A Human Microphysiological System Characterizes Renal Autologous Cell Therapy Candidate Rilparencel-Mediated Responses in a CKD Injury Model

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INTRODUCTION

- Coordinated multicellular interactions drive metabolic and inflammatory injury in CKD but are difficult to resolve using reductionist culture systems.
- Co-culturing human renal proximal tubule epithelial cells (RPTEC) and glomerular microvascular endothelial cells (GMVEC) within a kidney-on-chip microphysiological system (MPS) reveals how cellular cross-talk drives compartment-specific injury mechanisms.
- Introducing rilparencel, composed of an autologous population of selected renal cells (SRC) currently in a Phase 3 registrational study (NCT05099770)[1], into this ongoing injury microenvironment provides a high-resolution humanized testbed to evaluate potential mechanisms of action (MoA) across epithelial and endothelial compartments.

METHODS

- Primary RPTEC and GMVEC were co-cultured in an OrganoPlate Graft on a collagen-I matrix interface for 5 days to establish mature lumens (Figure 1).
- Chips were exposed to injury media (table 1) for 7 days, and co-cultured with or without National Disease Research Interchange (NDRI)-derived SRC in the grafting chamber (Figure 2) for 4- or 9-days.
- Single-cell RNA sequencing (scRNA-Seq) and functional assays were used to characterize multicellular injury states and responses following NDRI-derived SRC treatment across control and injury conditions (*n=16* chips pooled per condition).

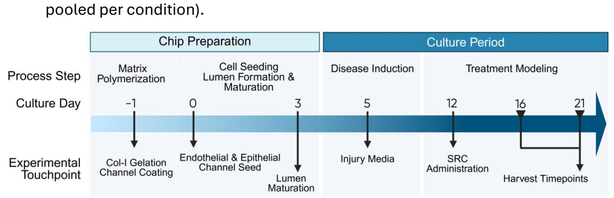

Figure 1: Experimental overview and timeline for *in vitro* kidney-on-chip ± NDRI-derived SRC co-culture.

- For 2D co-culture functional validation, RPTEC or GMVEC were exposed to injury media for 3 days and co-cultured with or without NDRI-derived SRC for 4 days followed by relevant functional assays.

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

KIDNEY-ON-CHIP MODEL

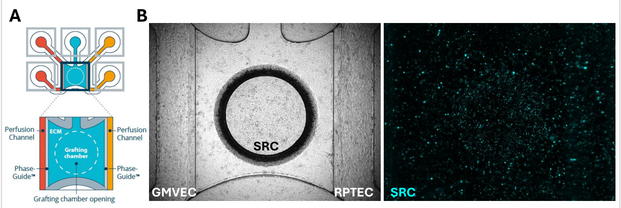

Figure 2: Kidney-on-chip. (A) Schematic for kidney-on-chip platform using OrganoPlate Graft. Image adapted from MIMETAS product documentation[2] (Mimetas BV, The Netherlands). (B) Representative brightfield (left) and QTracker-labeled SRC (right) administered into the grafting chamber.

RESULTS (SRC Promote Stress Adaptive Cellular Crosstalk)

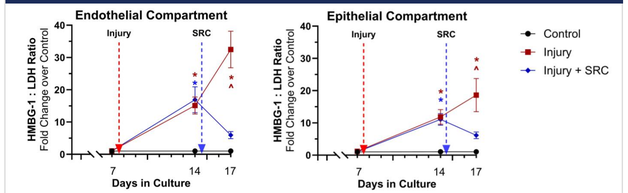

Figure 3: SRC attenuate DAMP accumulation within the dual-compartment microenvironment. Under uniform injury conditions, DAMP secretion is skewed toward the endothelial channel, as indicated by an elevated HMGB1-to-LDH ratio relative to the epithelial compartment. (Mean ± SD; *n*=4 chips with *n*=2 donors; Two-way ANOVA with Tukey's post-hoc; * *p*<0.05 vs. Control, ^ *p*<0.05 vs Injury + SRC).

RESULTS (SRC Induce Epithelial Cytoprotective Response)

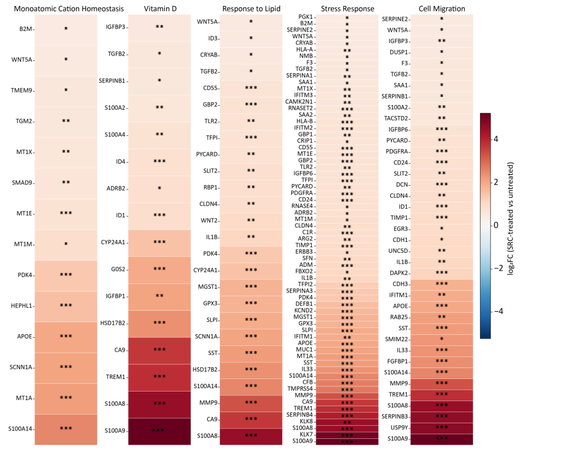

Figure 4: Pathway analysis of proximal tubule epithelial responses to SRC co-culture under continuous injury. Pseudobulk differential expression analysis depicting log2 fold change for SRC-treated versus untreated RPTEC, controlling for timepoint. SRC administration activates a coordinated cytoprotective and metabolic adaptive program in injured RPTEC, characterized by the upregulation of metal ion homeostasis, intracrine Vitamin D axis, enhanced antioxidant defenses, and activation of pathways involved in epithelial cell survival, migration, and membrane stabilization.

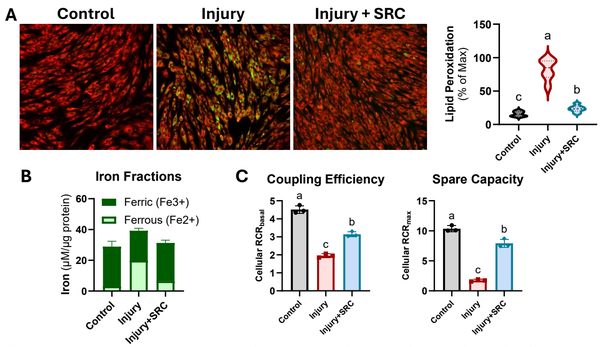

Figure 5: Functional confirmation of SRC-mediated cytoprotection and metabolic adaptation. SRC co-culture (A) reduces lipid peroxidation byproducts accumulation, (B) suppresses expansion of the reactive ferrous (Fe^{2+}) pool, and (C) mitigates injury-induced declines in mitochondrial fitness (Agilent Seahorse Mito Stress Test). Dissimilar letters indicate p<0.05 via One-Way ANOVA (Mean ± SD; *n*=3-8 experiments with *n*=3-6 donors).

RESULTS (SRC Support Endothelial Junctional Integrity):

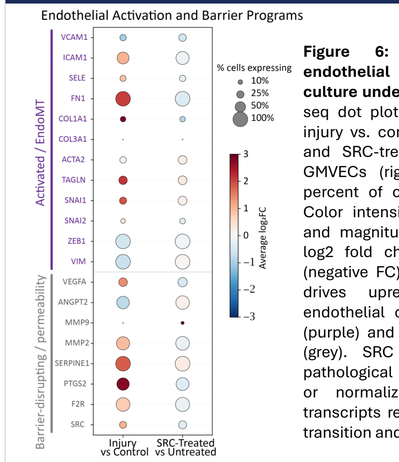

Figure 6: Pathway analysis of endothelial responses to SRC co-culture under continuous injury. scRNA-seq dot plot comparing GMVECs under injury vs. control media conditions (left) and SRC-treated vs. untreated injured GMVECs (right). Dot size reflects the percent of cells expressing each gene. Color intensity represents the direction and magnitude of the mean single-cell log2 fold change, spanning from blue (negative FC) to red (positive FC). Injury drives upregulation of pathological endothelial cell activation and EndoMT (purple) and barrier disrupting pathways (grey). SRC co-culture mitigates this pathological response, with a reduction or normalization (blue / white) of transcripts responsible for mesenchymal transition and barrier disruption.

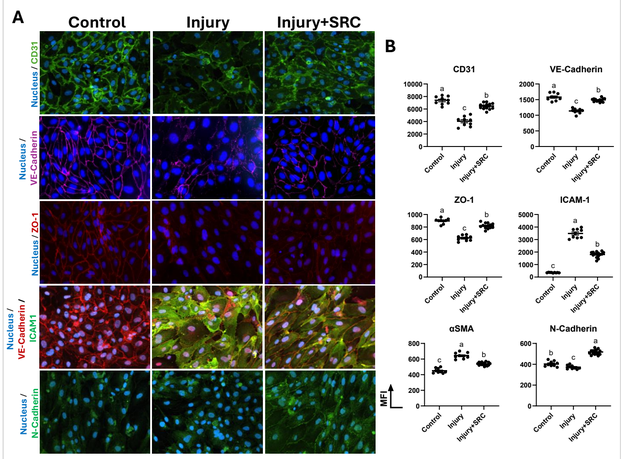

Figure 7: SRC co-culture promotes restoration of endothelial junctional integrity under continuous injury conditions. (A) Representative immunofluorescence images and (B) quantification of microvascular endothelial cells evaluating relevant protein expression and localization. Dissimilar letters indicate p<0.05 via One-Way ANOVA (Mean ± SD; *n*=9 chips with *n*=3 donors).

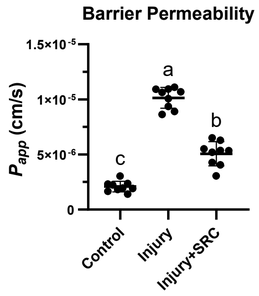

Figure 8: SRC improves endothelial barrier function under continuous injury conditions. Barrier integrity and function was quantified by measuring the flux of FITC-dextran (70 kDa, Equation 1) across the endothelial cell monolayer in 24-well Transwell inserts 4 days following SRC addition in the basolateral chamber. Dissimilar letters indicate p<0.05 via One-Way ANOVA (Mean ± SD; *n*=3 donors).

Equation 1

$$P_{app} = \frac{\frac{\Delta Q}{\Delta t}}{A \times C_0}$$

ΔQ/Δt = flux of FITC-Dextran
A = surface area (0.33 cm²)
C_0 = initial [FITC-Dextran]

RESULTS (Potential Mechanistic Targets):

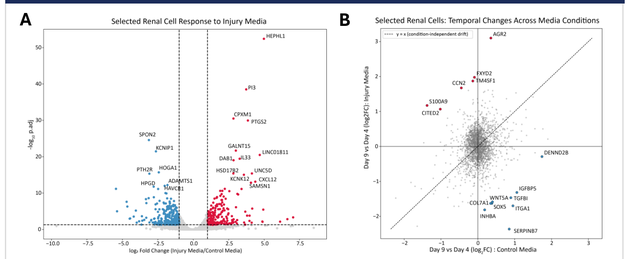

Figure 9. Identification of high-confidence DEGs provides a roadmap for systematic functional genomics to dissect SRC MoA. (A) Volcano plot showing differentially expressed genes (DEG) in pseudobulk scRNA-seq data from SRC exposed to injury media versus control media. (B) Comparison of temporal transcription responses between day 4 and day 9 culture conditions. Key DEGs are labeled by name. SRC upregulate a network of acute cytoprotective and chemotactic pathways, while progressively increasing the expression of chronic tissue repair support factors as the injury model advances. Integrating these differentially expressed targets into a multiplexed gene editing platform will demonstrate whether the identified DEGs are active or passive drivers of potency, effectively transforming correlative transcriptomic data into definitive, causative MoA validation.

STUDY LIMITATIONS

- The OrganoFlow perfusion system utilizes bidirectional, gravity-driven fluid shear stress rather than unidirectional, continuous flow.
- The engineered injury system utilizes specific factors relevant to DKD/CKD to ensure high experimental control but is inherently limited in its ability to model the holistic, multi-organ cross-talk that characterizes clinical CKD.
- While capturing the epithelial-endothelial interface, this model currently excludes circulating immune cells and interstitial fibroblasts, which play critical roles in driving the chronic inflammatory cascades and fibrotic remodeling characteristic of advanced CKD.

SUMMARY & CONCLUSIONS

- These findings highlight the value of advanced human MPS for elucidating mechanistic responses of the renal autologous cell therapy candidate, rilparencel, to CKD-relevant injury in a complex, multicellular model system.
- High-dimensional transcriptomics allow us to map global gene expression changes in a human multicellular injury model, uncovering additional downstream pathways to refine our understanding of rilparencel's putative MoA.
- CKD-relevant injury elicits immunogenic signaling and broad cellular dysfunction across epithelial and endothelial cell populations in a human kidney MPS.
- SRC co-culture mitigates these effects by promoting stress-adaptive regulation that preserves cellular functions and alleviates inflammatory and metabolic damage.

REFERENCES

1. NCT05099770; Proact: A Study of REACT in Subjects with Type 2 Diabetes Mellitus and Chronic Kidney Disease (REGEN-006). ClinicalTrials.gov
2. OrganoPlate® Graft Product Brochure. Brochure. MIMETAS; 2026.

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