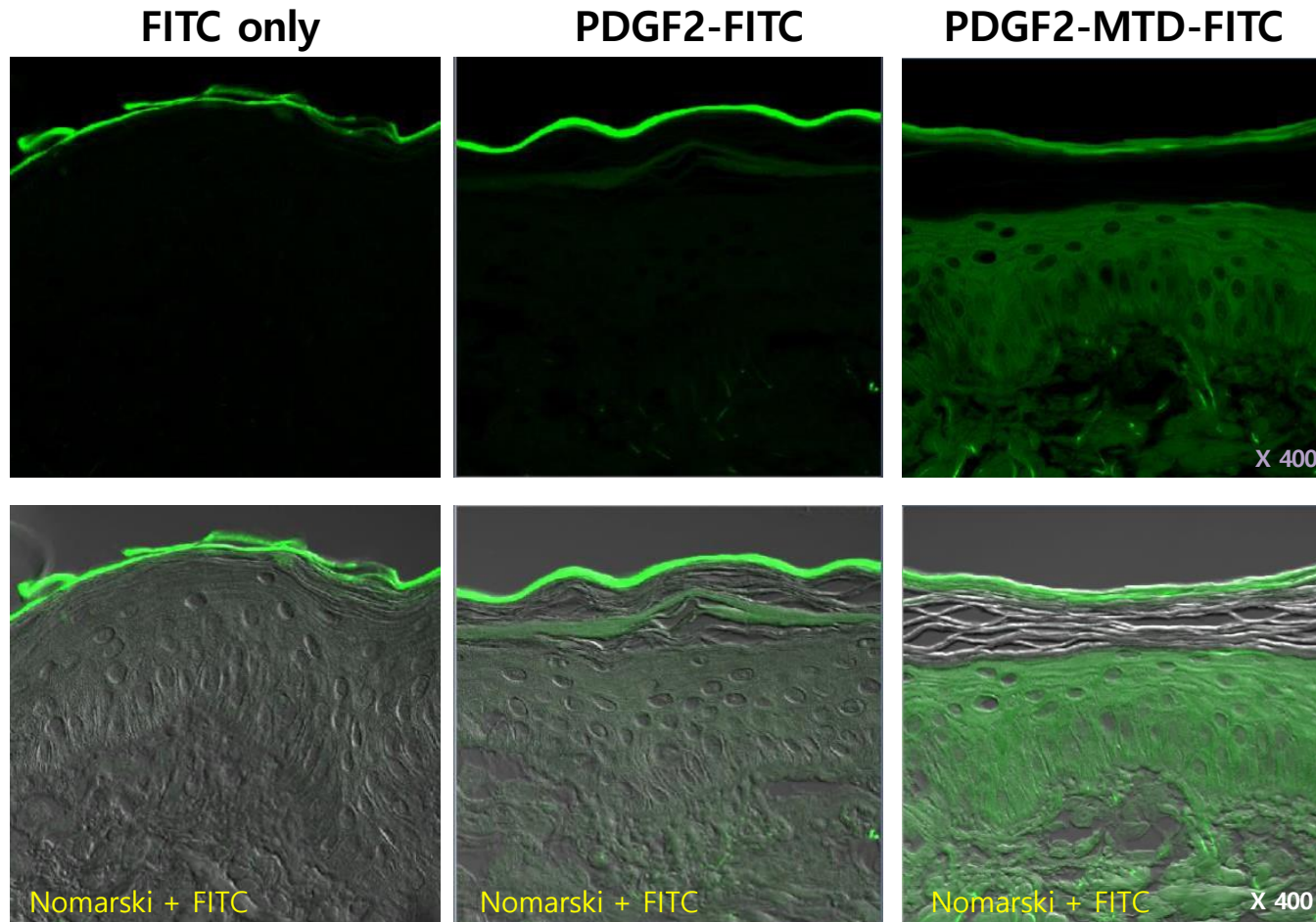
ZEISS LSM 700



10 μ g of each FGF1 and FGF1-MTD in 50 μ L of PBS(pH 7.0)
for 24 hours at 37 $^{\circ}$ C on 1cm 2 porcine(mini pig) skin



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 $^{\circ}$ C on 1cm 2 porcine(mini pig) skin

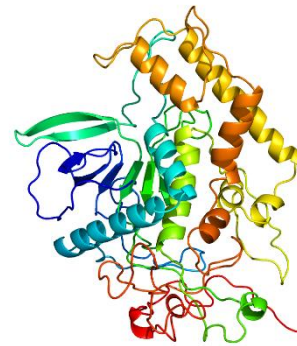


10 μ g of each FITC labeled PDGF2 and PDGF2-MTD in 50 μ L of PBS (pH 7.0) treatment for 24 hours at 37 $^{\circ}$ C on 1cm 2 porcine(mini pig) skin

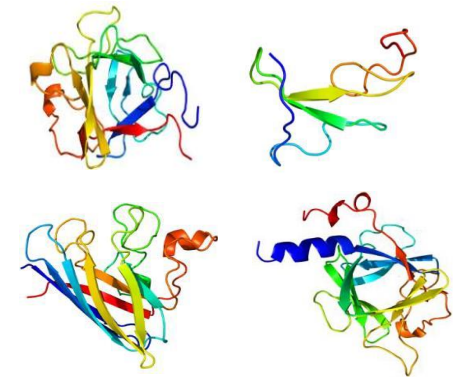
BPMed
COSMETIC



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 Efficay

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