TARGETING IN SITU AND IMAGING MULTIPLE INFLAMMATORY BIOMARKERS WITH QUANTUM DOTS IN DSS MODEL OF COLITIS

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OBJECTIVE

Develop appropriate biomarkers for quantification of inflammation. Demonstrate the use of Quantum Dots (QDs) conjugated to antibodies against Myeloperoxidase (MPO), Interleukin-1 (IL-1a) and Tumor Necrosis Factor (TNF-a) to detect and quantify acute inflammation in the DSS model of colitis.

INTRODUCTION

- Current measurement techniques for extracting MPO, IL-1a and TNFa to monitor disease progression are excruciatingly cumbersome, time consuming, and inefficient.
- We have developed an easy to use, nanosize based fluorescence assay using antibody-bound Quantum Dots (QDs) to follow disease progression and its response to treatment with therapeutic agents.
- QDs are fluorescent nanosize semiconductor particles with a size tunable emission from 495 nm to 705 nm.
- QDs offer advantages of high quantum yield, narrow emission spectrum, and extreme photostability, making them great tools for dynamic monitoring of disease progression markers

Figure1: Size tunable emission of QDs

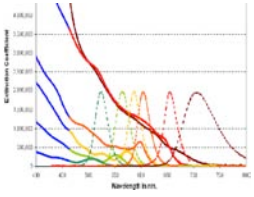


Figure2: Extinction and Emission Plot for QDs

MATERIALS & METHODS

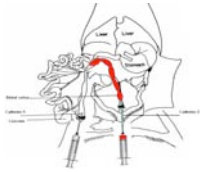


Figure 3. Surgical preparation for introduction of QDs in the colon of a mouse

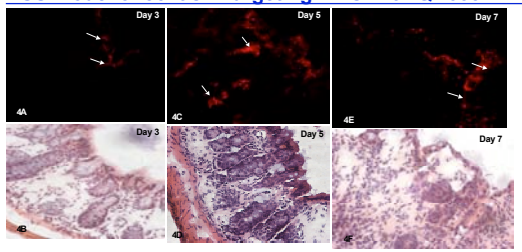
Animal Studies and Imaging for multiple markers

Dextran Sulfate Sodium (DSS) model of colitis

- Colitis was induced by feeding 4% DSS ad libitum and daily monitoring of Disease Activity Index (DAI) was performed.
- Animals were sacrificed depending on the DAI.
- QD conjugates were locally introduced (150nM) in the colon for 15 minutes.
- After 15 minutes, colon was washed, sectioned, processed and imaged under confocal microscope. Parallel histo- pathological sections were stained with H&E for comparison.

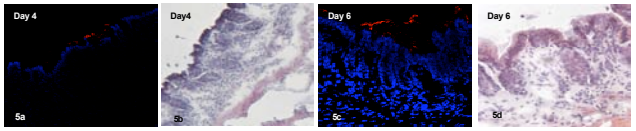
RESULTS AND DISCUSSIONS

DSS model of colitis : Targeting MPO with QD655-MPO conjugates



Adjoining images are from various days in DSS induced colitis model. Images 4A & 4B show the fluorescence and H&E stained images on the Day3 of DSS feed. On Day3 there is minimal inflammation and hence minimal expression of MPO. On Day5 there is increased inflammation, shortening of crypts (Image 4D) and increased intensity of MPO expression (Image 4C). On Day8 animal showed florid inflammation, ulceration, total loss of crypts (Image 4F) and hence even more intense labeling of the tissue with QDs (Image 4E).

DSS model of colitis : Testing the specificity of the QD conjugates



- QD- Anti-testosterone AB to test specificity of assay
- Non-specific antibody is not detected in the lamina propria

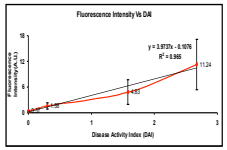


Figure7a: DAI increases with QD flow intensity. Severity of inflammation is proportional to intensity of targeted biomarker

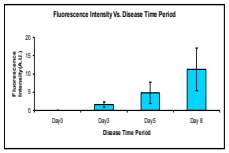
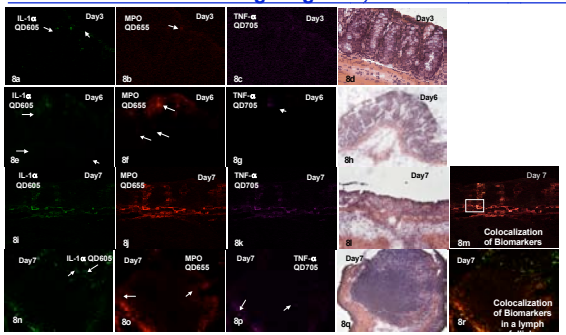


Figure7b: Flourescence increases with Disease Time Period (DTP). Severity of inflammation is proportional to intensity of targeted biomarker

DSS model of colitis : Targeting IL1α, MPO and TNFα with QD conjugates



Images depict targeting and simultaneous imaging of three different markers Il1-a, MPO and TNFα in the acute phase of DSS model of colitis. Images 8a-8r show increasing presence of markers with the increase in severity of inflammation. Image 8r shows selective targeting of these three markers in a lymphoid follicle.

CONCLUSIONS

- **Quantum dots labeled with antibodies were successfully used in situ to detect biomarkers of inflammation in an established model of experimental colitis.**
- **Animal studies showed that the *intensity* of biomarkers *correlated* well with the *disease severity and progression*.**
- **The developed assay is *specific*: QD conjugates to non-specific antibody failed to show any binding in the crypts of lamina propria in diseased animals.**
- **QD conjugates were *able to target multiple biomarkers in vivo* in the DSS model of colitis.**