Use of Second Harmonic Generation (SHG) and 2-Photon Emission (2PE) Imaging to Quantify and Describe the Structure of Tissue Fibrosis in the Rat TNBS Intestinal Fibrosis Model

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BACKGROUND

Intestinal fibrosis is the dominant cause of complication and surgery in Crohn’s disease.

- Strictures have increased collagen content, but collagen structure and networks in fibrotic intestine are poorly understood.
- Architectural changes in the collagen matrix induce and propagate fibrosis.
- However, with current methods, it is difficult to quantify collagen organization in tissue.
- Trichrome staining is the histological standard to identify collagen. However, it is not amenable to quantification (or automation).
- Second Harmonic Generation (SHG) imaging and two-photon excitation fluorescence (2PE) imaging enables:
  - Stain-free and label-free imaging
  - Preserves tissue microarchitecture
  - Can analyze standard histological sections (i.e. FFPE)
  - Extremely sensitive (0.39 μM resolution at 20x)
  - Quantification of tissue collagen content
  - Quantification of collagen fiber organization

AIMS

1) To determine whether SHG, 2PE can detect changes in tissue collagen associated with the progression of intestinal fibrosis
2) To determine if collagen fiber organization correlates with biochemical and histological fibrosis
4) To determine whether SHG and 2PE can differentiate between normal, inflammed, and fibrotic intestine

METHODS

Rat TNBS colitis and fibrosis model
- Female Lewis rats
- Intracolonic TNBS (2,4,6-Trinitrobenzenesulfonic acid)
- acute (d2, d4, d7) single, 15 mg dose
- chronic (d14, d28) weekly, escalating doses (15 mg to 60 mg)
- Outcomes: Fibrotic gene expression (coll1A1, coll III, IGF-1, TGFβ)
  - Inflammatory gene expression (IL-1β, TNF-α)
  - Tissue ROIs delineating the epithelium, submucosa (lamina propria) and muscularis
  - ROI selections were reviewed by a pathologist (D.M., blinded to the experimental groups)

SHG and 2PE Imaging
- Unstained, deparaffinized FFPE slides
- GENESIS R 200 imaging system (HistoIndex), high (0.39 μM resolution)
- Two optical methods
  - SHG Second Harmonic Generation method (transmission), 350 nm (collagen I, III)
  - Two photon fluorescence (2PE), 550 nm (NADPH, NADH)
- Tissue ROIs delineating the epithelium, submucosa (lamina propria) and muscularis
- ROI selections were reviewed by a pathologist (D.M., blinded to the experimental groups)
- Image Analysis (proprietary algorithms/software) of collagen
  - Collagen content
  - Retraction index (%) = # of branching points/total length of the skeleton.
  - Tortuosity = walking (geodesic) length/distance between the fiber end points.

SHG collagen objects, tortuosity, and reticulation
- Histological comparison of SHG to Trichrome staining
- Gene expression
  - Fibrotic genes: col1A1, coll III, TGFβ, IGF-1
  - αSMA protein expression
- Analysis
  - Assayed full thickness, submucosa, muscularis and epithelial ROIs
  - Rationale for tissue sub-analysis
  - Epithelial loss due to ulceration (acute phase) or scarring (fibrotic phase)
  - Fibrosis initially develops from activated lamina propria myofibroblasts
  - Muscularis hypertrophy and architectural distortion via collagen infiltration
  - R package (Pearson correlation) cox-test

RESULTS

Figure 1. SHG, 2PE imaging and identification of regions of interest (ROIs) in rat distal colon.

Figure 2. SHG collagen imaging corresponds to Trichrome collagen staining.

Figure 3. Collagen structural changes (tortuosity and reticulation) precede myofibroblast activation in the submucosa.

Figure 4. SHG tortuosity and reticulation correlate with biochemical markers of fibrosis, not inflammation.

CONCLUSIONS

- SHG imaging reproduces Trichrome staining for collagen.
- SHG detects collagen I and III structural changes in fibrotic intestinal tissue.
- Simple direct measurement of collagen content is insufficient to quantitate tissue fibrosis.
- However, measurement of collagen morphometrics correlates with biochemical metrics of tissue fibrosis, including col1A1, col III gene expression, αSMA protein expression and histological scoring.
- SHG tortuosity and reticulation are independent of inflammatory gene expression.
- Collagen structural changes (as evidenced by increased tortuosity and reticulation) precede induction of αSMA protein and MF activation.
- Future work will evaluate these image analysis tools for assessment of strictures in human Crohn’s disease.