GPX4-Associated Cytoprotective Activity of Renal Autologous Cell Therapy Candidate Rilparencel in Human *ex vivo* and *in vitro* CKD Models

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INTRODUCTION

- In CKD/DKD, chronic metabolic stress drives tubular iron accumulation and lipid peroxidation, causing localized nephron loss once the primary GPX4 defense pathway is overwhelmed.[1,2]
- Rilparencel, an autologous population of selected renal cells (SRC) currently in a Phase 3 registrational study (NCT05099770), potentially preserves renal function in patients with advanced CKD and T2D.[3,4] However, SRC-mediated modulation of the GPX4-regulated lipid peroxidation cascade remains unexamined.
- This exploratory pipeline pairs humanized *ex vivo* and 3D *in vitro* models with complementary 2D co-cultures to screen for pathway-level changes.

METHODS

Ex Vivo Kidney Biopsy

- Biopsies from National Disease Research Interchange (NDRI) donor kidneys were cultured with or without NDRI-derived SRC for 10 days in air liquid interface followed by scRNA-seq.

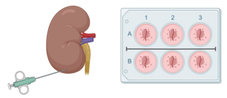

Figure 1: *Ex vivo* kidney biopsy ± SRC co-culture platform overview.

3D *In Vitro* Kidney-on-Chip

- Primary renal proximal tubule epithelial cells (RPTEC) and glomerular microvascular endothelial cells (GMVEC) were cultured on a collagen-I matrix interface to establish mature lumen.
- Chips were exposed to injury media (table 1) for 7 days, and co-cultured with or without NDRI-derived SRC in the grafting chamber (Figure 2) followed by scRNA-seq (*n*=16 chips pooled per condition) or functional assays (*n*=3-4 chips per condition).

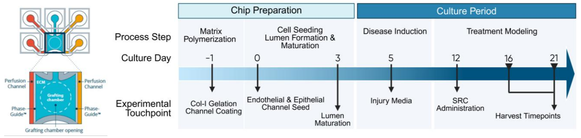

Figure 2: Experimental overview and timeline for *in vitro* kidney-on-chip ± SRC co-culture using OrganoPlate Graft.[5] Left panel image adapted from MIMETAS product documentation (Mimetas BV, The Netherlands).[6]

Table 1. Injury media formulation (prepared in cell-specific basal media)

Injury Inducer	Components	Concentration
Glucose		4.5 g/L (25 mM)
CytoMix	IL-6, IL-1β, TNFα, MCP-1, CXCL8, TGF-β1	0.0325 - 2.5 ng/mL
LipidMix	arachidonic, linoleic, linolenic, myristic, oleic, palmitic, stearic acid, cholesterol	2 – 20 ng/mL
Uremic toxins	p-cresyl sulfate, indoxyl sulfate	10 µM

2D *In Vitro* Co-Culture (Primary RPTEC)

- Glutathione peroxidase 4 (GPX4) activity was pharmacologically modulated using RSL3 (inhibitor) and PKUMDL-LC-101-D04 (1D4, activator).
- Time-resolved responses were evaluated for oxidative stress (GSH:GSSG, TFCH, BODIPY) and mitochondrial fitness (TMRM, Seahorse bioanalyzer) across control and injury conditions ± NDRI-derived SRC ± RSL3 (Figure 3).

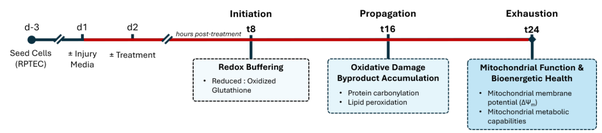

Figure 3: Experimental overview and timeline for acute *in vitro* functional assays.

RESULTS (SRC Mediate Iron and Lipid Homeostasis):

Ex Vivo Kidney Biopsy

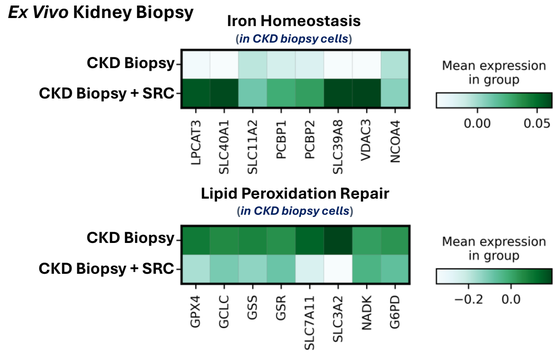

Figure 4: Transcriptional modulation of iron-handling and lipid peroxidation markers following SRC co-culture. Pilot SRC co-culture induces an increase in iron homeostasis gene expression (top panel) with a corresponding downregulation of lipid peroxidation repair transcripts (bottom panel), consistent with a stabilized cellular environment and attenuated oxidative stress profile.

3D *In Vitro* Kidney-on-Chip

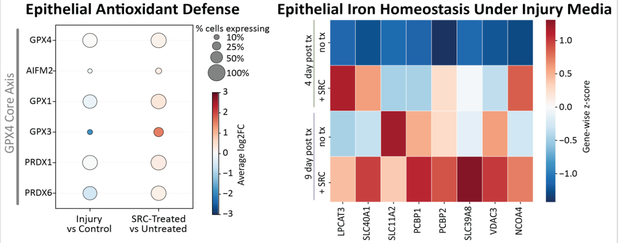

Figure 5: Pathway analysis of epithelial responses to SRC co-culture under continuous injury. (A) Single cell RNA-seq dot plot comparing RPTEC cultured in injury vs. control media conditions (left) to SRC-treated vs. untreated injured RPTEC (right). Injury drives downregulation of cytoprotective pathways related to lipid peroxidation repair while SRC administration activates a coordinated cytoprotective and metabolic adaptive program in injured RPTEC. (B) Mean log-normalized gene expression was calculated and visualized as gene-wise z-scores. Values were standardized independently for each gene across the four injury groups. SRC-treatment increased expression of several genes in the iron homeostasis pathway over the duration of co-culture.

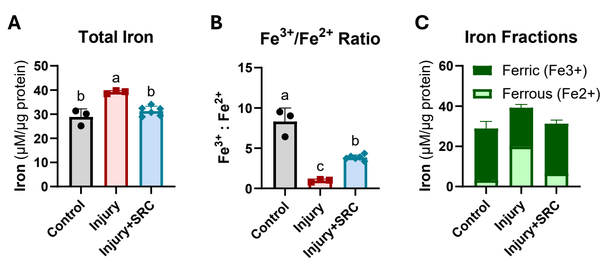

Figure 6: Total and fractional iron pool quantification. (A) Injury-induced cellular iron accumulation is restored to baseline levels by cell therapy. (B) Homeostatic Fe^{3+}/Fe^{2+}ratio collapses post-injury and is partially but significantly recovered with SRC co-culture. (C) Injury response is driven by expansion of the reactive ferrous (Fe^{2+}) pool, which SRC co-culture selectively suppresses. Values normalized to total protein; dissimilar letters indicate $p<0.05$ via One-Way ANOVA (Mean ± SD; *n*=4 chips pooled per condition; *n*=3 donors).

RESULTS (SRC Promote Glutathione Turnover)

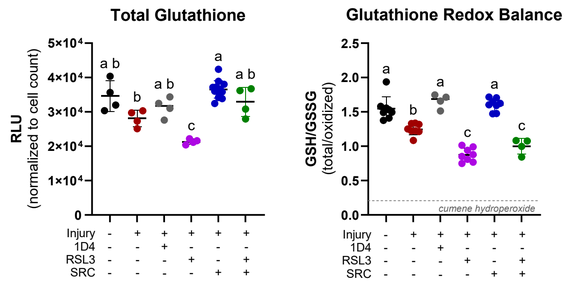

Figure 7: Glutathione Redox Balance. Continuous injury initiates a shift in the glutathione redox equilibrium and is driven by a kinetic bottleneck within the glutathione axis. SRC co-culture bypasses the injury-induced bottleneck in a GPX-4 dependent manner and is capable of acute restoration of proximal tubule epithelial host cell antioxidant turnover. Dissimilar letters indicate $p<0.05$ via One-Way ANOVA (Mean ± SD; *n*=3 donors).

RESULTS (SRC Reduce Intracellular Oxidative Burden)

Figure 8: Oxidative Byproduct Accumulation. Continuous injury leads to early accumulation of oxidative byproducts. SRC co-culture confers broad protection that is not exclusively mediated through GPX4 activity and can reduce the proximal tubule epithelial host intracellular oxidative burden. Dissimilar letters indicate $p<0.05$ via One-Way ANOVA (Mean ± SD; *n*=3 donors).

RESULTS (SRC Preserve Mitochondrial Function)

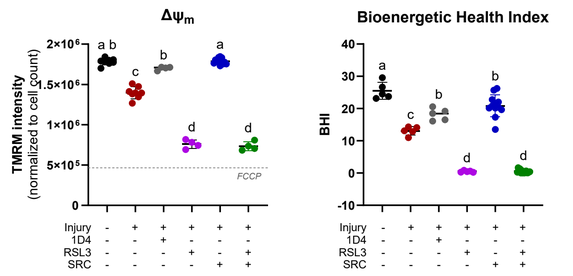

Figure 9: Bioenergetic Attrition. Lipid peroxidation is the primary driver of bioenergetic collapse under continuous injury conditions. SRC-mediated lipid preservation is an essential component for proximal tubule epithelial host mitochondrial stabilization. Dissimilar letters indicate $p<0.05$ via One-Way ANOVA (Mean ± SD; *n*=3 donors).

STUDY LIMITATIONS

- In a multi-scale co-culture system, utilizing a single mixed media formulation across diverse cell types represents a metabolic compromise that may obscure cell-specific nutrient requirements or baseline survival cues.
- Direct co-culture in a 2D system obscures individual cell responses, as bulk assays generate a confounded, blended signal of both host RPTEC and SRC signals.
- Direct co-culture in a 2D system is restricted to immediate, short-term interactions rather than long-term or chronic responses.
- While the selected small molecule doses maintain >85% viability, they can still induce sub-lethal cellular stress that alter the phenotypic readout independently of a target-specific effect.
- Future studies will employ genetic knockouts or overexpression to definitively validate possible mechanisms of action, eliminating the potential for sub-lethal small molecule stress.

SUMMARY AND CONCLUSIONS

- This multi-scale pipeline successfully leverages humanized *ex vivo* and advanced *in vitro* platforms alongside 2D co-cultures to screen for complex pathway-level responses without relying on single-cell reductionist models.
- *Ex vivo* exposure to SRC resulted in an overall reduction in expression of lipid peroxidation repair genes with a concomitant upregulation of iron homeostasis regulation genes, indicating that cells transition away from an active injury cascade once stabilized.
- Advanced 3D *in vitro* modeling data suggest SRC administration directly mitigates cellular oxidative burden by modulating iron fractions and stabilizing the Fe^{3+}/Fe^{2+} ratio, thereby limiting the pool of reactive iron available to drive oxidative damage.
- SRC administration reduces proximal tubule epithelial intracellular oxidative burden and preserves mitochondrial function by actively promoting glutathione antioxidant turnover.
- SRC-mediated lipid preservation serves as an essential component for proximal tubule epithelial host cell mitochondrial stabilization, restricting active oxidative damage and supporting adaptive cell survival.
- Parallel pharmacological studies confirm that these cytoprotective effects are not exclusively mediated through GPX4 activity, strongly supporting that SRC operate via adaptive, multimodal and multifactorial mechanisms of action.

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Presented at: ASN Kidney Week; October 24th 2026; Denver, CO

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