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TRANSPORT OF SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS) ACROSS MUCUS AND CELLULAR INTERNALIZATION

Arshad Mahmood

PhD student

Institute of Pharmacy

Department of Pharmaceutical Technology

University of Innsbruck.

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Introduction

- For gene therapy, the target sites are mostly inside the cells, in the cytoplasm or the nucleus.
- Two main types of vectors that are used in gene therapy;
 - Viral
 - non-viral
- Safe and efficient delivery of DNA drugs into targeted cells is still a major task in pharmaceutical research.



- Biological barriers on oral route include
 - rapid enzymatic/lysosomal degradation of DNA drugs
 - poor cellular uptake
- Ongoing research on liposomes, polymer-based nanoparticles, self-nanoemulsifying drug delivery system (SNEDDS)
 - improve bioavailability
 - permeation enhancing
 - protective effect against enzymatic degradation



Aim of study

The aim of this study was to investigate SNEDDS as a carrier system for targeted delivery of drugs and/or genes to mucosal epithelial cells.



Methods & Results

Formulation 1 [SNEDDS]

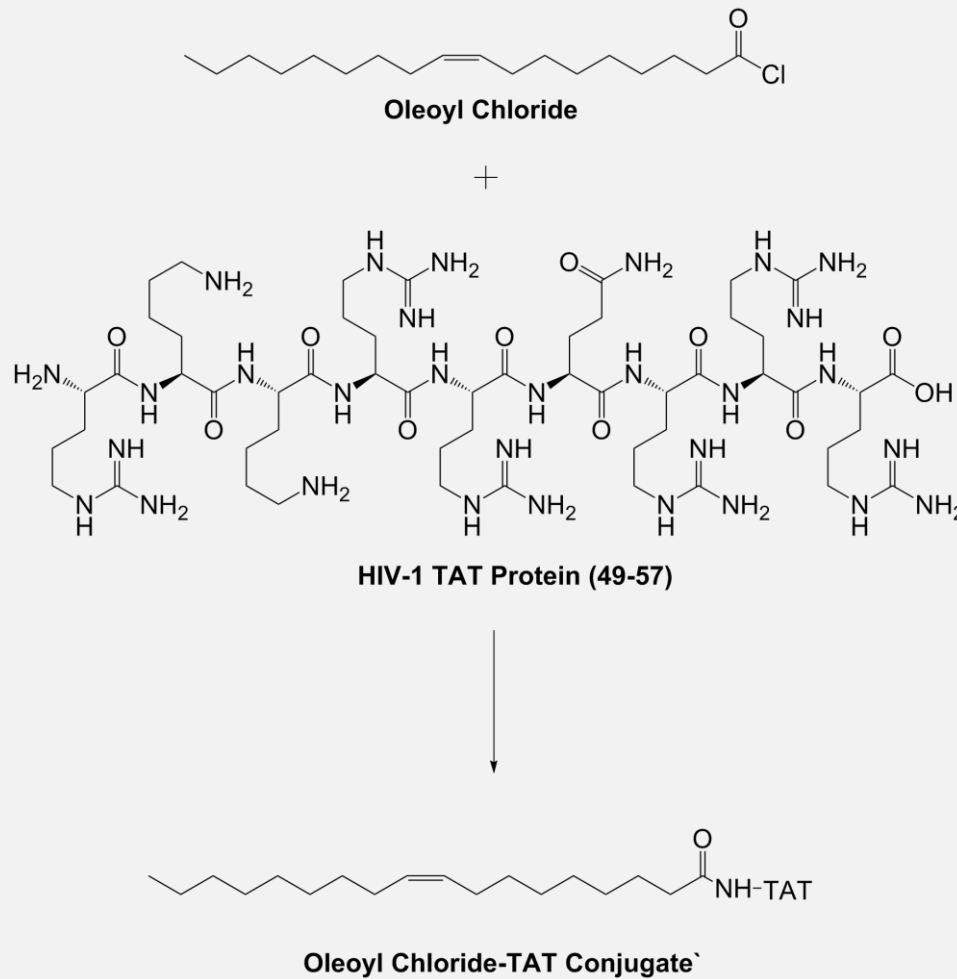
Cremophor EL	30% (m/m)
Capmul MCM	30% (m/m)
Crodamol	30% (m/m)
Propylene glycol	10% (m/m)

Formulation 2 [SNEDDS-TAT]

Cremophor EL	29.7 % (m/m)
Capmul MCM	29.7 % (m/m)
Crodamol	29.7 % (m/m)
Propylene glycol	9.9 % (m/m)
Oleoyl chloride-TAT	1.0 % (m/m)

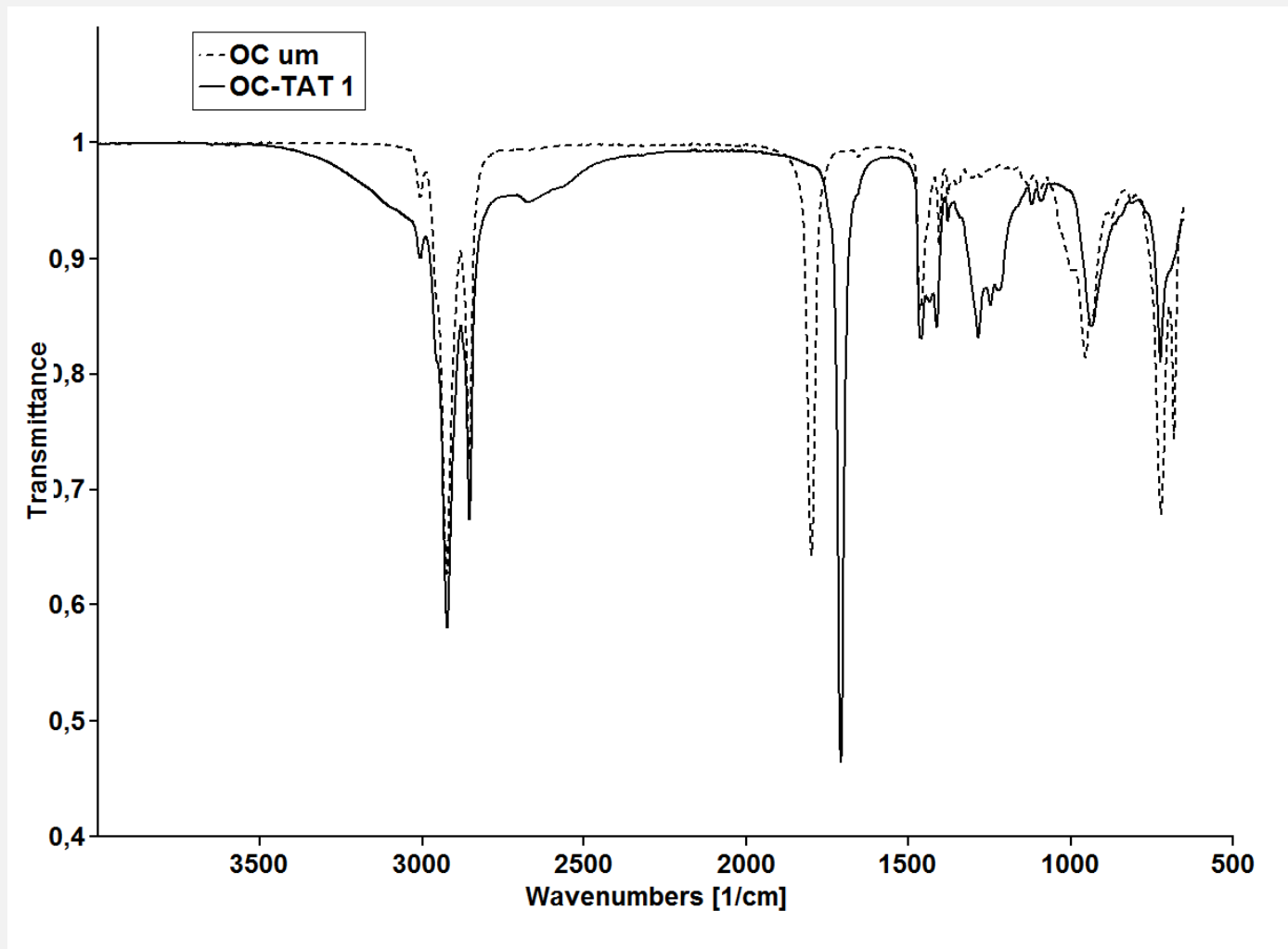


Synthesis of lipophilic TAT conjugate





FTIT-ATR analysis





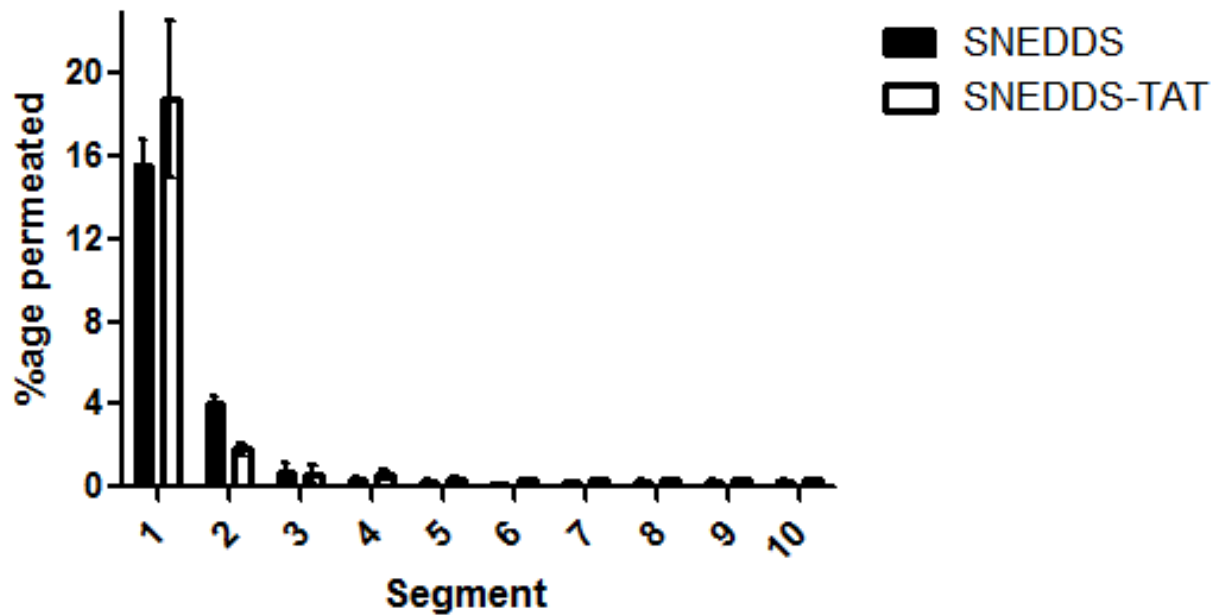
Methods & Results

Characterization

	Mean diameter (nm)	Zeta potential
Formulation 1 [SNEDDS]	35.5 ± 8.37	-0.52 mV
Formulation 2 [SNEDDS-TAT]	37.7 ± 9.07	-2.23 mV

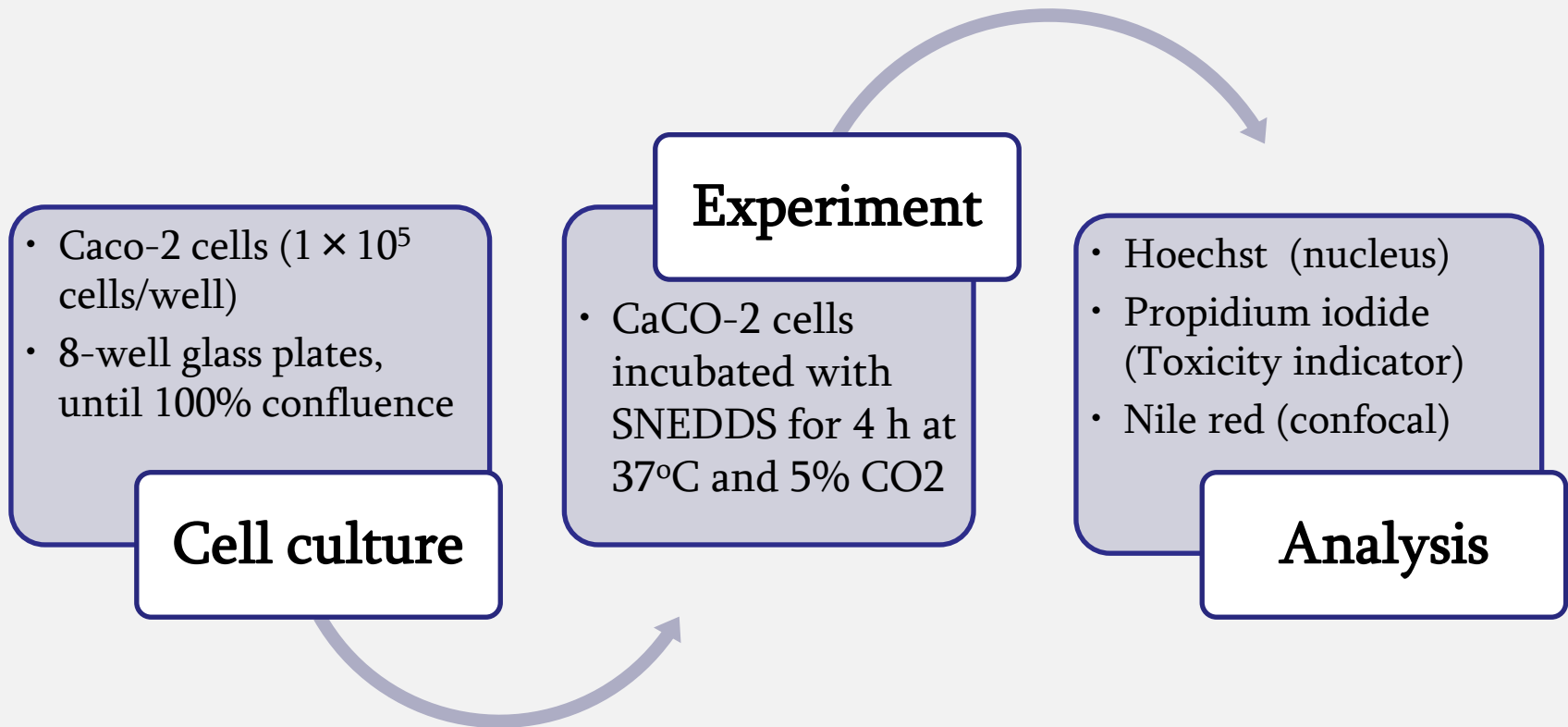


Diffusion through mucus using silicon tubes



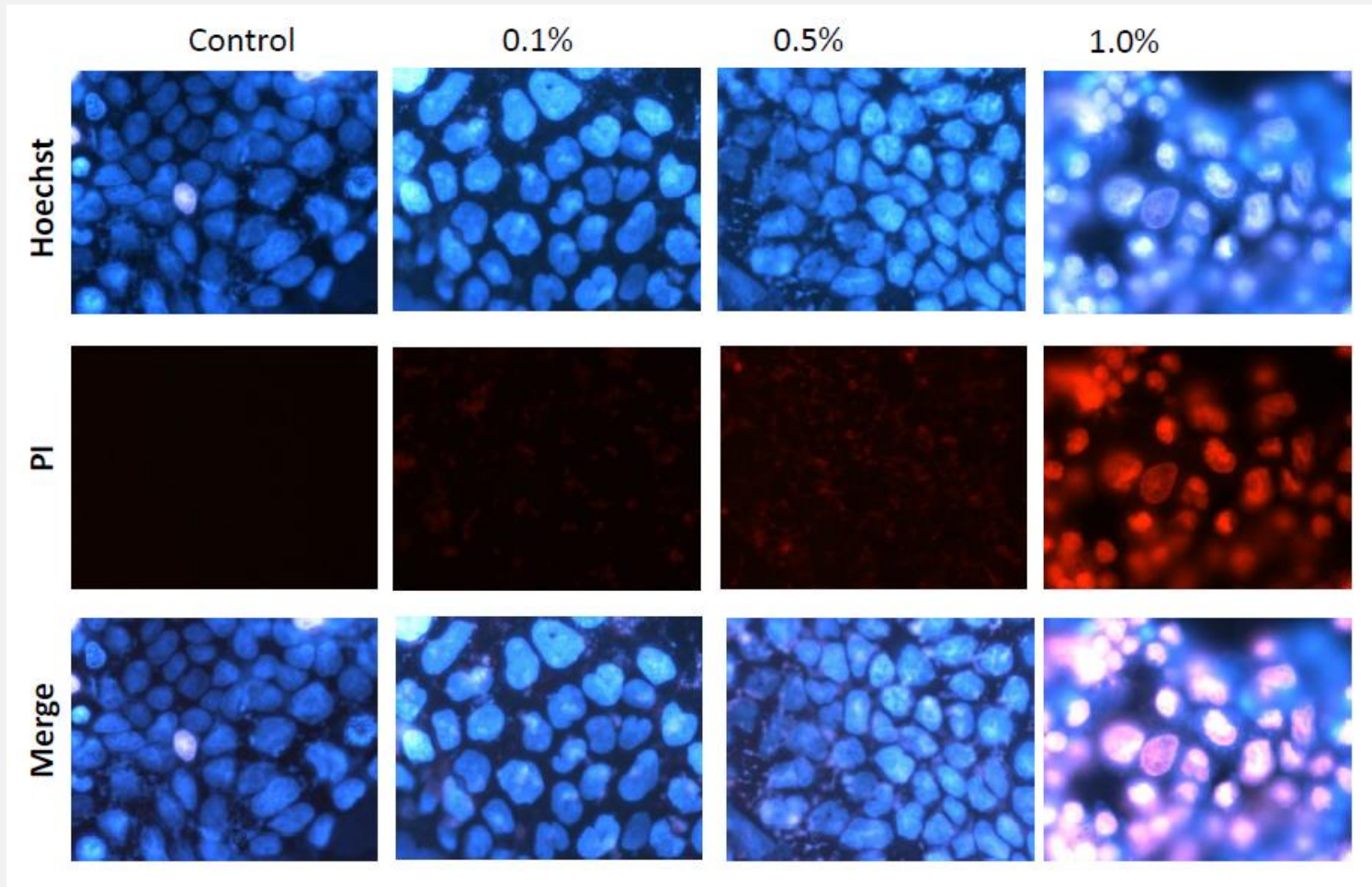


Fluorescent and confocal microscopy





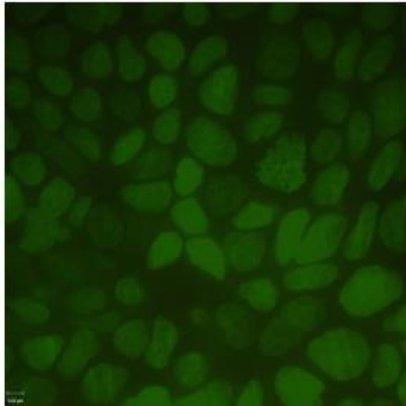
Fluorescent microscopy: concentration gradient viability



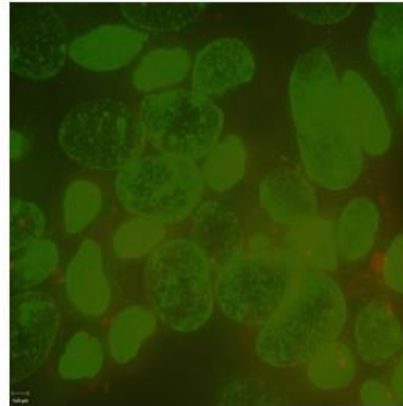


Confocal microscopy

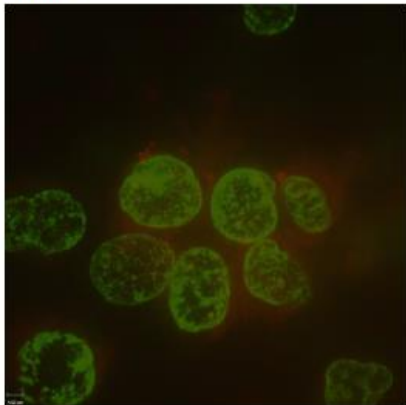
Control



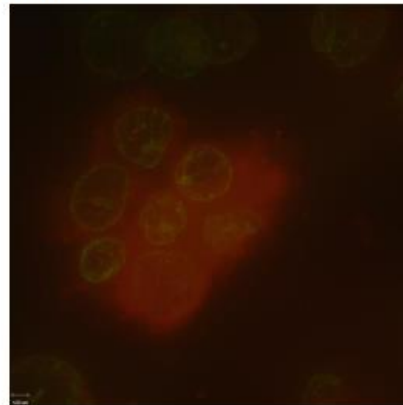
Time-1



Time-2

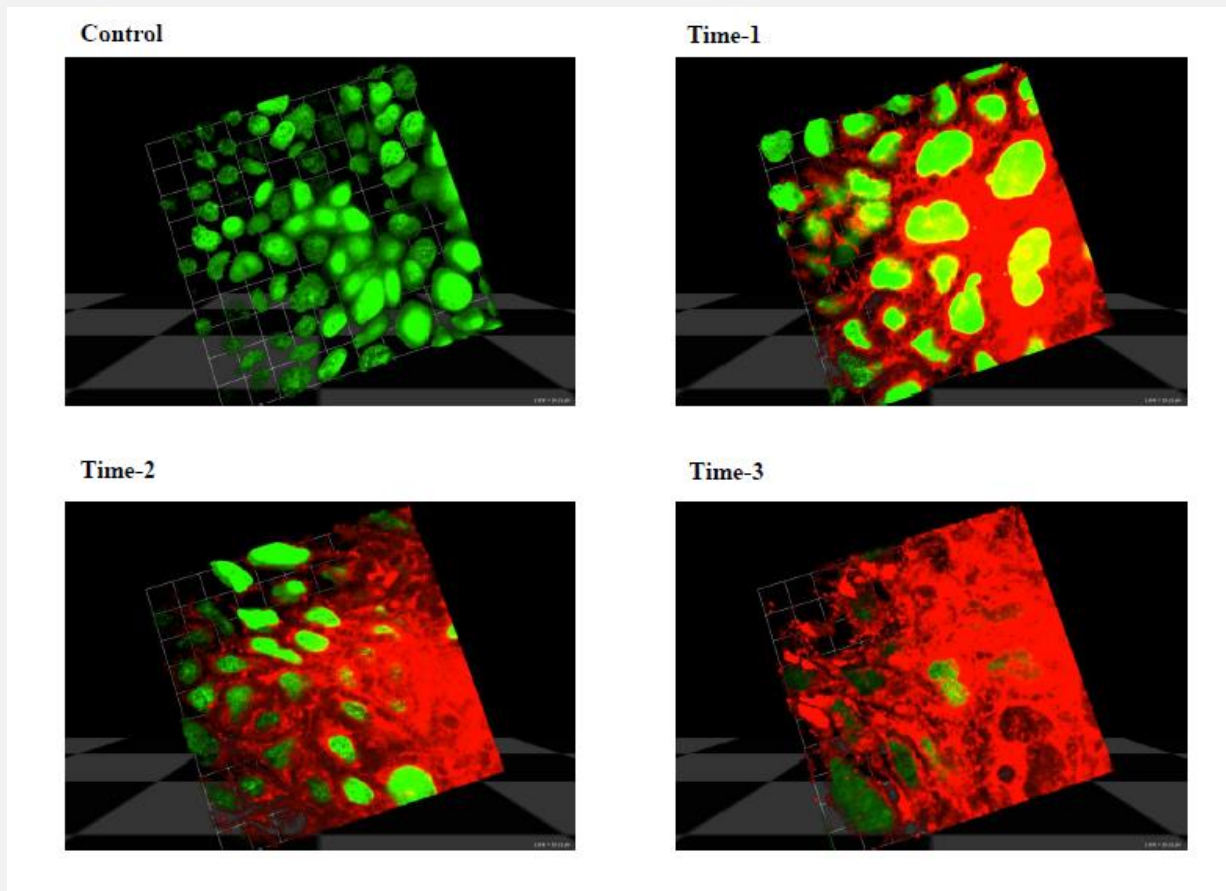


Time-3



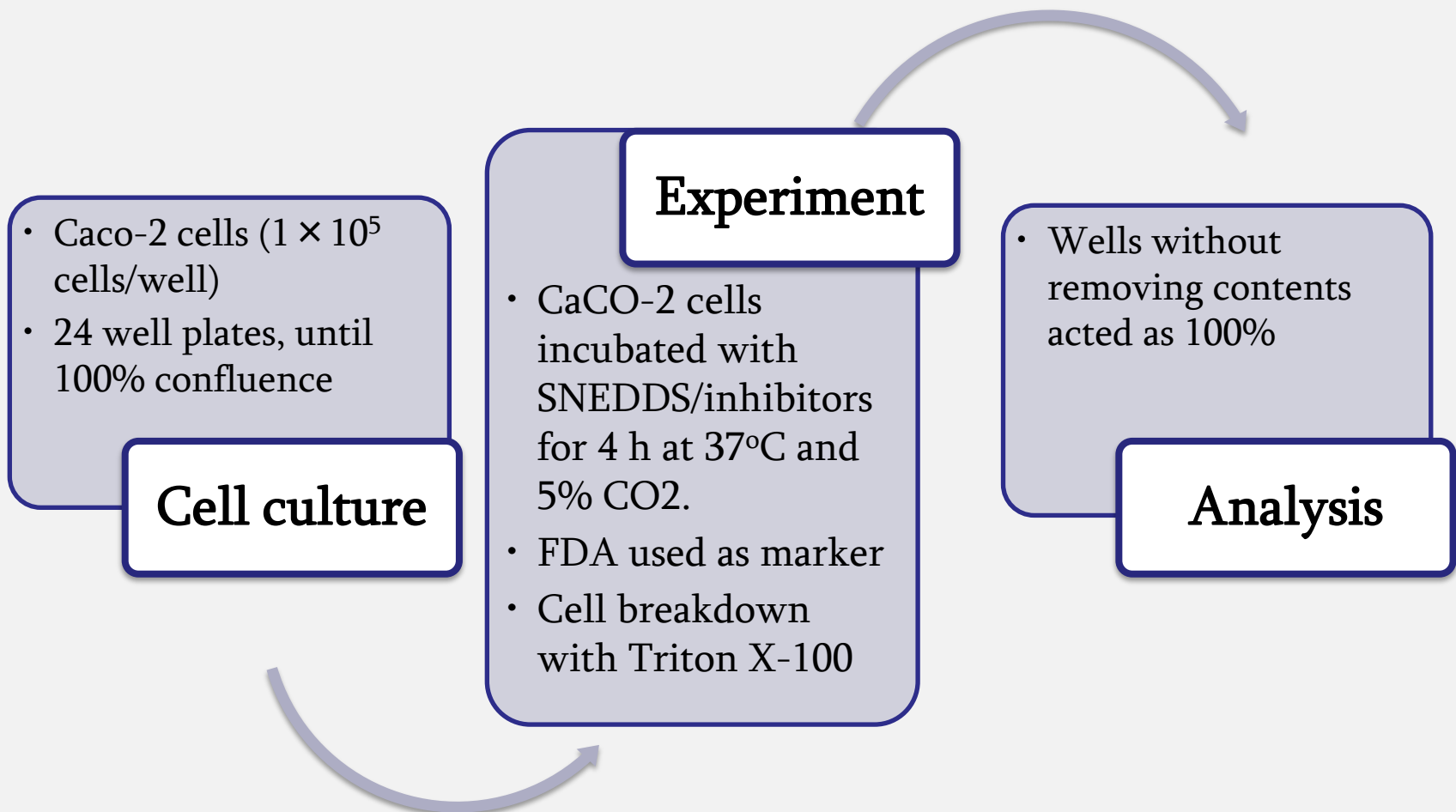


Confocal microscopy: 3D view



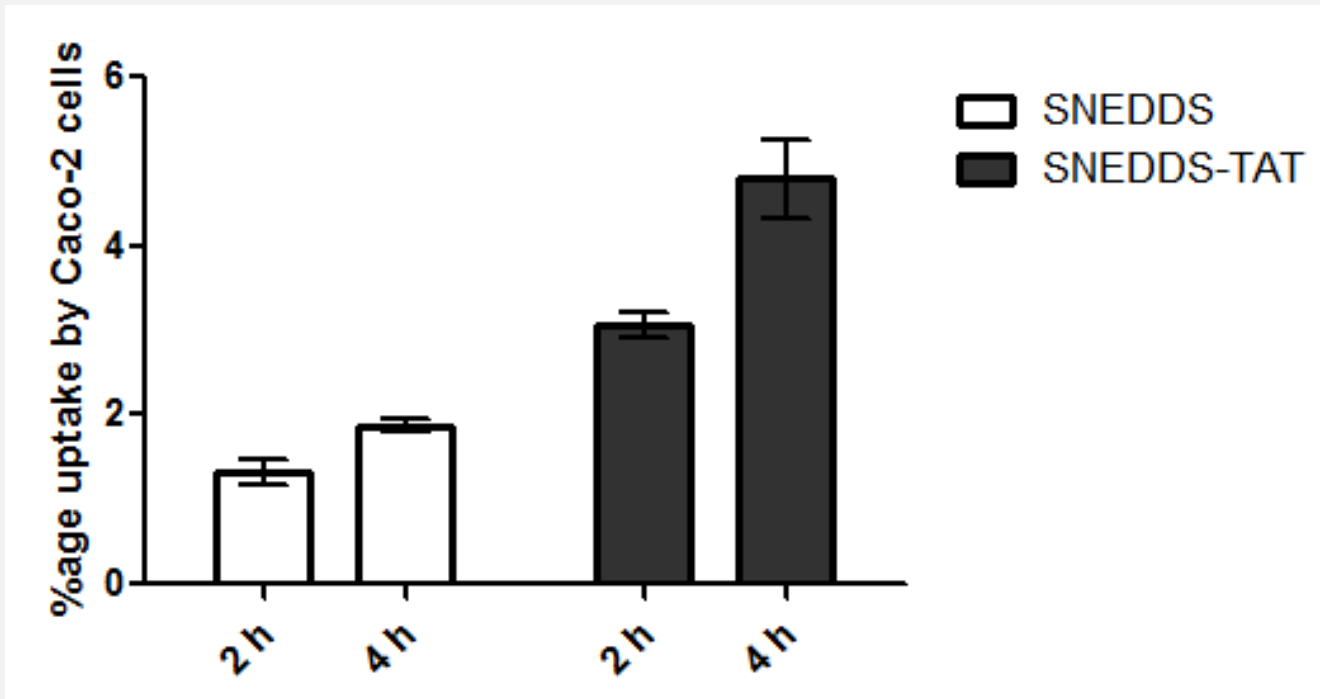


Cellular uptake and pathway determination





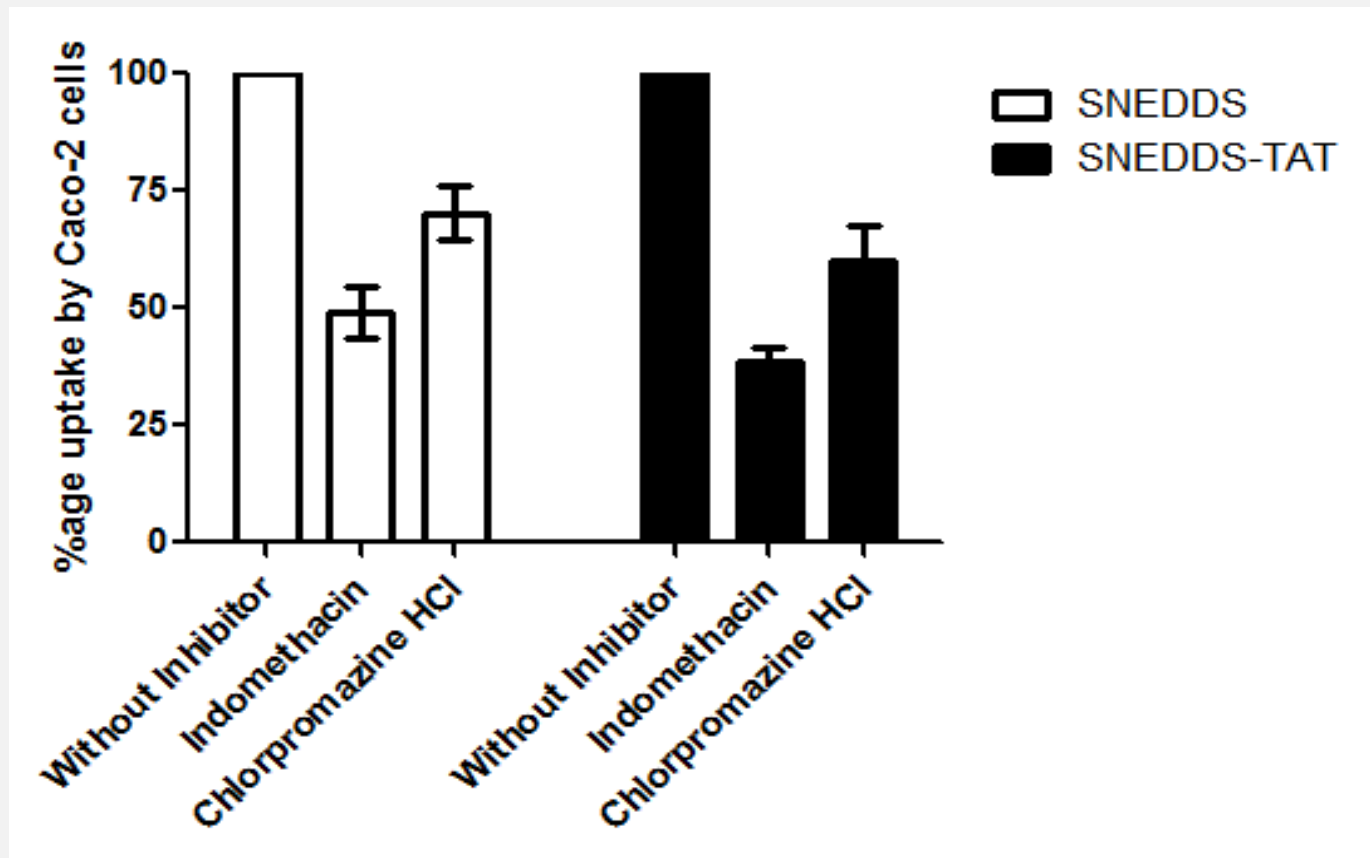
Cellular uptake



Internalization of SNEDDS and SNEDDS-TAT conjugate into Caco-2 monolayers.



Determination of uptake pathway using pharmacological block model



Internalization of SNEDDS and SNEDDS-TAT conjugate into Caco-2 monolayers treated with different endocytosis inhibitors. Indomethacin 300 μ M and Chlorpromazine 10 μ g/ml



Conclusion

- SNEDDS are promising carrier system for mucosal epithelial delivery.
- SNEDDS permeate the mucus gel layer and reach the cytoplasm after being transported by multiple endocytosis pathways predomoinently by caveolae mediated and clathrin pathway.
- Combination of SNEDDS with cell penetrating peptides significantly increased internaliztion.



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