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Tissue Clearing: A Novel Tool for Visualization of Collagen Deposition in Injured Kidney

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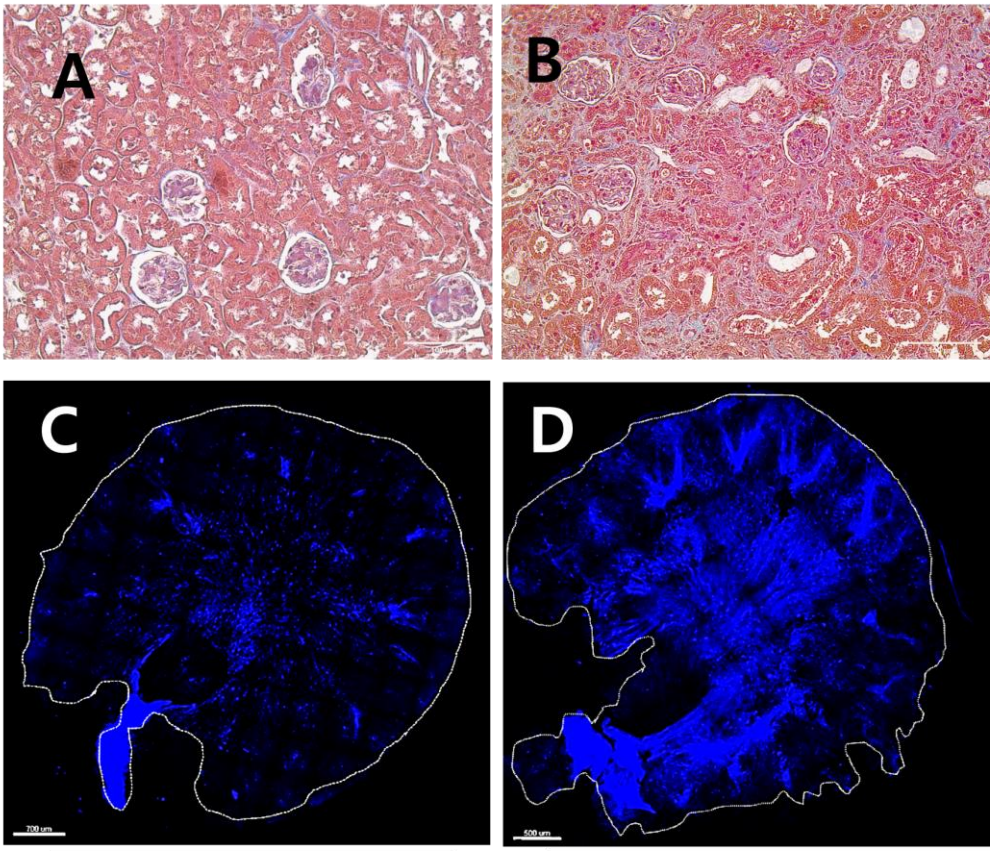
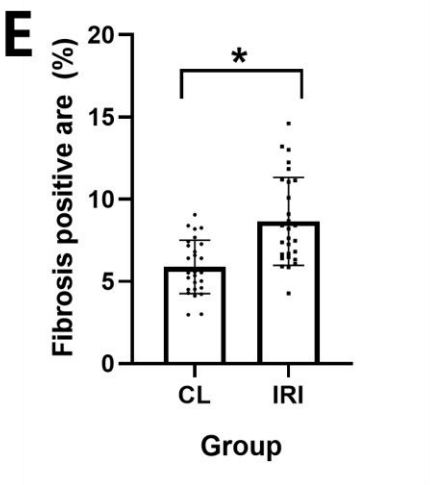
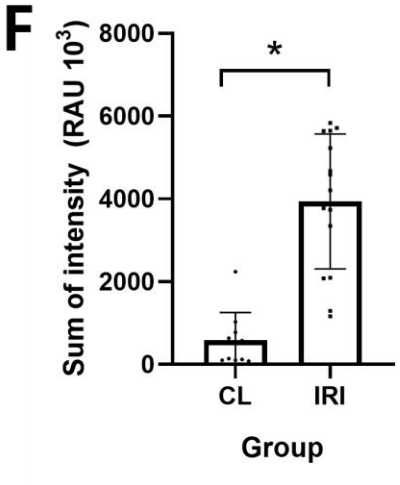
Objectives : Fibrosis and excessive deposition of extracellular matrix is a key mechanism of acute kidney injury progression to chronic kidney disease. Second-harmonic generation (SHG) multiphoton microscopy is a useful tool for label-free imaging of repetitive extracellular structures, namely collagen. In this study, we investigated the utility of label-free SHG multiphoton microscopy for examining kidney fibrosis via pathogenic collagen deposition in whole mouse kidney.
Methods : Ischemia-reperfusion injury (IRI) was induced by unilateral clamping of the left renal artery for 45 minutes on 12-month-old mice (n=32). After 3 weeks, mice were sacrificed and the injured kidney, defined as IRI, and non-injured kidney, defined as contralateral (CL), were isolated and processed for Masson-Trichrome (MT) staining or tissue clearing. Fibrosis with MT staining was quantified by calculating the area positive for fibrosis under 100X magnification light microscopy with Image J software. Whole mouse kidney samples were cut into 1-2mm sections for tissue clearing and collagen deposition was imaged using multiphoton microscopy. Fibrosis was quantified by summing the signal intensity of collagen deposition in each sample.
Results : Compared to CL kidney, IRI kidney showed significantly more fibrosis on MT staining (Figure 1A-1B). Although statistically significant, difference in fibrosis positive area was within 5% (Figure 1E). Multiphoton microscopy showed deposition of normal collagen through label-free imaging of whole mouse kidney (Figure 1C). Quantification of collagen deposition showed significantly more fibrosis in IRI kidney, predominantly at the corticomedullary junction (Figure 1D). Multiphoton microscopy was much more sensitive for quantification of fibrosis, showing a 6.5-fold difference between groups (Figure 1F).
Conclusions : Analysis of collagen deposition using SHG multiphoton microscopy is not only well correlated with histopathologic examination, but more sensitive to collagen deposition and reveals additional information about geographic distribution of fibrosis without the need for tissue staining, proving it a useful tool for visualization of collagen deposition in injured kidney.

Poster figure 1.png


A
B
C
D


E
Fibrosis positive are (%)
20
15
10
5
0
*
CL
IRI
Group


F
Sum of intensity (RAU 10^3)
8000
6000
4000
2000
0
*
CL
IRI
Group