Quantitative platform for optically cleared tissue validated in inflammatory kidney disease

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Crescentic nephritis is a highly complex form of inflammatory kidney disease, characterized by formation of pathological lesions (crescents) in the kidney filters (glomeruli). While the development of crescents remains incompletely understood, major features of crescentic nephritis include capillary injury, podocyte injury, parietal epithelial cell activation, and interstitial inflammatory infiltrates and fibrosis, all of which clearly highlight the complexity of this disease.

There has been a recent surge in the development of optical clearing methods. However, their application to kidney tissue has proven challenging. Features of experimental crescentic nephritis were investigated using our quantitative platform for optically cleared tissue, which combines lineage tracing, optical clearing, advanced light microscopy and 3D morphometry.

Tissue collection was performed after perfusion with physiological saline via aortic cannulation under controlled pressure. Multiple optical clearing methods were optimized for kidney tissue, including aqueous-based (hyperhydration; SCALE), hydrogel-based (CLARITY), and solvent-based (Ethyl Cinnamate). Simple and cheap in-house made imaging chambers were used for both up-right and inverted microscopy systems. Image acquisition was based on whole structures (ie. glomeruli) or random 3D fields of view for analyses of interstitial inflammatory deposits and fibrosis.

Crescentic kidneys had signs focal podocyte loss. Pathological glomeruli showed higher numbers of parietal epithelial cells, and a reduction in capillary volume. Glomerular integrity (ie. patent tubular connection) and intraglomerular location of lesions were identified in 3D. Crescentic kidneys showed increases in interstitial inflammatory infiltrates and interstitial fibrosis compared to controls. Tissue de-clarification allowed co-registration between 3D and 2D analyses via immunolabelling and classical histopathology.

In conclusion, this platform provides new tools for the study of pathophysiology and validation of potential therapies in complex disease settings.