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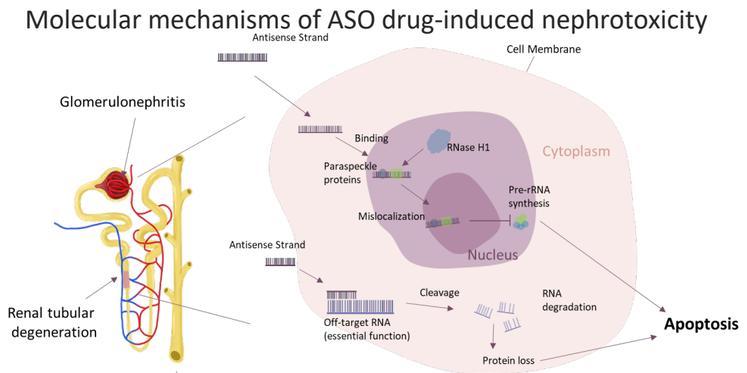
Comparative Evaluation of 2D- and 3D-Cultured Human RPTEC for Renal Toxicity Assessment of Oligonucleotide Therapeutics

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Background and Objective

The kidney, particularly the renal proximal tubule, is a major site of oligonucleotide accumulation and toxicity. For antisense oligonucleotides (ASOs), intracellular accumulation, lysosomal burden, and subsequent cellular stress responses have been implicated in renal injury; however, the mechanisms underlying nephrotoxicity remain incompletely understood. Because renal toxicity is likely influenced by complex cellular and microenvironmental factors, physiologically relevant *in vitro* models are needed to improve early safety assessment. Although conventional two-dimensional (2D) cultures of renal proximal tubule epithelial cells (RPTECs) are widely used for nephrotoxicity screening, three-dimensional (3D) RPTEC spheroids may better recapitulate key features of the *in vivo* renal microenvironment. In this study, we compared oligonucleotide-induced cellular responses in 2D and 3D RPTEC models to evaluate the potential utility of the 3D model for nephrotoxicity assessment.

Molecular mechanisms of ASO drug-induced nephrotoxicity



Adapted from Wu et al. Current Opinion in Toxicology 2022, 32: 100373

Materials and Methods

Human RPTECs for the 2D model were purchased from Lonza Bioscience and cultured as monolayers, whereas 3D RPTEC spheroids were obtained from Nikkiso Co., Ltd. Oligonucleotides, including ASOs, small interfering RNAs (siRNAs), and an RNA aptamer, were introduced using a transfection reagent. The oligonucleotides were tested at concentrations of 10, 30, and 100 nM in the 2D model and 50, 500, and 5000 nM in the 3D model. RPTECs were maintained for 48 h (Day 2) in the 2D model or 7 days (Day 7) in the 3D model after transfection. Cell viability was assessed by ATP levels, cytotoxicity by LDH release, and apoptosis by Caspase-3/7 activity. The experimental design of the 3D RPTEC assay was based on the manufacturer's recommendations and the protocol described by Morimura et al. (2026).

References
Morimura K, Takahashi E, Maeda H, Nishioka Y, Araki A, Mizumoto H, Jimbo Y. Predicting nucleic acid drug-induced nephrotoxicity using a 3D human renal proximal tubule spheroid model. J Toxicol Sci. 2026;51(1):75-87.

Information on the Evaluated Oligonucleotides

No.	Test articles	Type	Nephrotoxicity reports	
			Non-clinical	Clinical
1	SPC5001	ASO (LNA)	Not reported	Reported
2	ISIS388626	ASO (2'-MOE)	Not reported	Reported
3	Mipomersen	ASO (2'-MOE)	Reported	Reported
4	Inotersen	ASO (2'-MOE)	Reported	Reported [a]
5	Givosiran	GalNAc-siRNA	Not reported	Reported [b]
6	Pegaptanib	RNA aptamer	Reported	No serious case reports*
7	Viltolarsen	ASO (PMO)	Reported	No serious case reports*
8	Nusinersen	ASO (2'-MOE)	Not reported	No serious case reports*
9	Inclisiran	GalNAc-siRNA	Not reported	No serious case reports*
10	Scrambled ASO	ASO (LNA)	Not reported	N/A

* No serious clinical nephrotoxicity case reported; does not indicate absence of renal effects.
[a] Glomerulonephritis. [b] Transient renal impairment. N/A: Not available.

References
Annie Moisan, et al. "Inhibition of EGF Uptake by Nephrotoxic Antisense Drugs In Vitro and Implications for Preclinical Safety Profiling." Molecular Therapy: Nucleic Acids Vol. 6 (2017) 89-105.

Results

3D RPTEC (Day 7)

- Most oligonucleotides showed limited changes in ATP levels.
- SPC5001 and givosiran increased LDH release relative to the scrambled ASO.

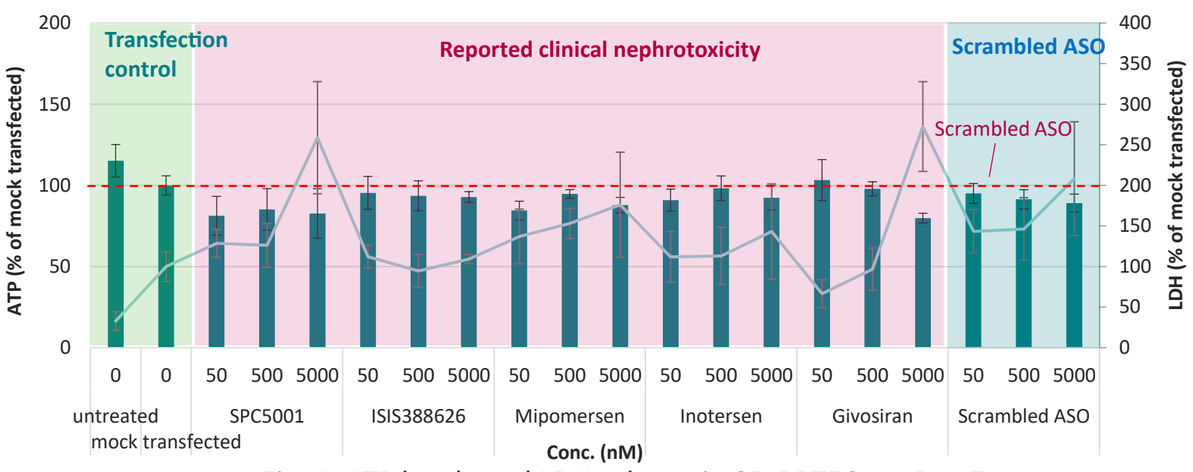


Fig. 1. ATP levels and LDH release in 3D RPTECs at Day 7.
Bars: ATP levels (%, left axis). Lines: LDH release (%, right axis).

- SPC5001, ISIS388626, and givosiran increased Caspase-3/7 activity.

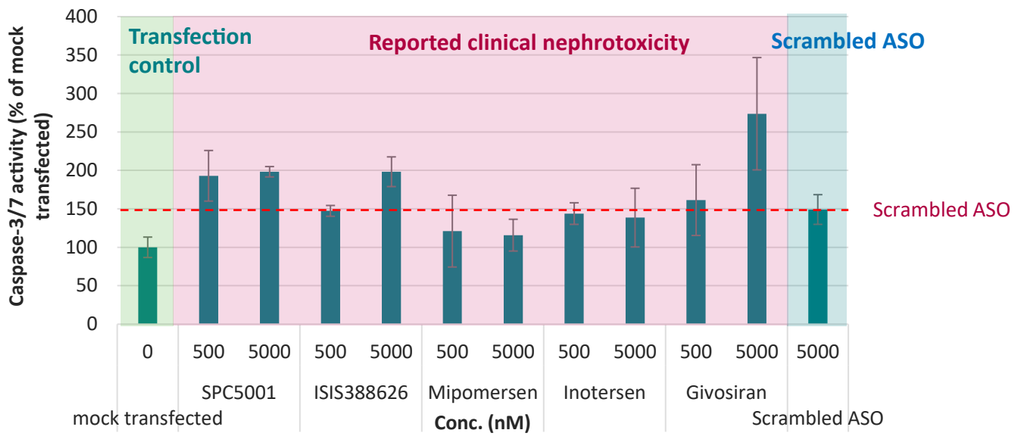


Fig. 3. Caspase-3/7 activity in 3D RPTECs at Day 7.

- ATP levels did not decrease for oligonucleotides without reported serious clinical nephrotoxicity.
- Pegaptanib, nusinersen, and inclisiran induced LDH release comparable to SPC5001.

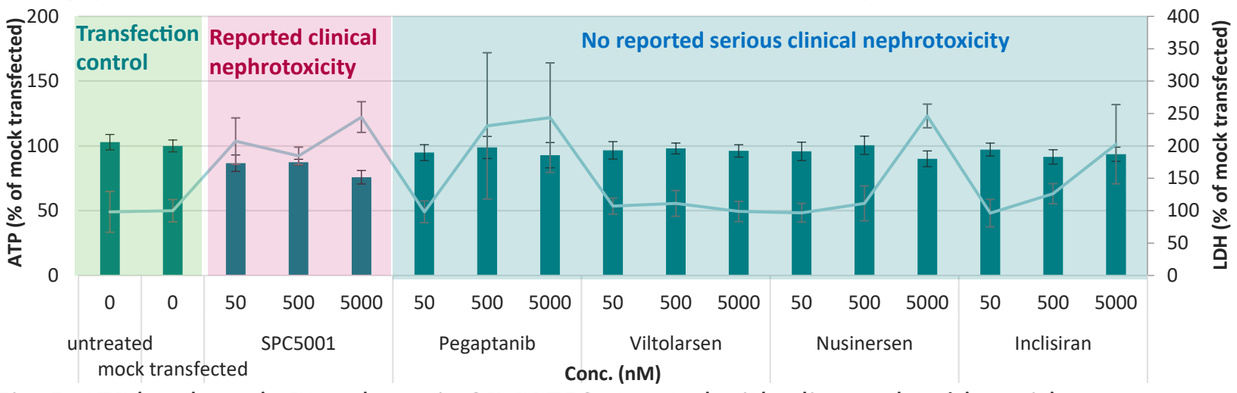


Fig. 5. ATP levels and LDH release in 3D RPTECs treated with oligonucleotides without reported serious clinical nephrotoxicity and SPC5001.
Bars: ATP levels (%, left axis). Lines: LDH release (%, right axis).

2D RPTEC (Day 2)

- Only SPC5001 reduced ATP levels below 75%.
- SPC5001, mipomersen, and inotersen increased LDH release relative to the scrambled ASO.

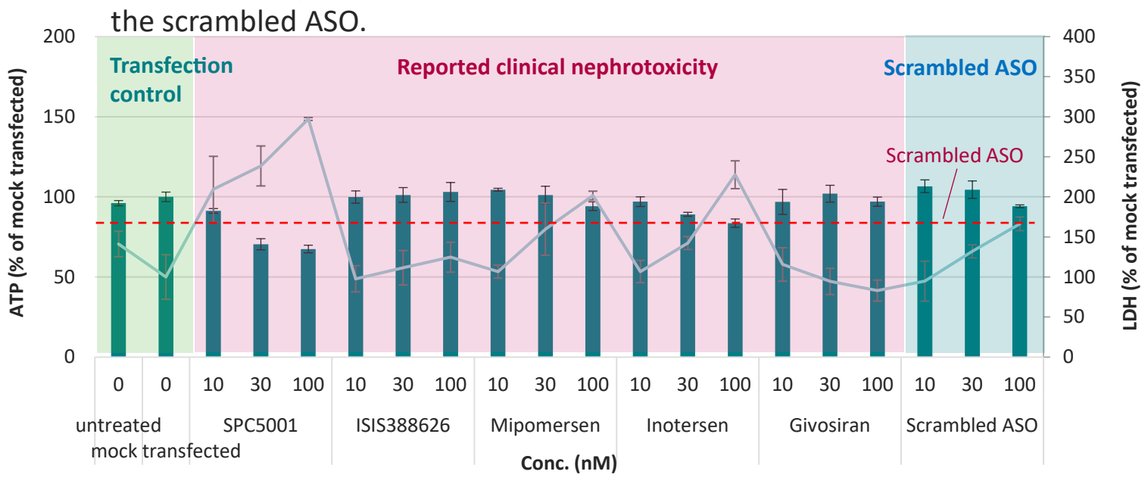


Fig. 2. ATP levels and LDH release in 2D RPTECs at Day 2.
Bars: ATP levels (%, left axis). Lines: LDH release (%, right axis).

- SPC5001 and mipomersen increased Caspase-3/7 activity relative to the scrambled ASO.

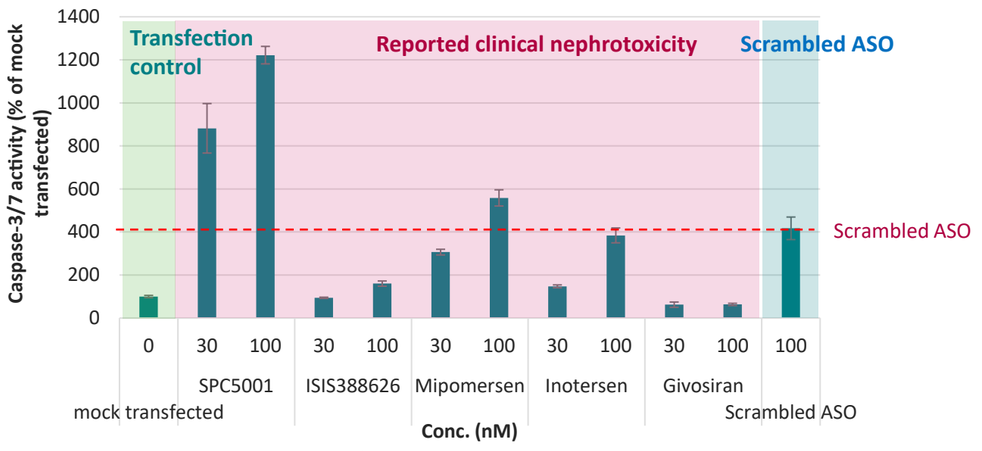
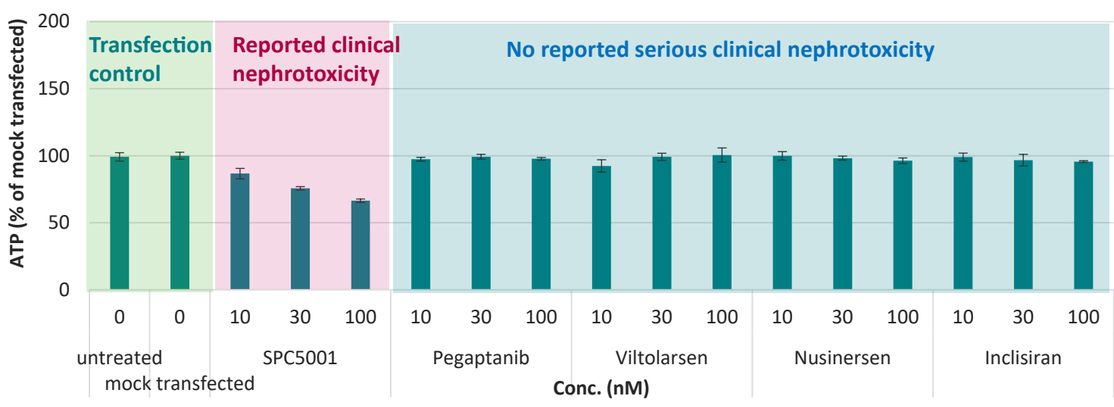


Fig. 4. Caspase-3/7 activity in 2D RPTECs at Day 2.

- SPC5001 reduced ATP levels in a concentration-dependent manner.
- ATP levels did not decrease for oligonucleotides without reported serious clinical nephrotoxicity.



∗ LDH data are not shown for this panel because measurements in the culture medium exceeded the upper limit of the calibration range.

Fig. 6. ATP levels in 2D RPTECs treated with oligonucleotides without reported serious clinical nephrotoxicity and SPC5001.

Conclusion

- The 2D and 3D RPTEC models showed complementary responses. Only SPC5001 reduced ATP levels below 75% in 2D, whereas 3D showed LDH release and Caspase-3/7 activity despite limited ATP changes.
- Additional Caspase-3/7 responses to ISIS388626 and Givosiran suggest that 3D may capture injury-related responses less apparent in 2D. LDH release alone did not clearly distinguish clinical nephrotoxicity groups.
- Combining ATP-based 2D screening with 3D assessment of LDH release and Caspase-3/7 activity provides complementary information on oligonucleotide-induced renal injury responses. Further studies are required to establish predictive utility for clinical nephrotoxicity.

Proposed Complementary Roles of 3D and 2D RPTEC Models

	3D RPTEC	2D RPTEC
Main findings	LDH and Caspase-3/7 responses with limited ATP changes	ATP below 75% with SPC5001
Interpretation	Cellular injury and apoptosis-related responses	ATP-based viability-associated changes
Proposed role	Complementary injury assessment	Initial screening