Kidney macrophages enhance vasculature formation in early phased nephrogenic zone organoid

Author: Kim Nguyen[1]; Isis N. Thomas[1]; Mandy J. Croyle[1]; Reagan S. Andersen[1]; and Bradley K. Yoder, PhD[1].

Author Affiliation: [1]Department of Cell, Developmental, and Integrative Biology, Heersink School of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, the United States of America.

Introduction: *Ex vivo* kidney organogenesis offers a potential replacement source of functional tissue for more than 100,000 Americans waiting for a kidney transplant. Nephrogenic zone organoids (NZOs) differentiated from primary mouse nephrogenic zone cells demonstrate cellular complexity, but their weak and unstable vascular network hinders NZO survival and function upon engraftment. During kidney development, resident macrophages have been shown to promote endothelial connections, but there is a lack of macrophages in NZOs generated from current protocols. Therefore, we hypothesize that adding macrophages into NZOs will enhance vasculogenesis, which could be critical for their survival and subsequent engraftment to the host to acquire blood flow and partially compensate for kidney function.

Methods: Bead-isolated $F4/80^{+}$ macrophages from $CX3CR1^{GFP}$ $CCR2^{RFP}$ reporter mice (for resident and infiltrating kidney macrophages respectively) were added into NZO cultures at day 0. Organoids were harvested on days 4 to 7 for immunofluorescent staining, including CD31, a marker for vessels.

Results: Organoids with macrophages showed a well-established vasculature compared to minimal vessels detected without macrophages through day 5. However, blood vessels started to disappear on day 6 and were almost undetectable on day 7 in both groups.

Conclusion: Our results supported the role of macrophages in promoting vasculature formation in early stage NZOs, but further studies are needed to maintain macrophage-based vasculogenesis in NZOs. Successful completion of this project will bring us closer to the goal of *ex vivo* generation of functional renal tissues for transplantation.

Funding Resources: This research was funded by the 5RC2DK125960-03 (BKY) by the National Institute of Health (NIDDK).