

## The use of agarose beads as spatial markers and bone wax to stabilize the kidney for repeated intravital renal evaluation in mice using an abdominal imaging window.

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Observation of renal cellular and physiological responses during injury and cystogenesis in vivo is important to advance our understanding of PKD pathogenesis and progression. One method to allow visualization of the kidney and tubule function in mouse models is the placement of an abdominal imaging window (AIW) and longitudinal intravital imaging. However, current approaches to immobilize the kidney lead to inflammation and fibrotic changes in the surrounding area complicating long term studies. Identifying the same region in the kidney is critical for this analysis.

We investigated several methods for kidney support and immobilization during AIW placement, as well as agarose bead placement under the kidney capsule as spatial markers for longitudinal studies of the same nephrons in adult mice. Previous studies have used sterile gauze to prevent kidney movement, but this resulted in fibrotic changes within the surrounding tissue and adherence of the kidney to the window making it difficult to visualize the kidney for more than 3 days. Use of bone wax to secure kidney placement in the window resulted in less fibrotic changes, allowing visualization of the kidney for up to 3 weeks. Additionally, coating the window with PEG prior to placement prevented adhesion of the kidney to the AIW. The placement of ~200  $\mu\text{m}$  beads under the kidney capsule permitted monitoring of the same region of the kidney over the length of the study.

We found that using bone wax, PEG coating, and beads greatly extends visualization of the same nephrons compared to other techniques. This longer time of visualization will allow us to analyze changes in tubule and vascular flow dynamics, ciliary responses, cellular movements and interactions, and pathways using biosensor reporters during cyst formation and progression.

Funding: R01DK122939, RC2DK125960 to BKY