Super-resolution ultrasound imaging approaches to non-invasively visualise changes in gut structure

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Context

- Gastrointestinal (GI) disease alters the release of cytokines and gut hormones[1]
- With current in vivo models of GI disease, animals are typically culled at predetermined timepoints for data collection
  - This limits the data that can be collected
  - It is difficult to study dynamic movement of tissue
  - Increases the number of animals needed for timepoints
- Contrast Enhanced Ultrasound (CEUS) imaging provides **non-invasive**, **dynamic** information on blood flow in the GI tract
  - Allows wide range of structural and dynamic parameters to be measured, including villi length and density, tissue perfusion and blood velocity
  - Enables **longitudinal monitoring** studies[2] and increase the power of the studies
  - **Reduces** the number of animals needed and **refines** the severity for each study

Project Hypothesis: CEUS imaging will reduce and refine animal use in the study of gut physiology and pathophysiology to aid the design of new therapies.

CEUS Facts

- **Contrasting agent:** Gas filled microbubbles
- **Size:** Smaller than red blood cell
- **Resolution with a Verasonics high frequency L22-14Vx probe :** 10µm in deep tissue
- **Administration:** Intravenous
- **Excretion:** Though lungs after 15 to 20 mins
- **Safety:** Excellent safety record, FDA approved

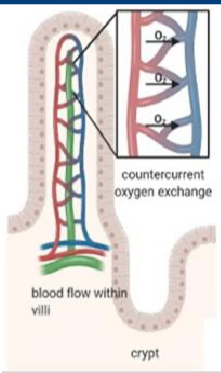

Figure 1: Schematic diagram of a villi showing the blood vessels[3]

Methods

Microbubbles filled with gas, their echo contrast to surrounding tissue as they travel through blood vessels

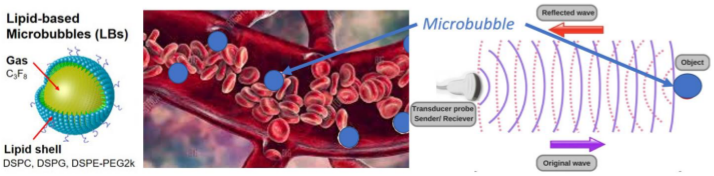

Figure 2: Schematic diagram of a microbubbles in blood vessels and the sounds waves to and from the ultrasound probe.

Singular value decomposition image of a mouse duodenum

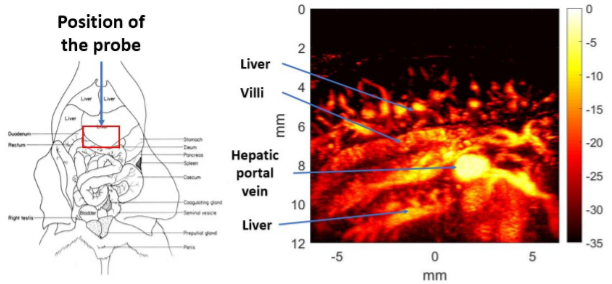

Figure 3: Diagram of mouse abdomen with exposed organs to illustrate the position of the probe. The image was acquired using a Verasonics high frequency L22-14Vx probe.

Methotrexate as an IBD model to study mild gut damage in male Wistar rats

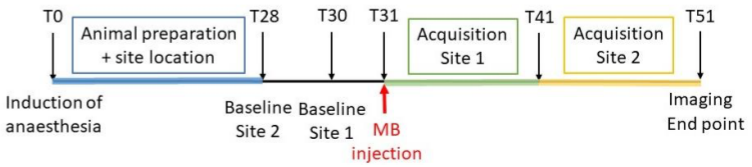

Figure 4. Timeline of the methotrexate (MTX) induced mild gut damage study in male Wistar rats. 6-day experiment starting with a baseline imaging of the duodenum followed by a once daily injection of MTX or saline for 3 days.

GLP2 analogue to induced gut growth in male Wistar rats

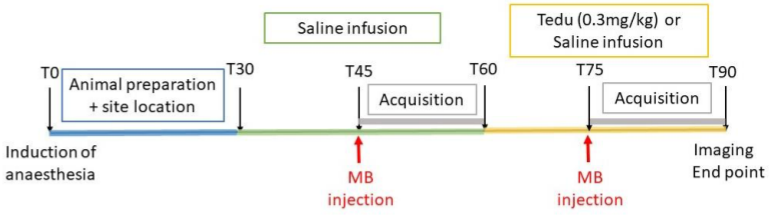

Figure 5. Timeline of the glucagon-like-peptide 2 (GLP2) analogue teduglutide (Tedu) induced gut growth study in male Wistar rats. In an 8-day experiment: baseline imaging of the duodenum then twice daily injection of tedu or saline for 6 days. Rats were imaged at end point on day 8.

Results

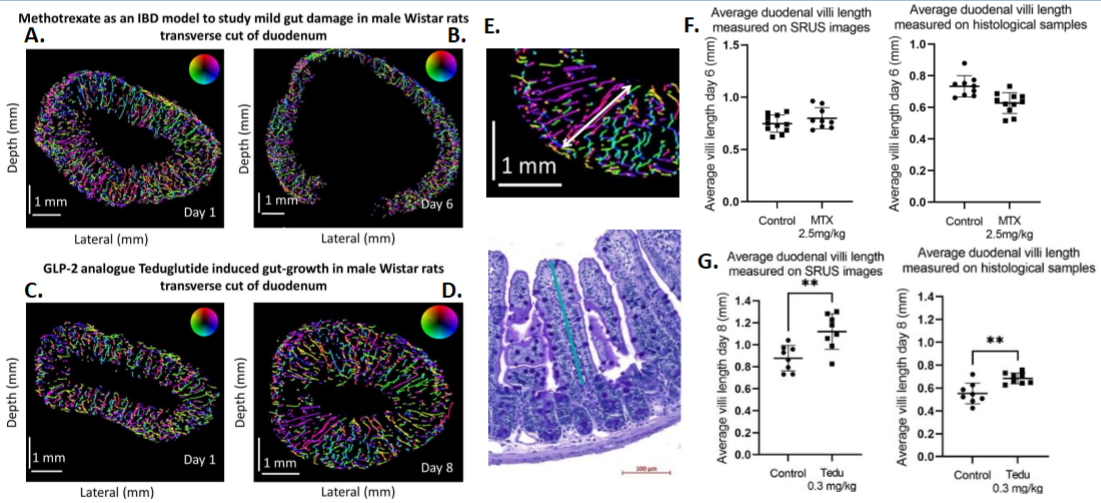

Figure 4. Super resolution ultrasound (SRUS) as a tool to study subtle structural changes in the duodenal gut wall in rats. Representative SRUS angle maps of the transverse section the duodenum taken at baseline (**A.**) and after three daily injection of methotrexate (MTX, 2.5mg/kg) causing mild blunting of the villi (**B.**). **C.** SRUS angle maps showing cuts of rat duodenum at baseline (**C.**) and the treatment of 6-day twice daily injections of the gut growth stimulating drug teduglutide (tedu, 0.3mg/kg) (**D.**). **E.** Representative images depicting villi measurements in SRUS and histology. Histological image by Fidelta, Croatia. **F.** There was no significant difference in villi length between the average villi length measured with SRUS images and on histological cut in the MTX study, likely due to the patchy effect of the drug, which it did not result in an average reduction in villi length as measured by CEUS or histology. **G.** There was a significant difference between the tedu treated group and the controls, a results that was found both in the SRUS and histological measurements. Statistical test: unpaired t-test, $P < 0.05$; MTX: methotrexate; Tedu: teduglutide

Future Work

- This data demonstrate the potential of CEUS as a tool to **non-invasively** investigate GI function and disease.
- **Longitudinally monitor gut pathology** and to better understand gut disease and response to treatment
- Study blood flow changes to the gut in response to stimuli (glucose, stress, macronutrients).

Acknowledgement

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