**Abstract: TH-PO763**

# Genome Engineering of Renal Epithelial Cells with Improved Function for an Implantable Artificial Kidney

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## Authors


- Wilson, Matthew H., VA Medical Center, Nashville, Tennessee, United States
- Luo, Wentian, Vanderbilt University Medical center, Nashville, Tennessee, United States
- Veach, Ruth Ann, Vanderbilt University Medical center, Nashville, Tennessee, United States
- Welch, Rick C., VUMC, Nashville, Tennessee, United States
- Roy, Shuvo, UCSF, San Francisco, California, United States
- Fissell, William Henry, Vanderbilt University Medical center, Nashville, Tennessee, United States



**Background**

Development of an implantable artificial kidney (IAK) will require renal epithelial cells capable of reabsorption of salt and water. We used genome engineering to bioengineer cells for improved $Na^+/H^+$ exchange and $H_20$ reabsorption. The non-viral *piggyBac* transposon system enables genome engineering cells to stably overexpress one or more transgenes simultaneously. The CRISPR/Cas9 system (dCas9-VP64) enables selective endogenous gene upregulation in human cells.

**Methods**

We generated epitope tagged human sodium hydrogen exchanger 3 (NHE3) and aquaporin-1 (AQP1) cDNA expressing *piggyBac* transposon vectors. *piggyBac* transposase expression enabled stable transposon integration and overexpression of NHE3 or AQP1 in cultured renal epithelial cells. The transposon system was also used to integrate dCas9-VP64 into human kidney cells.

#### Results

As a proof-of-principle, we generated renal epithelial (MDCK) cells stably expressing a cumate inducible NHE3 and confirmed induced overexpression via Western blot and immunofluorescence analysis. We also generated MDCK cells stably overexpressing AQP1. Cell surface delivery of NHE3 and AQP1 was confirmed using cell surface biotinylation assays. Importantly, MDCK cells expressing AQP1 and cumate inducible NHE3 demonstrated increased cellular transport. We subsequently used a high-throughput flow cytometry analysis to generate a library of MDCK clones with varying expression of AQP1 and NHE3 to determine the best ratios for optimized transport function. We tested and validated various guide RNAs (gRNAs) for targeted upregulation of endogenous AQP1 and NHE3 in human kidney cells at both the mRNA and protein level.

#### Conclusion

Our results reveal that genome engineering can enable improved cellular transport via stable overexpression of AQP1 and NHE3 in polarized kidney epithelial cells. We are currently attempting to extend these studies to human renal proximal tubular epithelial (RPTEC) cells. Additionally, the dCas9-VP64 system enables upregulation of endogenous AQP1 and NHE3 in human kidney cells. These studies will allow us to determine the optimal genome engineering approach and renal epithelial cell type for maximal function in the IAK.


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