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Ultrasound capsule endoscopy

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Running Title: Ultrasound Capsule Endoscopy (USCE)

Ultrasound Capsule Endoscopy: Sounding out the future

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Abstract (300)

Video capsule endoscopy (VCE) has been of immense benefit in the diagnosis and management of gastrointestinal disorders since its introduction in 2001. However, it suffers from a number of well recognized deficiencies. Amongst these is the limited capability of white light imaging, which is restricted to analysis of the mucosal surface. Current capsule endoscopes are dependent on visual manifestation of disease and limited in regards to transmural imaging and detection of deeper pathology. Ultrasound capsule endoscopy (USCE) has the potential to overcome surface only imaging and provide transmural scans of the gastrointestinal tract. The integration of high frequency microultrasound into capsule endoscopy would allow high resolution transmural images and provide a means of both qualitative and quantitative assessment of the bowel wall. Quantitative ultrasound can provide data in an objective and measurable manner, potentially reducing lengthy interpretation times by incorporation into an automated diagnostic process. The research described here is focused on the development of USCE and other complementary diagnostic and therapeutic modalities. Presently investigations have entered a preclinical phase with laboratory investigations running concurrently.

Keywords

Capsule Endoscopy, Diagnosis, Gastrointestinal, Ultrasound, Ultrasound Capsule Endoscopy (USCE)

1) Text

Introduction

The introduction of video capsule endoscopy (VCE) has been a technical boon to the diagnosis and management of gastrointestinal (GI) disorders (1) with the ability to non-invasively image the mucosa of the entire GI tract. This is especially true for the small bowel (SB) which has previously been difficult to image directly. Despite the obvious benefits, VCE suffers from a number of limitations including the inability to biopsy, poor capsule/lesion localization and dependency on gut peristalsis for locomotion (2). In addition to these well recognized impediments is the restriction to analysis of only the superficial mucosa due to VCE's reliance on visible light for imaging (3).

Imaging Limitations

Visible light rays range between 400-700 nm and are strongly attenuated by tissue at depths of 100 - 1000 μm (3) with a diminished return of light to the camera. Thus only the mucosal surface can be analyzed and subsurface pathology cannot be imaged and evaluated. Reliance on superficial manifestations of disease opens interpretation to a number of pitfalls regarding lesions that are visually obscure or occult, variable in appearance, patchy in distribution and/or occurring in microfoci (4,5). Furthermore, pathologic mucosal visual changes often cannot be considered specific due to visual overlaps between diseases (6) and sensitivity declines when encountering lower grade diseases, as visible manifestation is less overt (7).

Non-optical Capsules

Attempts have been made to develop capsule endoscopes that do not rely on visible light and allow subsurface visualization. C-Scan® Cap (Check-Cap Ltd, ISR), for instance, is being developed as an X-ray based imaging capsule (8). Gora and colleagues have

developed an endomicroscopy capsule (9,10). Designed to detect metaplastic and dysplastic changes associated with Barrett's esophagus, this tethered capsule employs optical coherence tomography to provide high resolution ($< 10 \mu\text{m}$) axial sections of the esophagus.

Ultrasound Capsule Endoscopy

After earlier research with limited outcomes (11,12), ultrasound capsule endoscopy (USCE) is under development in several groups including those led by Khuri-Yakub at Stanford University (USA) (13) and Qiu at Shenzhen (CHN). The largest such activity (Sonopill, UK EPSRC reference GR/K034537/2), is a multi-institutional programme with the ultimate aim to incorporate microultrasound (μUS) and video modalities into a 10 mm diameter by 30 mm long capsule, as depicted in Figure 1. This will allow simultaneous optical mucosal visualization and transmural μUS imaging in a manner similar to conventional endoscopic ultrasound (EUS). However, USCE will have full GI tract transit with a higher μUS spatial resolution with ultrasound (US) imaging limited to the bowel wall. To accomplish this within the volume restrictions of an ingestible capsule, the μUS transducer array and associated electronics must be: microscale reducible, biologically safe and cost effective single-use device. As a means of imaging that is already clinically established, μUS met the above criteria in terms of miniaturization (14, 15), safety (i.e. nonionizing radiation) and relatively low manufacturing costs (16).

An important aspect of USCE development is to incorporate an imaging modality capable of transmural visualization with higher resolution than conventional EUS. To achieve this, μUS has been considered as the modality of choice. Microultrasound frequencies are more typically a factor at least $1\frac{1}{2}$ times higher than standard clinical frequencies which generally operate at a maximum of 20 MHz (17). Nevertheless, μUS operates under the

same physical principles as conventional clinical US. It is sometimes called ultrasound biomicroscopy (UBM) with reference to potential micrometer axial and lateral resolution. Early results in UBM from Sherer et al. (18) demonstrated it to be capable of non-invasively imaging subsurface structures and tumor spheroids with a 100 MHz US transducer and μ US has consequently become established in clinical applications such as ophthalmology (19), dermatology (20) and intravascular ultrasound (21).

Ultrasound Physics

Ultrasound waves are produced by the application of electrical voltage to a piezoelectric material such as quartz or piezoceramic. The electrical signal causes a material deformation which generates a high frequency pressure wave. Ultrasound waves generated by an array of piezoelectric transducers and waves are transmitted through tissue and echoic reflections from tissue interfaces and other features return to the probe. The echoes are detected by the transducer array and are converted to electrical signals which are combined to generate images based on a time - distance relationship.

With μ US, the improved axial and lateral resolution is the result of greater US wave interaction with microscopic tissue components. As US frequency is increased, the wavelength shortens and microstructures normally too small to generate a distinct signal at conventional frequencies become acoustically manifest at higher frequencies. This allows for improved discrimination between adjacent microstructures. The tissue echoes can be reconstructed in a brightness-mode (B-mode) image or other conventional image display formats.

The trade-off for increasing frequency and hence spatial resolution is a decrease in depth of US penetration. Figure 2 illustrates the phenomena of changing resolution and tissue penetration depth as a function of US frequency (22). In general, US attenuation is a result

of interactions between the US wave and tissue resulting in signal scattering and absorption. Both are strongly related to frequency and higher frequencies will experience increased signal loss. However, this relationship has the potential to be beneficial for limiting the region of interest (ROI) to the bowel wall itself. Hence, despite the potential for user complaints regarding the lack of penetration (23), this could reduce the amount of superfluous and potentially confounding data gathered as is for example, in transabdominal sonography (TABS) assessment of bowel inflammation (24).

Qualitative Aspects

The ability of μ US to characterize GI tissue with a high degree of agreement with histological analysis has been established (22, 23). Part of the strong correlation between μ US images and histology stems from the ability of μ US to provide high resolution images, as noted in the previous section. High frequency catheter mini-probes have been developed for upper and lower GI examination in conjunction with standard endoscopy, employing frequencies ranging from below 20 MHz to greater than 30 MHz (27). Primary indications include use for local 'T' staging and establishing the feasibility of endoscopic mucosal resection (EMR) (25, 26).

Standard EUS frequencies usually generates a five-layer image that correlates with the lumen to gut wall interface and the cardinal transmural layers consisting of the mucosa, submucosa, muscularis propria and serosa. Higher frequency sonography can depict bowel wall structure with additional details and layers (22, 23, 27). This additional detail makes μ US well suited for imaging the gut wall for subsurface and transmural defects.

Results using a single element μ US probe have also revealed a high degree of correlation between μ US and small bowel histology (31). The authors' work illustrated in Figure 3 demonstrates the degree of correlation between a 47.7 MHz scan of a section of explanted

porcine SB and corresponding histology¹. The μ US scan demonstrates three distinct layers corresponding to the combined mucosa/submucosa, muscularis propria and serosa as opposed to the four distinct layers of the histology slide consisting of mucosa, submucosa, muscularis propria and serosa. The lack of differentiation between the mucosa and submucosa may be due to insufficient change in the acoustic impedance between these layers in the *ex vivo* samples.

Existing data suggest that μ US imaging has could allow direct imaging of mucosal and/or transmural pathology in a way that is not possible with conventional frequencies. For instance, TABS imaging of coeliac disease and inflammatory bowel disease (IBD) can be achieved with conventional US frequencies (29–31), but the findings of increased luminal fluid, luminal dilation, mural thickening, mesenteric lymphadenopathy and increased peristalsis, are generally nonspecific (30, 32). Other issues with TABS include assessment that can be hindered by a large body habitus and is a generally non-continuous scan of the GI tract. Furthermore, distinguishing adjacent bowel loops from mural thickening can be difficult (34). Direct bowel imaging using μ US in capsule form has the potential for direct imaging of bowel wall pathology whilst avoiding the shortcomings associated with TABS.

Additionally, the ability to analyze a lesion *in situ* using μ US and other combined diagnostic modalities has the potential to further develop the concept of *in vivo* pathology or virtual histology (36,37). As noted earlier, a marked limitation of VCE is its inability to obtain tissue for analysis. This deficiency leads to a requirement for conventional endoscopic or surgical follow-up if a biopsy or intervention is deemed necessary. The ability to characterize a lesion *in situ* and differentiate between malignant and benign, at a minimum, possibly reducing the need for invasive follow-up.

Frequency Choice

As noted earlier, μ US operates at frequencies minimally greater than 20 MHz and typically greater than 30 MHz to achieve improved lateral and axial resolution. This indicates that there is a wide frequency spectrum in which to adopt USCE. The importance of this property relates to the balance between adequate resolution for diagnostic yield and data generated as it relates to interpretation times. The issue of already lengthy reading times has been addressed in the literature (38) and the addition of a second modality to a capsule, such as μ US, has the potential to significantly increase interpretation time. Therefore determination of a frequency that meets the needs for diagnostic accuracy without overburdening the clinician with data is an area of active research (31).

Quantitative Aspects

A notable aspect of US imaging is the data acquisition method used to reconstruct an image. Echoes generated by tissue are affected by tissue density and the speed of wave propagation. The qualitative images typical of US are formed from these reflections but this image also contains quantitative information about the physical properties of tissue examined (39). Calculation of the physical or acoustic properties of tissue from the reflected signals is termed quantitative ultrasound (QUS) and this adds objective and measurable parameters to US data (40).

Tissue undergoing pathologic changes has the potential to affect the acoustic properties as demonstrated by Fatehullah et al (41). This paper concluded that tissue architectural changes could be detected with both qualitative and quantitative US prior to being detectable with conventional histological means. Work in combined biologic/inorganic and organic phantoms using QUS with μ US has been conducted on porcine SB¹ (42).

Figure 4 illustrates the quantitative and qualitative results of a scan of porcine SB¹ infused with hyperechoic microspheres at an ultrasound frequency of 47.7 MHz with graphic overlay indicating quantitative changes in MRayl attenuation. As the scan passes from unperfused tissue to regions containing microsphere aggregates there is a noticeable change in signal attenuation. In this analysis, only the first 100 μm depth of the sample was analyzed quantitatively for changes. Data from below that depth were ignored which included a polystyrene fiducial marker. The ability to choose the tissue depth to be analyzed allows focusing on ROI restricted to layers of user interest.

Further work is under way on to measure qualitative and quantitative mucosal changes in porcine esophagus to detect transition from the stratified squamous to simple columnar mucosal lining at the gastroesophageal junction. Figure 5 shows a full thickness scan again at 47.7 MHz for an explanted porcine esophagus as it transitions into the stomach¹. The overlaid graph of MRayl attenuation changes as the scan progresses from proximal to distal at the area of the gastroesophageal junction.

Computer Assisted Diagnosis

A major advantage of the quantitative aspect of μUS is its potential to be adapted to computer assisted diagnosis (CADx). Means of reducing the time commitment to review the clinical data is ranked high on the clinician's 'wish list' of VCE improvement due to the lengthy interpretation times currently experienced (43). QUS-based image analysis has already been studied in intravascular ultrasound (IVUS) (44) where Timmins et al demonstrated quantifiable changes in coronary atherosclerotic plaques and postulated that automated quantitative methods could improve and accelerate lesion analysis. The previously discussed ROI control and the quantitative factors of μUS demonstrate promise for adapting QUS to an automated interpretation process. An automated QUS

diagnostic method based on the acoustic property differences between healthy and diseased GI tissue could be developed where abnormal quantitative results can possibly be used to direct physician attention to particular areas of concern.

Conclusion

Efforts are under way to develop USCE with the inclusion of μ US to allow for high resolution transmural imaging of the gut wall. Given the close proximity of the US probe and the relevant tissue, μ US could be able to provide direct evidence of subsurface and transmural pathology. This will overcome the issues associated with white light imaging, where there is a reliance on visual disease manifestation. It will also address issues associated with TABS, in regards to relying less on nonspecific signs of inflammation, and overcome problems associated with transcutaneous US. Furthermore, μ US can be potentially adapted to automated diagnosis by applying the quantitative aspects of US. By ascertaining the acoustic properties of tissue, data can be presented in an objective and measurable way.

Laboratory experiments continues with investigations into various aspects of capsule development. This includes a study to determine which μ US frequency provides optimal diagnostic yield. Other work is also considering the development of the electronic hardware and software necessary for the capsule functionality. This includes microchip design, μ US array development, integration of the functional sub-elements and capsule shell functionalization. While conceived primarily as an US capable diagnostic capsule, other sensing modalities are under consideration, including fluorescent imaging (45). Development of therapeutic capsules is another area of active research (46).

Current research efforts have entered the translational phase with large animal trials being conducted at the Roslin Institute, University of Edinburgh². Tethered versions of single

modality capsules are being tested in the upper GI tract and small bowel of anaesthetized pigs (results not shown). These trials are designed to address fundamental questions regarding the USCE development. Chief amongst the investigations is to determine if there is adequate coupling between USCE transducer array and mucosa to facilitate US imaging. Additional experiments have examined the thermogenic profile of an USCE device to aid in power budgeting. Translational trials will continue with further refinement of USCE and also test other diagnostic and therapeutic modalities.

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5) Footnotes

1. Laboratory work was conducted on porcine small bowel and esophageal samples that were abattoir obtained (Medical Meats Supplies, Oldham UK).
2. This study was approved by the Animal Welfare and Ethical Review Board of the Roslin Institute, Roslin, Midlothian, EH25 9RG and is being carried out under Home Office (UK) License PPL 7008812.

6) References (Vancouver)

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7) Supplementary Material

None

8) Figure Legends

Figure 1. A schematic of the Ultrasound Capsule Endoscopy (USCE) under development in the Sonopill programme. The 10 mm diameter x 30 mm long capsule, with spherical ends, will contain both ultrasound (Component 2) and optical modalities (Components 6 and 11). The ultrasound array is being developed as a high frequency or microultrasound transducer (> 20 MHz) to facilitate transmural high resolution imaging of the bowel wall. Optical modalities include both white light imaging (component 11) and fluorescent imaging (component 2). Development of the fluorescent imaging cube is being conducted by Al-Rawhani and colleagues and is detailed in a separate publication (45). Additional development concerns other aspects of USCE including electronic circuitry (components 3, 4 and 10) and power budget.

Figure 2. A schematic of ultrasound resolution and tissue penetration depicted from the lumen of the bowel outwards. There is a twofold effect as the ultrasound frequency is increased in terms of enhancing axial and lateral resolution with a proportional loss in depth of beam penetration (22) as indicated by scaled purple arrows. The diminished tissue penetration is a result of increased signal attenuation as a result of enhanced ultrasound wave to tissue interaction as frequency is increased. Conversely, the enhanced interaction results in improved axial and lateral resolution as finer structures become acoustically manifest and allows for improved discrimination between structures. The major advantages of using microultrasound in USCE are the provision of high resolution images coupled with decreased penetration providing images pertaining directly to the gut wall.

Figure 3. A single element scan at 47.7 MHz and 40X magnification optical image of a hematoxylin and eosin (H&E) slide of porcine small bowel¹. The top image is across the short axis of an explanted small bowel section scanned *in vitro*. The mesenteric vessels have been cannulated and perfused with phosphate buffered saline (PBS) (28). The bottom image is a magnification of the microultrasound scan at 18 - 21 mm accompanied by an H&E image to demonstrate the fidelity in which microultrasound can reconstruct tissue architecture. The scan depicts three distinct layers; namely the mucosa/submucosa, muscularis propria and serosa. The H&E slide depicts the four major layers with the mucosa and submucosa visibly separate. One reason for the lack of distinct upper layers in the scan may be the diminished interface difference between the mucosa and submucosa in the *in vitro* PBS perfused tissue.

Figure 4. A single element 47.7 MHz scan across the short axis of an explanted porcine small bowel section¹ post infusion with phosphate buffered saline and hyperechogenic glass microspheres. The microspheres have accumulated subsurface (marked with red arrows) and have been detected qualitatively by the ultrasound transducer. Quantitative detection is indicated by the overlaid graph and indicates acoustic impedance (MRayl) changes specifically at the areas of microsphere aggregation. The infiltration of microspheres has resulted in a physical (i.e. acoustic) property change in the tissue allowing for quantitative detection of disruption. Additionally, there is a qualitative detection of the 90 μm polystyrene microsphere fiducial marker at 11 mm (red *) (Polysciences, USA) but there is a lack of quantitative signal. This is attributed to the depth of the marker lying below the region of interest segmentation of 100 μm . Of note is the qualitative imaging of the dilated capillaries (red +) lying below the aggregated microspheres which were used to infiltrate the glass microspheres.

Figure 5. A camera image and a 47.7 MHz scan of an explanted porcine esophagus and stomach¹. The scan is across the long axis of a full thickness porcine esophageal/gastric section at the gastroesophageal junction. The image and scan are not in scale with one another. The camera image illustrates the change from smooth stratified squamous lining of the distal third of the esophagus. The esophagus then keratinizes before transition into the stomach proper. The overlaid graph indicates attenuation changes across the scan as it passes from the esophagus to the stomach. There is a large change in the region of the gastroesophageal junction (red *) with an increase in attenuation at the area of cornification (red +). Scan results of the stomach are not shown.

9) Tables

None

10) Figures



