Development of the nephrotoxicity evaluation system by nucleic acid drugs using 3D-cultured human renal proximal tubule epithelial cells

3次元培養ヒト近位尿細管上皮細胞を用いた核酸医薬品による腎毒性評価系の構築

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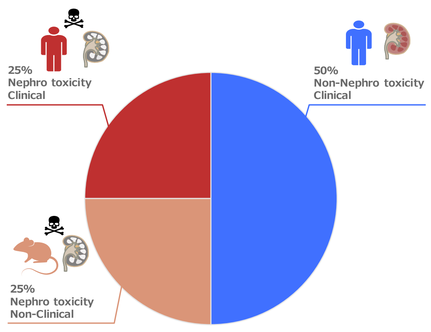
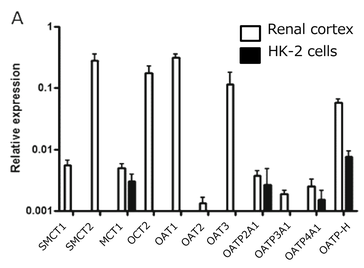
Introduction

In recent years, development of new modality drugs such as nucleic acid drug, gene therapeutic, and cell therapy has been progressing. Among them, nucleic acid drugs are relatively high specificity, but are known to cause thrombocytopenia, complement activation, hepatotoxicity, and nephrotoxicity in many cases(1).
Their toxicity of antisense oligonucleotide(ASO) are considered to be the class effects, and the development of methods to detect their toxicity in advance is desired.
In particular, hybridization-dependent off-target toxicity is difficult to detect in animal studies.
Currently, there are the reports that hepatotoxicity could be reduced by modifying the sequence or modifications using the result of *in vitro* studies(2). However, studies on the detection of nephrotoxicity have not progressed sufficiently due to the lack of well-functioning kidney cells(3).



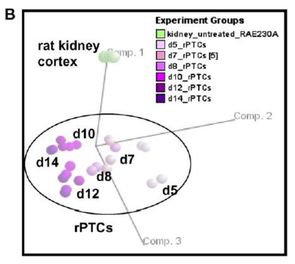
(1) Nephrotoxicity rate of nucleic acid drug (Clinical)

Gene expression of SLC transporters



Jenkinson et al., Eur J Physiol. (2012) 464:601–611

Primary rat kidney cells



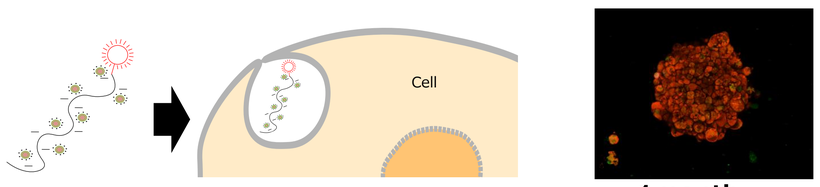
C Weiland et al., Toxicology in Vitro (2007)21:466–491

(2) Function of commercially available kidney cells

Conclusion

1. 標識した核酸医薬品の3D-RPTEC内への導入を確認

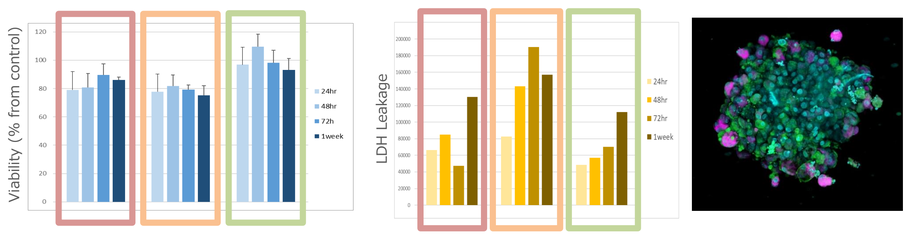
- Lipofection法だけでなく、Nakedでも導入が確認された。
- 導入試薬の毒性域を確認し、無毒性域内での核酸医薬品導入を確認した。
- 核酸医薬品の長期的な暴露を確認。2Dでは約１週間で排出された。



1month

2. 漏出したマーカーやHCAによって毒性が評価できた。

- LDHで漏出マーカーの上昇を検出した。
- HCAにおいて小胞体などへの毒性が検出された。



問い合わせ先

3D-RPTEC®についてご興味やご質問のある方は,以下または取扱代理店までお問い合わせ下さい。

日機装株式会社
https://www.nikkiso.co.jp/products/industrial/3drptec/

Mail: 3D-RPTEC@nikkiso.co.jp

オリエンタル酵母工業株式会社
https://www.oyc.co.jp/bio/ADME_Tox/3d-rptec.html

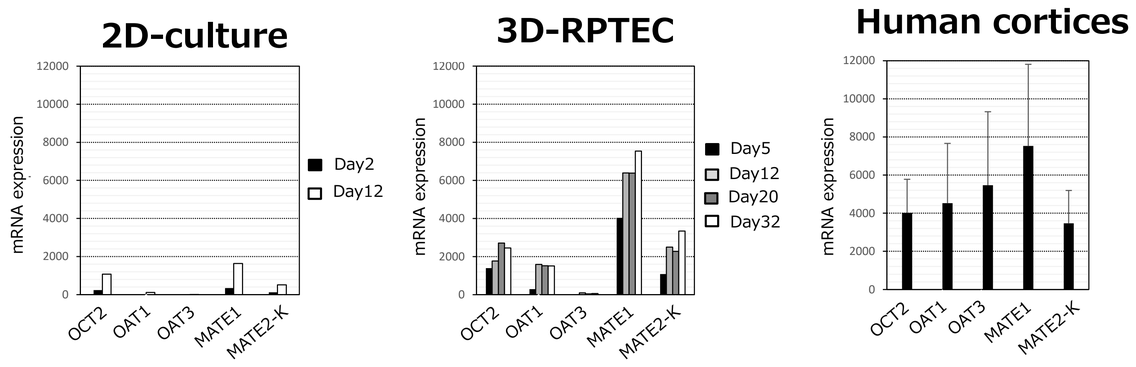
富士フイルム和光純薬株式会社
https://labchem-wako.fujifilm.com/jp/category/03232.html

1. Features of 3D-RPTEC

3D-RPTEC® : RPTECs were cultured as spheroids in ultra-low attachment 96-well culture plate.
The medium was changed once every 2-3 days.
3D-RPTEC® can be available from Nikkiso.

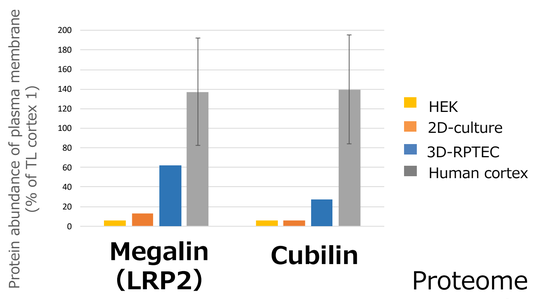
3D-RPTEC® PRODUCT

(1-A) Drug transporter (Microarray)

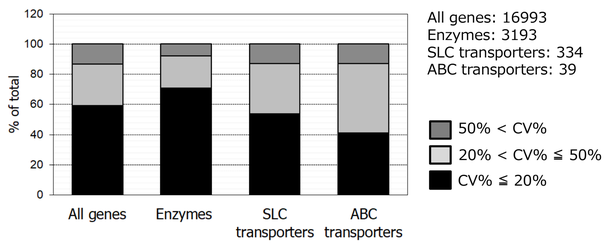


3D-RPTEC showed improved expression of drug transporters and was maintained in long-term culture.(Fig.1-A).

(1-B) Endocytosis (Proteomics)

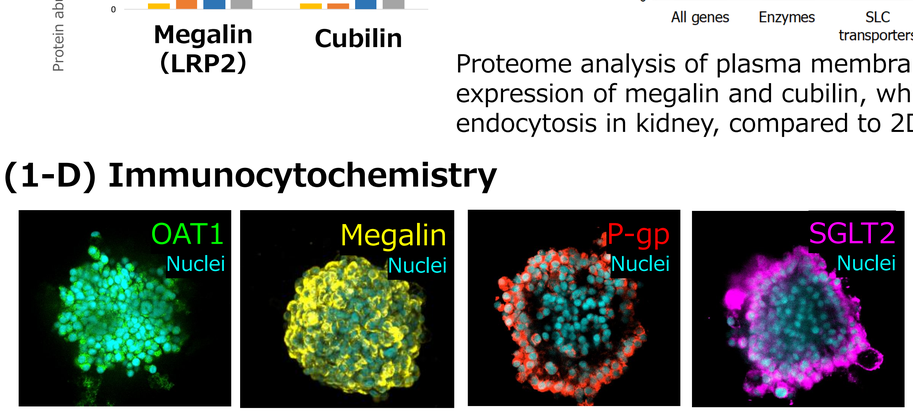


(1-C) Donor-to-donor variability



Proteome analysis of plasma membranes showed higher expression of megalin and cubilin, which are responsible for endocytosis in kidney, compared to 2D-culture (Fig.1-B).

(1-D) Immunocytochemistry



Antibody
anti-OAT1(Transgenic, KE038)
anti-LRP2(Novus Biologicals, NBP1-85292)
anti-Pgp (abcam, ab129450)
anti-SGLT2(Proteintech, 24654-1-AP)
Photo using CQ-1

3D-RPTECs showed the expression of drug transporters in immunocytochemistry.
Apical transporters (P-gp or SGLT2) and plasma membranes (Megalin) were more strongly localized to the outside of the spheroid than basolateral transporter (OAT1) (Fig.1-D).

2. Transfection of Nucleic Acid

(2-A) Transfection of Nucleic acid

Nucleic Acid: SPC5001(Gene Design Inc.)
Labeled with Cy5

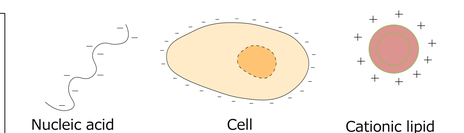
Number of base pairs: 14-nucleotide
(MW is 4689.85 g/mol)

Modification: LNA, PS

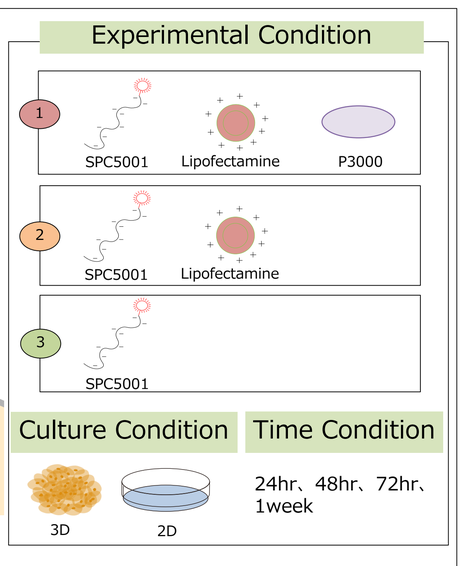
Indicated: Hypercholesterolemia

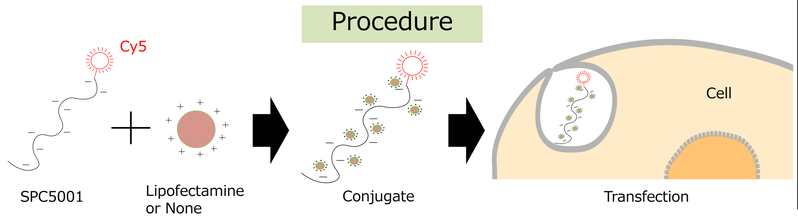
Sequence: 5'-TGmCTACAAAACmCmCA-3'

Drug Delivery: Naked



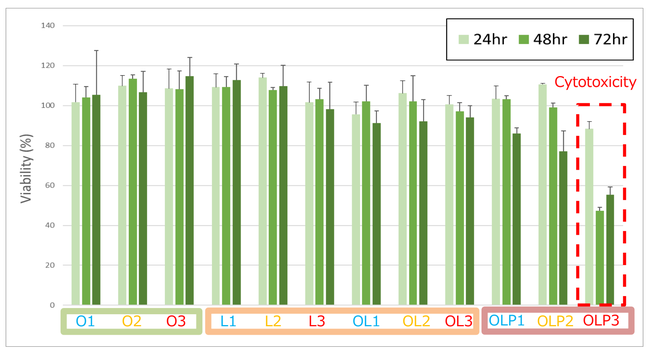
Both nucleic acids and cells are negatively charged. Therefore, nucleic acids are usually difficult to introduce into the cell.





First, we examined the uptake of labeled nucleic acid into 3D-RPTEC and the toxicity of the transfection reagent itself.

(2-B) Toxicity of transfection reagent



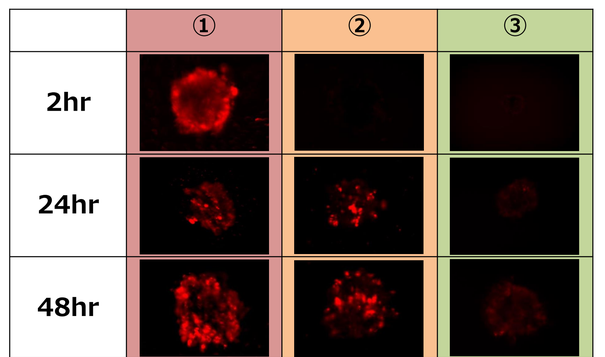
O1: Opti-MEM Low (3μL)
O2: Opti-MEM Middle (10μL)
O3: Opti-MEM High (30μL)

L1: Lipofectamine Low (0.1μL)
L2: Lipofectamine Middle (0.3μL)
L3: Lipofectamine High (1μL)

OL1: Opti-MEM Low (3μL) +Lipofectamine Low (0.1μL)
OL2: Opti-MEM Middle (10μL) +Lipofectamine Middle (0.3μL)
OL3: Opti-MEM High (30μL) +Lipofectamine High (1μL)

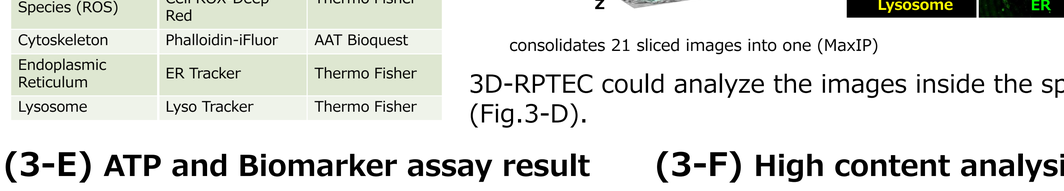
OLP1: Opti-MEM Low (3μL) +Lipofectamine Low (0.1μL) +P3000 Low (0.1μL)
OLP2: Opti-MEM Middle (10μL) +Lipofectamine Middle (0.3μL) +P3000 Middle (0.3μL)
OLP3: Opti-MEM High (30μL) +Lipofectamine High (1μL) +P3000 (0.3μL)
※ Cytotoxicity

(2-C) Results of transfection



Transfection reagents showed cytotoxicity at high concentrations (Fig.2-B).
Transfection was confirmed in 3D-RPTEC without transfection reagents(Fig.2-C).

(2-D) Comparison of transfection efficiency and duration



3D-RPTEC maintained for a longer period of time after transfection(Fig.2-D).

3. Toxicological Assay Result

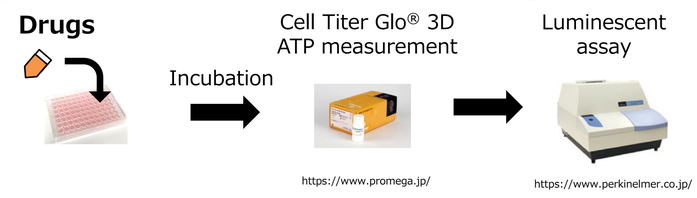
(3-A) Nucleic acid drugs

① SPC5001 (Gene Design Inc.)
Antisense oligonucleotides (ASOs)
Modification: LNA, PS

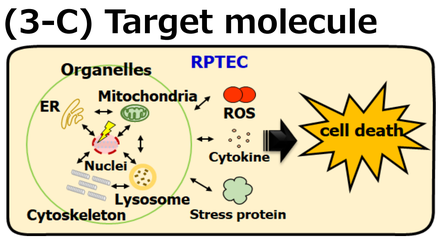
② Givosiran (MedChemExpress)
siRNA

③ Viltolarsen (MedChemExpress)
Antisense oligonucleotides (ASOs)

(3-B) ATP and Biomarker assay



(3-C) Target molecule

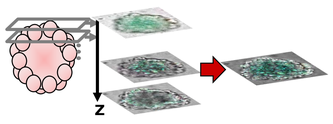


Target	Dye	Manufacturer
Nucleus	Hoechst 33342	Thermo Fisher
Mitochondria	Mito Tracker	Thermo Fisher
Reactive Oxygen Species (ROS)	Cell ROX-Green Cell ROX-Deep Red	Thermo Fisher
Cytoskeleton	Phalloidin-iFluor	AAT Bioquest
Endoplasmic Reticulum	ER Tracker	Thermo Fisher
Lysosome	Lyso Tracker	Thermo Fisher

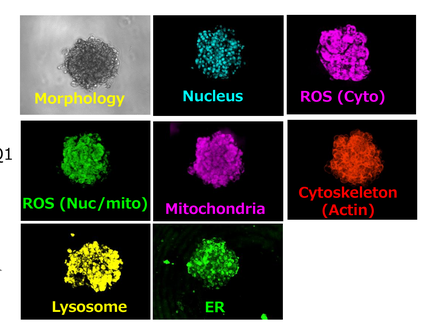
(3-D) Image analysis

YOKOGAWA

Confocal Quantitative Image Cytometer, CQ1

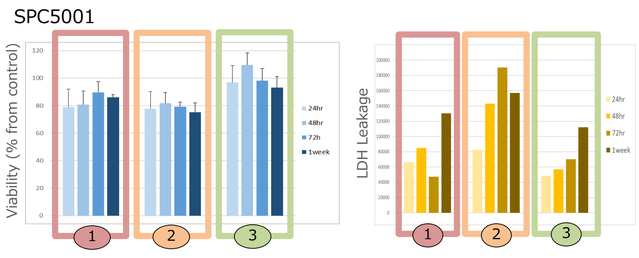


consolidates 21 sliced images into one (MaxIP)



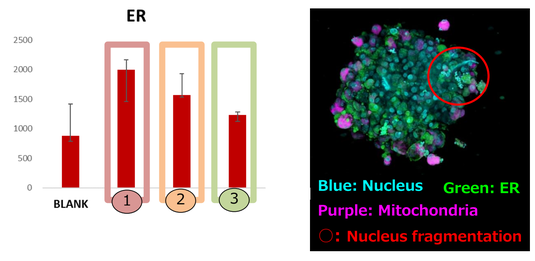
3D-RPTEC could analyze the images inside the spheroid (Fig.3-D).

(3-E) ATP and Biomarker assay result



Toxicity of nucleic acid drugs was not detected by ATP assay. However, a dose-dependent increase in LDH leakage was confirmed.
Other Nucleic acid drugs showed similar results.

(3-F) High content analysis (HCA)



HCA showed the nuclear fragmentation and toxicity to the endoplasmic reticulum with long-term transfection (2week).

COI Disclosure Information

I have no COI regarding this presentation.
Presenting author: Kaoru Morimura
Organization: Nikkiso Co. Ltd

Acknowledgement

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References

(1) Jenkinson et al., Eur J Physiol. (2012) 464:601–611
(2) C Weiland et al., Toxicology in Vitro (2007)21:466–491