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Abstract—**Current clinical standards in the endoscopic diagnosis of gastrointestinal diseases are primarily based on the use of optical systems. Ultrasound has established diagnostic credibility in the form of endoscopic ultrasound (EUS), however it is limited to examination of the upper gastrointestinal tract (oesophagus, stomach and upper (proximal) small bowel). Access to the remainder of the small bowel is currently limited to optical capsule endoscopes and a limited number of other modalities as these capsules are restricted to visual examination of the surface or mucosa of the gut wall. Ultrasound capsule endoscopy has been proposed to integrate microultrasound imaging capabilities into the existing capsule format and extend examination capabilities beyond the mucosa.**

To establish the ability of high frequency ultrasound to resolve the histological structure of the gastrointestinal tract, *ex vivo* scans of pig and human tissue were performed. This was done using 25 and 34 MHz single element, physically focused composite transducers mechanically scanned along the tissue. Tethered prototype devices were then developed with 30 MHz physically focused polyvinylidene fluoride (PVDF) single element transducers embedded for use in initial translational trials in the small bowel of porcine subjects. B-scan images from the *ex vivo* model validation and the *in vivo* trials are presented.

Index Terms—**endoscopy; microultrasound; prototyping; pre-clinical; capsules; porcine; animal models**

I. Introduction

Endoscopy and colonoscopy are the clinically accepted means of imaging and diagnosis of diseases of the gastrointestinal (GI) tract. Conventional endoscope based approaches combine optical and ultrasound imaging to allow both optical imaging of the surface and ultrasound facilitated full thickness imaging of the bowel wall and adjacent organs. These approaches are limited in their ability to access the full length of the GI tract either due to scope length or technical challenge. This is particularly true for the anatomically remote small bowel. Wireless video capsule endoscopy (VCE) devices have been designed to transit the entire GI tract, but they are currently limited to optical imaging of the superficial mucosal surface. Work is thus under way to implement additional capsule endoscopy (CE) functionality through the development of devices incorporating both optical and ultrasound imaging.

Examining the literature for ultrasound imaging of the small bowel, it can be seen that the current clinical standard for small bowel imaging is based on external (transabdominal) ultrasound systems at standard clinical frequencies ($\leq$15 MHz) [1]–[3]. Some internal imaging has been achieved using EUS and double-balloon enteroscopy [4], however, work at higher ultrasound frequencies (microultrasound) is extremely limited [5], [6]. The Sonopill project looks to address this limitation with the development of an ultrasound capsule endoscopy (USCE) device operating in the microultrasound range.

As the proposed device is highly complex, there was a need for both benchtop and in vivo models for the validation of the proposed imaging frequencies and configurations. To this end, a suitable animal model was sought that would match human tissue characteristics at the histologic level and allow device prototyping in translational trials.

This paper presents the evaluation of a porcine model for microultrasound imaging in the GI tract for use in benchtop and translational trials and the results of preliminary trials of USCE devices in the model.

II. MICROULTRASOUND IMAGING OF THE GASTROINTESTINAL TRACT

Microultrasound imaging has been proposed as a viable solution to the clinical need for real-time, non-invasive monitoring of the GI tract [3] given its ability to distinguish sub-millimeter tissue characteristics. The thinnest layers of human GI tissue measured were on the order of 100 μm [7], mandating ultrasound centre frequencies above 20 MHz to fully visualise the layer structure and permit accurate diagnosis of pathology in those layers. While microultrasound faces reduced penetration depths due to increased attenuation when compared to conventional frequencies, GI tissue only requires penetration of 1-3 cm provided that the ultrasound probe can be brought into proximity with the tissue under observation. This matches well with the proposed capsule-based modality, but raises the question of suitable tissue analogues for the verification of capsule prototypes prior to clinical trials. To assess the suitability of the porcine model for this modality, human and porcine GI tissue samples were obtained and mechanically scanned with microultrasound transducers, then clinically assessed. All *ex vivo* scans reported here were performed with a previously established mechanical scanning system [8] in conjunction with 25 and 34 MHz single-element, physically focused piezocomposite transducers (AFM Ltd, Birmingham, UK).

A. Human Imaging

To establish a baseline for the analysis of the suitability of the selected animal model, initial *ex vivo* scans were performed on ethically obtained human colon tissue at multiple microultrasound frequency ranges. The human tissue sample trial was approved by the Tissue Bank Committee and supplied by the Tayside Biorepository (Study Number: TR000442 Microultrasound characterization of gastrointestinal tissue).

Tissue was collected post-surgical excision and delivered to pathology for processing. The excised colon was transected along the long axis and inspected. A presumed healthy 3×3 cm sample from each colon was supplied by the pathologist for mechanical scanning with single-element microultrasound transducers.

Samples were taken from areas remote (~20 cm) to the primary tumor and remained unfixed during the entire experiment. Prior to scanning, the sample was pinned (25G microlance, Becton-Dickson) on 1% agar (Fisher) above embedded sound absorber within a plastic dish. A thin layer of ultrasound coupling gel (1 - 2 mm) separated the sample from the agar thus ensure distinct ultrasound signals. The dish was then filled with degassed phosphate buffered saline (dPBS). A

sufficient fluid level above the sample was ensured to provide sufficient acoustic coupling throughout the scanning procedure.

Scanning was done running proximal to distal along the long axis of the sample. Scans in the X direction (proximal to distal) were performed using 0.02 mm steps for a total of 15 mm. Each X slice was separated by a 1 mm Y step (left to right on the short axis). This was performed to provide at least one viable scan slice per sample. All collected data was then stored using custom LabVIEW software (National Instruments, Austin, TX, USA) and post-processed using MATLAB (Mathworks, Natick, MA, USA) to generate grey-scale B-scans for comparisons.

B. Porcine Model

As an established model of the human GI tract in regards to histological similarity, pigs were chosen as the model for translational work. They are well suited both in their comparative physical dimensions as well as the availability and ethical considerations of obtaining access to both *in vivo* and *ex vivo* tissue from the selected animal. Porcine tissue is well-matched to the human GI tract due to their similarity of diet and lifespan [9] and it has the additional advantage of healthy tissue being readily available *ex vivo.*

Porcine oesophageal tissue samples were sourced from an abattoir (Medical Meat Supplies Ltd, Oldham, UK). Samples were supplied frozen and considered fit for human consumption, so they were assumed to be a suitable to provide a healthy baseline. To prepare for scanning, sealed samples were thawed for 20 minutes under running tap water. They were then rinsed prior to being transected down the longitudinal axis, allowing the tissue to be opened up and placed on the same agar substrate as described earlier. The tissue was prepared with the same approach as that used in the human tissue scans i.e. head to toe LabVIEW and MATLAB were again used to store and process the data, and the B-scans were compared to the human scans and assessed by a clinician for diagnostic quality and alignment of the porcine and human anatomy.

III. TRANSLATIONAL TRIALS

Once the porcine model had been established, preliminary translational trials were undertaken with a set of tethered ultrasound capsules which were previously developed [10], featuring four 30 MHz PVDF single element transducers and an internal receive amplification circuit comprising 4 channel transmit/receive protection (MD0101, Microchip Technology, Chandler, USA) and operation amplifiers (ADA4807, Analog Devices, Norwood, USA) set for 12 dB of gain. Multiple identical capsules were prepared prior to the trials, with 2 capsules being used in the final experiment. System control was achieved with a combination of custom Labview software in conjunction with a myRIO acquisition device (myRIO-1900, National Instruments Inc., Austin, TX, USA). A commercial ultrasound pulser/receiver (DPR500, JSR
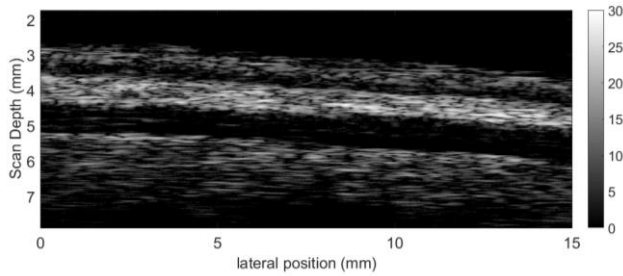
Fig. 1 Human colon mechanically scanned with a 25 MHz composite transducer physically focused at 8 mm. Scan is from the proximal colon (0 mm) to the distal colon (15 mm)

Fig. 3 Porcine oesophagus scanned with a 25 MHz composite transducer physically focused at 8 mm. Scan is from the oesophageal mouth (0 mm) towards the stomach (15 mm).

Fig. 2 Human colon mechanically scanned with a 34 MHz composite transducer physically focused at 6.5 mm. Scan is from the proximal colon (0 mm) to the distal colon (15 mm)
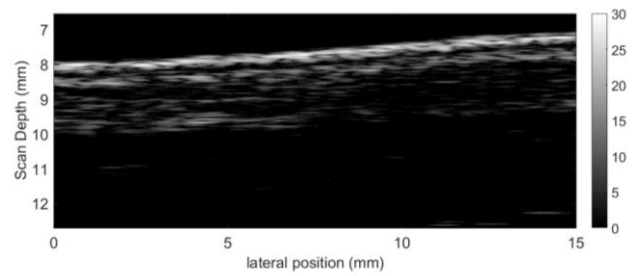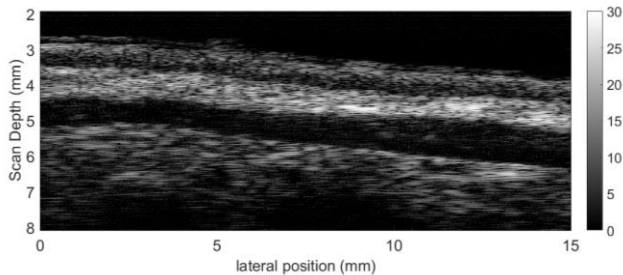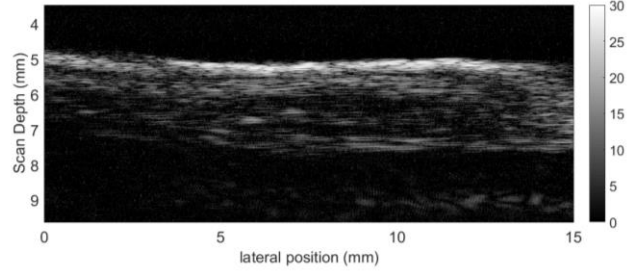

Fig. 4 Porcine oesophagus scanned with a 34 MHz composite transducer physically focused at 6.5 mm. Scan is from the oesophageal mouth (0 mm) towards the stomach (15 mm).

Ultrasonics, Pittsford, USA) and a 2 GS/s oscilloscope (MDO3024, Tektronix, Beaverton, USA) were used to fire the transducers and observe the returning echoes. All protocols were repeated for both capsules on both animals.

The described study was conducted under Home Office (UK) License (PPL: 70/8812) in accordance with the Animal (Scientific Procedures) Act 1986. Two female Landrace pigs, aged 4 months, were supplied by a local breeder/supplier. The pigs were maintained in groups of no less than two animals in licensed housing (PEL 60/4604), bedded on straw and fed 'ABN Pig Rearer Pellets' ad lib. Environmental variables were maintained within the limits detailed by PEL. Food was withheld for 12 hours before anesthesia, but access to water was maintained until pre-anesthetic medication. This was: azaperone (1 mgkg^{-1}; "Stresnil 40 mg/ml Solution for Injection", Elanco Animal Health, Hampshire), ketamine (2 mgkg^{-1}; "Ketamidor 100 mg/ml Solution for Injection", Chanelle UK, Berkshire), midazolam (0.5 mgkg^{-1}; "Hypnovel 10mg/2ml Solution for Injection", Roche, Hertfordshire) and morphine (0.25 mgkg^{-1}; "Morphine Sulphate 30mg/ml", Martindale Pharmaceuticals, Buckinghamshire) combined in one syringe and injected intramuscularly. Anesthesia was induced with isoflurane ("Isoflo" Zoetis, Surrey), vaporized in oxygen and nitrous oxide administered via a Bain breathing system and facemask. A cannula was placed in the auricular vein. Complications with capsule introduction arising from the endotracheal tube were circumvented by the oro-oesophageal insertion of a modified, wide diameter endotracheal tube.

Access to the remote small bowel was enabled via an artificially created stoma under general anesthesia immediately prior to the experiment. The experiment and subject were monitored throughout the experiment by an experienced veterinary anesthetist. When the experiments were complete, the animals were euthanized without recovery using pentobarbital.

During the study, each capsule was introduced 60 cm into the small bowel via the stoma and moisture levels were maintained with a saline drip placed in the mouth of the stoma. Each transducer was then activated in sequence and the capsule pulled back 12 cm by the certified operator over the course of 30 seconds. Following each pull-back, the capsule was returned to the original position with the assistance of physical marks on the tether of the capsule. The protocol was then repeated for each transducer three times, then for the other capsule.

IV.   RESULTS

A.   Human Tissue

High quality tissue B-scan images were obtained from the human colon scans at 25 MHz (Fig. 1) and 34 MHz (Fig. 2), with good layer definition and tissue texture having been noted by the clinical team member. Individual layers were noted at approximately 1 mm in thickness, with the full
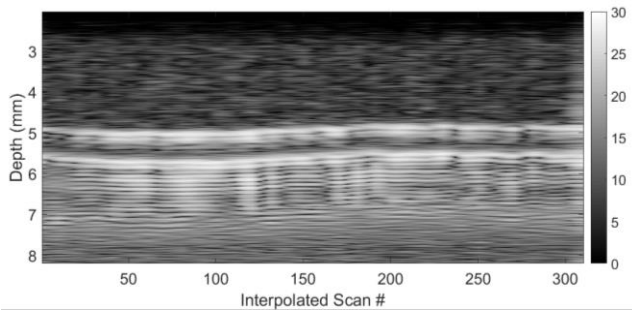
Fig. 5 Porcine small bowel tissue scan *in vivo* obtained with a 30 MHz PVDF transducer physically focused at 6 mm. Scan was obtained via a 12 cm pullback through the small bowel accessed via a surgically inserted stoma.

thickness of the tissue measuring approximately 4.5 mm. These were then treated as benchmarks for the assessment of the porcine tissue images.

B. Porcine ex vivo Tissue

Grey-scale images were obtained from the porcine small bowel at both 25 MHz (Fig. 3) and 34 MHz (Fig. 4) and compared to the human tissue scans in Fig. 1 and 2. Good agreement was noted between the physical layer thicknesses as well as in relative tissue contrast and image quality.

The esophageal samples do show thinner upper tissue layers when compared to the human colon samples, however this variance is in keeping with normal anatomical variation for these tissue types [9].

C. Porcine in vivo

Good acoustic coupling was achieved in the small bowel during *in vivo* trials with the saline drip acting as the sole artificial coupling agent. As seen in Fig 5, layer differentiation was successfully achieved and tissue dimensions matched those seen in the *ex vivo* scans were observed. Degradation of the image and layer structure is noticeable, in comparison to Fig. 3 and 4, at least some of which can be attributed to the reduced ultrasound power provided by the PVDF transducers. Examination of the frequency content of the *in vivo* data also shows attenuation in the higher frequency bands, with a resulting downshift in the centre frequency of the echoes. This may reflect increased frequency-dependent attenuation in the live model.

V.   CONCLUSIONS AND FUTURE WORK

This study was able to establish good agreement between human GI tissue and porcine GI tissue for microultrasound frequencies of 25 and 34 MHz. Similarity of layer structure

and thickness was observed and clinical assessment was that the porcine tissue offered a suitable and ethical analogue for human tissue for the purpose of assessment of microultrasound scanning. Based on the assessment of the *ex vivo* tissue and *in vivo* experiments, microultrasound can provide a viable means of subsurface gut (GI) examination in capsule form.

Microultrasound images were subsequently successfully obtained in the porcine small bowel during initial trials of the capsule endoscopy prototype using saline as a coupling agent. This represents the first *in vivo* trial of a capsule-based ultrasound endoscopy prototype and validates both the device design concept as well as the ability of the selected frequency range to differentiate the tissue layers. Future work will examine the extension of this work to linear arrays and the integration of additional modalities into the capsules to better replicate the desired final device. Additional receive signal processing will be integrated within the capsule to alleviate some of the signal loss seen in the *in vivo* experiments to date.

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