A pilot study for the real-time detection of colorectal cancer using electrical impedance spectroscopy

Angela Pathiraja[1], Paul Ziprin[1], Arsam Shiraz[3], Reza Mirnezami[1], Andrew Tizzard[2], Brian Brown[4], Andreas Demosthenous[2] and Richard Bayford[2,3]

[1]Imperial College London, UK

[2]Middlesex University, London, UK.

[3]University College London, UK.

[4]Sheffield University, UK.

Emails: angela.pathiraja12@imperial.ac.uk and r.bayford@mdx.ac.uk

ABSTRACT

Colorectal cancer is the fourth most common cancer worldwide, with a lifetime risk of around 20%. Current technology available at endoscopy and peri-operatively do not allow the clinician to provide an indication of the likelihood of malignancy. There is, therefore, a need for novel diagnostic techniques to detect malignant lesions at an early stage. Given the new developments in minimally invasive diagnostics and management strategies, real-time point-of-care technology represents a potential area for further research into improving diagnosis and influencing management of colorectal cancer. Electrical impedance spectroscopy (EIS) has been shown to accurately discriminate malignant lesions in other cancers. We present a feasibility study for the real-time detection of malignant colorectal tissue using EIS. This prospective ex-vivo pilot study used a Zedscan device to obtain EIS measurements from excised human colorectal tumour tissue and matched normal colonic mucosa in 22 freshly resected specimens following elective surgery for colorectal cancer. Histopathological examination was used to confirm the final diagnosis. Statistical significance was assessed with the Wilcoxon signed rank test. Twenty-two colorectal resection specimens were tested with EIS. Three were excluded as final histopathology did not identify colorectal adenocarcinoma. EIS was able to discriminate cancer with statistically significant results when applying frequencies over 152KHz ($p < 0.05$). At a cut-off level of 350 Ω, the area under the receiver operating characteristic curve was 0.7097. This preliminary study demonstrates that EIS changes in colorectal tissue are detectable and statistically significant, suggesting that EIS has the potential as a novel non-invasive point of care test for detecting colorectal cancer.

Keywords: cancer, colorectal, electrical impedance spectroscopy (EIS), diagnosis, real-time

1 Introduction

Colorectal cancer (CRC) is a leading cause of global cancer-related mortality in men and women with over 1 million new cases diagnosed annually worldwide (Cunningham *et al*., 2010). It is the fourth most common cancer worldwide, with a lifetime risk of around 20%. Complete surgical resection remains the only curative option for management of CRC and is combined with chemo and/or radiotherapy in selected cases (Andre N *et al,.* 2005). The past 30 years have seen a significant shift towards increasingly 'minimal access' surgical approaches (minimally invasive surgery, MIS), and laparoscopic surgical techniques are now routinely employed in the operative management of cancers of the colon (Jayne DG *et al.***,** 2007) and rectum (Chand Chand M *et al*., 2012). Endoluminal cancer excision is an extension of this philosophy and represents the next major objective on the MIS road-map, offering a further reduction in surgical invasiveness by permitting cancer lesion removal by completely 'scarless' means. Here, tumours are approached and removed via the bowel lumen using an operating endoscope, such as in endoscopic mucosal resection (Woodward TA *et al*., 2012, Waye JD *et al*., 2001) or a proctoscope in transanal endoscopic microsurgery (De Graaf EJ *et al*., 2002). These approaches are technically demanding but appear to result in reduced post-operative morbidity (De Graaf EJ *et al.,* 2009). There have been recent advances in endoscopic technology that have been working towards providing more real-time information for the clinician, such as chromoendoscopy, narrow-band imaging and, most recently, the iKnife (Mcgill *et al*, 2013 and Balog *et al,* 2013). However, a reliable, quantitative measure of tissue abnormality at initial endoscopy or that of intraoperative resection margins which is tissue sparing remains to be achieved. Histopathological assessment of excised tissue samples therefore remains the gold standard. However, as this assessment relies on various steps which each take time (Loughrey *et al,* 2014), histopathological assessment does result in a time delay prior to a definitive tissue diagnosis.

1.1 Electrical Impedance Spectroscopy (EIS)

The main focus of this paper evaluates the use of electrical impedance spectroscopy (EIS) to assess whether bioimpedance measurements could be used to differentiate colorectal cancer from benign colonic mucosa. Of particular interest to our clinical work is the possibility of the detection of these types of cancer during endoscopic and surgical procedures as a predictor of recurrence, including lymph node involvement, poor differentiation, perineural invasion, and vascular invasion. A major advantage of this approach over previously proposed methods is the ability to provide instant information regarding the state for the tissue, equivalent to a biopsy, without requiring any alteration to the tissue being measured. The detection of cancer pathology in the tissue using bioimpedance measurements, and particularly cervical cancer, has been reported in literature (S. Abdul *et al,* 2005, Abdul *et al,* 2006 and JA Tidy *et al*, 2013) in which tetrapolar EIS measurements were performed on the wall of the cervix for the detection of cervical intraepithelial neoplasia, and the distinction from normal epithelium using a custom-made four electrode portable probe. No study has been reported (to the authors' knowledge) in the case of colorectal cancer. It is well known that the magnitude of the impedance and its dependence on frequency are a function of tissue composition. There have been demonstrations that different tissue structures are associated with different frequency bands within an impedance spectrum. At high frequencies (>1 GHz) molecular structure is the determining factor, whereas at low frequencies (<100 Hz) charge accumulation at large membrane interfaces dominates. At frequencies of a few kHz to 1 MHz, sometimes referred to as the dispersion region, cell structures are the main determinant of tissue impedance. Within the dispersion region, low-frequency current can be thought of as passing through the extracellular space. The current passes around the cells; the resistance to flow depends on the cell spacings and their arrangement. At higher frequencies, current can penetrate the cell membranes and hence passes through both intracellular and extracellular spaces. (Duck FA. 1990, Foster KR and Schwan HP 1989). It is, therefore, hypothesised that the resistivity of the epithelial surface is diminished with precancerous development, hence this dispersion becomes smaller with advancing pathology.

Conventionally, EIS is performed by applying sinusoidal currents of different frequencies to a pair of electrodes and recording the voltage drop across two other electrodes in the vicinity. The ratio of

the voltage and current yields a complex transfer impedance. This impedance is complex due to the fact that living tissues demonstrate capacitive and resistive properties. The former demonstrates a polarisable medium with respect to charges, while the latter indicates a direct flow of charge carriers. This leads to the recorded voltage having a phase difference with the applied current. Having separate current-carrying and voltage-recording electrodes, the effect of contact impedance which would otherwise dominate the recording in small electrodes is suppressed. In biological tissues, given that polarisation is not instantaneous and varies at different frequencies, the concepts of relaxation and dispersion may be introduced to explain the impedance profiles seen. Thus, to mathematically demonstrate the origin and nature of the complex impedance, in the presence of *N* frequency dispersions, the complex relative permittivity is given by (Gabriel *et al,*1996) :

$$\epsilon_c = \epsilon_\infty + \frac{\sigma_i}{j\omega\epsilon_o} + \sum_{n=1}^{N} \frac{\Delta\epsilon_n}{1 + (j\omega\tau_n)^{(1-\alpha_n)}} \quad (1)$$

where ω is the angular frequency, ϵ_c is the complex relative permittivity, ϵ_∞ is the value of complex relative permittivity as $\omega \to \infty$, σ_i is the static ionic conductivity, ϵ_o is the permittivity of free space, $\Delta\epsilon_n = \epsilon_s - \epsilon_\infty$, where ϵ_s is the value of complex relative permittivity when $\omega \to 0$, $j = \sqrt{-1}$, τ_n is the corresponding relaxation time constant and α_n is the corresponding broadening parameter in a given dispersion. Considering the geometrical capacitance, ϵ_c may be used to derive the complex impedance. In experiments, multiplying the recorded voltage by in-phase and quadrature signals, the real and imaginary components may be found. The magnitude of impedance can then be found by vector addition of the components.

1.2 Competing Solutions

In recent years, several methods have been proposed for real-time intra-operative tumour margin assessment including diffusive reflectance (Wilke *et. al*, 2009) radiofrequency-based detection (Allweis *et al*, 2008), and targeted fluorescence imaging (Van Dam GM *et al*, 2011). Each of these techniques is subject to particular strengths and limitations. However, a general consensus has yet to be reached on the optimal strategy.

Of particular clinical importance is the possibility to predict negative distal resection margin and negative circumferential resection margins. This can be considered especially for locally-advanced poorly-differentiated rectal tumours which have undergone neoadjuvant (preoperative) chemoradiation therapy. A major advantage of our proposed approach over previously proposed methods is the ability to provide instantaneous information regarding the state for the tissue, equivalent to a biopsy. Table 1 compares various methods for intraoperative tumour margin assessment with our proposed approach.

Table 1: Comparison of Intraoperative Methods

Method	**Advantage**	**Disadvantage**
Radiofrequency-based detection (e.g. MarginProbe)	• Real-time measurement	• Technology not small enough to mount on an endoscope • No evidence that it will work on colorectal (only studies have been on breast)
Real-time near infrared targeted fluorescence imaging	• Good sensitivity and specificity in tumour margin detection • Compatible with minimally invasive cancer therapy	• Limited detection depth • Requires fluorescent conjugate to be injected intravenously
Intelligent Knife (iKnife)	• Gives instant reading in the	• Requires tissue ablation

	form of a mass spec profile	before measurement can be carried out
Bioimpedance	• Can detect surface as well as depth, highly suitable for CRC • Small physical size • Technology is low cost • Does not require tissue alteration in order to obtain measurements	• Technology for effective characterisation of the colorectal tissue does not currently exist

2 Materials and Methods:

22 colorectal tissue samples were obtained directly from theatre from patients diagnosed with an advanced-stage colon cancer who had undergone a surgical procedure for its removal. Visual inspections of the specimen by a pathologist were used to identify the two sites to be measured: the site of the cancer and approximately 10 cm away from the affected site, which was considered to be healthy non-cancerous tissue (Figure 1).

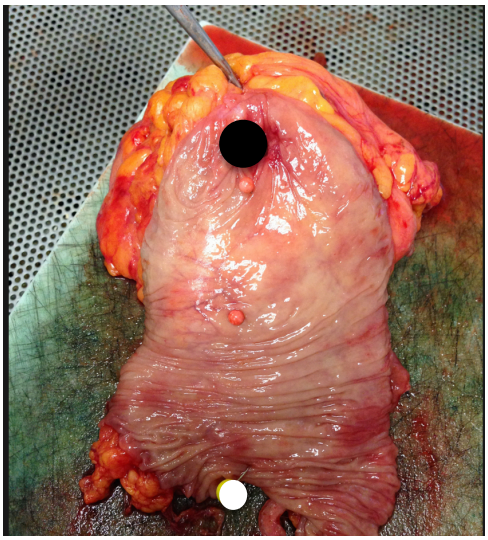

Figure 1: Fresh colorectal specimen with identified sites marked by pins (colorectal cancer lesion marked by black pin and normal mucosa marked by white pin).

EIS measurements were made by applying sinusoidal current of 12 μA amplitude at fourteen different frequencies from 76 Hz to 625 kHz with the ZedScan device (Zilico Ltd, Manchester, UK) (Figure 2) in test mode. The ZedScan device at the tip of the sensing area consists of a square arrangement of 4 gold electrodes, each 1 mm in diameter where the adjacent electrodes are 1.65 mm apart. Each measurement was repeated three times and then averaged to provide a more accurate measure of trans-impedance. The purpose of obtaining measurements with the ZedScan device was primarily to determine the range of frequencies required for colorectal cancer and to generally evaluate the viability of the concept. The measurements were then downloaded on a personal computer using the software supplied with the ZedScan device. The collected data were then imported to Microsoft Excel software for analysis.

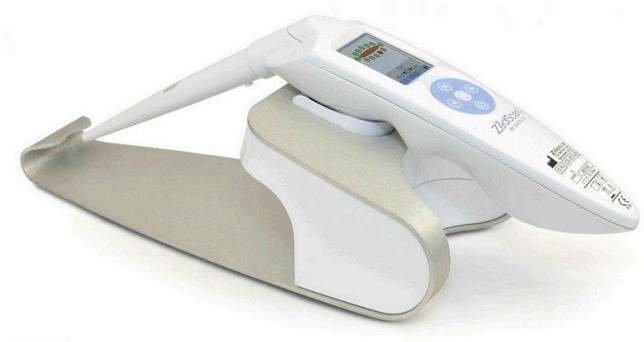

Figure 2: The ZedScan probe placed within its docking station.

The two pre-identified areas (of the malignant lesion and normal mucosa) for each of the 22 samples subsequently underwent formal histopathological analysis as per the normal clinical protocol. This consisted of formalin fixation, tissue processing, sectioning and subsequent haematoxylin and eosin staining and cover-slipping, thereby enabling microscopic analysis and histopathological confirmation of tissue type (Loughrey *et al,* 2014). Histopathological status of the 22 tissue specimens were matched with corresponding impedance results for data analysis and correlation with tissue histology. SPSS (IBM, UK) was used to analyse the measurement data. As the recorded measurements were not normally distributed (as determined by Shapiro-Wilk test) the Wilcoxon signed-rank test for paired non-parametric data of small sample sets was used to test for differences between matched normal and malignant tissue at each frequency. A p-value less than 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curve and the area under the ROC curve (AUROC) were calculated by plotting the sensitivity against 1-specificity of the median results at the 14 different frequencies, in order to quantify the overall reliability of discrimination between normal and malignant colorectal tissue.

3 Results:

Data from 22 specimens (17 males, median age 77, age range 48-85) were originally obtained. 3 specimens' data sets were excluded from final data analysis as 2 were found to have no residual microscopic evidence of cancer following chemoradiotherapy ("complete responders" – samples 13 and 18), and 1 was found to be non-malignant tissue ("benign villous adenoma" -sample 19) following formal histopathological assessment. This left 19 specimens for data analysis. Patient demographics and histopathological characteristics for each patient are summarized in Table 2.

Sample no.	Age	Sex	Site of Cancer	Type of Cancer	T stage	N stage	Ischaemic time
1	77	F	Rectosig.	Mod. diff. adenoCa	3	0	20
2	48	F	Rectum	Poorly diff. inv adenoCa	4	2	35
3	54	F	Sigmoid	Mod. diff. inv. adenoCa	1	0	15
4	40	M	Splen. Fl.	Poorly diff. adenoCa	3	0	45
5	78	M	Sigmoid	Mod. diff.adenoCa	4	0	30
6	55	M	Asc.col	Undifferentiated adenoCa	3	0	30
7	85	M	Caecum	Mod. diff. adenoCa	3	0	20
8	72	F	Caecum	Poorly diff. adenoCa.	4	2	20
9	73	M	Rectum	Mod. diff. adenoCa.	2	0	30
10	69	M	Rectum	Mod. diff. adenoCa	2	0	35
11	73	F	Rectosig.	Poorly diff. adenoCa	1	2	25
12	75	M	Caecum	Mod. diff. adenoCa	3	0	20

13	41	M	Rectum	COMPLETE RESPONDER	N/A	N/A	25
14	68	M	Asc.col	Mod.diff. inv.adenoCa	1	0	30
15	64	M	Rectum	Mod. diff. adenoCa	3	0	35
16	38	M	Asc. col	Poorly diff. adenoCa	4	1	45
17	79	M	Sigmoid	Mod. diff. adenoCa	3	0	30
18	72	M	Rectum	COMPLETE RESPONDER	N/A	N/A	20
19	69	M	Asc.col	VILLOUS ADENOMA	N/A	N/A	25
20	51	M	Rectum	Poorly diff. adenoCa	4	1	15
21	71	M	Rectum	Poorly diff. adenoCa	3	1	20
22	65	M	Rectum	Mod. diff. adenoCa	3	0	25

Table 2: Patient demographics and histopathological characteristics of the 22 specimens (Mod. = moderately, diff. = differentiated, inv. = invasive, adenoCa = adenocarcinoma)

The impedance spectra that were produced ranged from 100 Ω to 1000 Ω over the 14 frequencies used. Mean and median impedances for the indicated 19 cancer sites and corresponding normal sites at each of the 14 different frequencies were calculated. Mean and median differences between cancer tissue and normal tissue bioimpedance across the 14 frequencies were calculated in all 19 specimens. The median impedance spectra results for the 19 cancers and corresponding normal tissue as well as the median impedances of the 2 complete-responder-specimens have been represented in figure 3. It was noted that although all 19 specimens did show differences between cancer and corresponding normal tissue impedances, 15 of the 19 specimens showed higher cancer impedances than corresponding normal tissue, whilst the remaining 4 specimens showed lower impedances than the normal tissue.

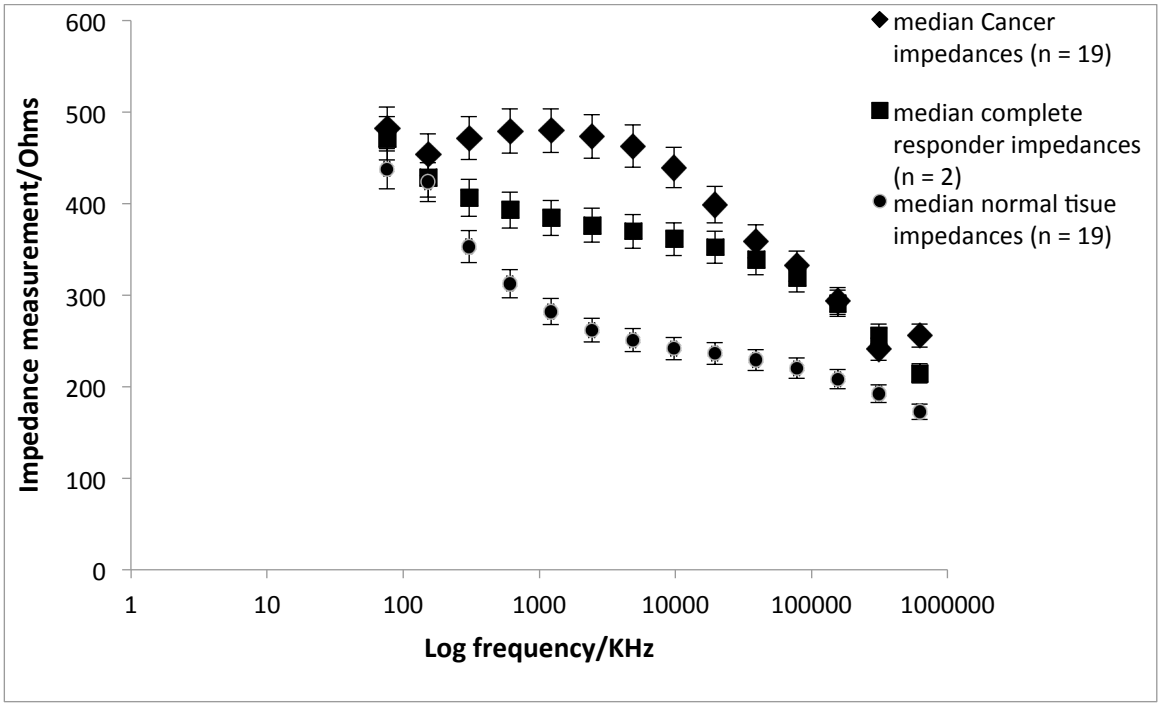

Figure 3: Median impedance measures (with 5% error bars) over 14 standard frequencies, for 19 cancerous samples, 2 complete responder samples and 19 normal tissue samples.

Table 3 shows the median impedance values along with the interquartile ranges at each of the 14 varying frequencies for the 19 colorectal cancer lesions and corresponding normal tissue. Wilcoxon signed-rank test P-values for the differences in median impedances have also been represented, with statistically significant P-values (less than 0.05) noted at frequencies between 152-625 KHz.

In order to create a ROC curve to display the effectiveness of the Zedscan probe at detecting colorectal cancer from the 19 adenocarcinoma samples (figure 4), a reasonable cut-off impedance value of 350 Ohms was chosen. This cut-off value was chosen as it represented the level below which the majority of normal tissue impedances occurred, as well as being the level above which the majority of cancer impedances occur. The area under the ROC curve (AUROC) was calculated to be 0.7097, (where the tool can produce a perfect discrimination between the two groups, AUROC is 1).

Applied Frequency / KHz	Median (+ IQR) of impedances for 19 cancer lesions'	Median (+ IQR) of impedances for 19 benign lesions'	**Wilcoxon test P-values for differences at each frequency**
76	482 (341-845)	438 (314-542)	0.1119
152	454 (325-766)	423 (314-456)	0.0382
305	472 (316-749)	353 (280-402)	0.0242
610	479 (313-734)	313 (247-354)	0.01
1220	480 (313-715)	282 (234-342)	0.0074
2441	473 (307-681)	262 (230-333)	0.0055
4882	463 (302-640)	251 (226-326)	0.0055
9765	439 (293-571)	242 (221-318)	0.0055
19531	399 (286-526)	236 (215-311)	0.0089
39062	359 (275-479)	229 (207-297)	0.0112
78125	332 (261-424)	220 (197-280)	0.0126
156250	294 (244-357)	208 (181-256)	0.0158
312500	241,221-279	192 (164-226)	0.0364
625000	256 (218-307)	173 (147-185)	0.0013

Table 3: Median impedance measures along with the interquartile ranges (IQR) for the 19 colorectal cancer (CRC) lesions and corresponding normal tissue. Wilcoxon rank test P-values for the differences in impedance have been stated.

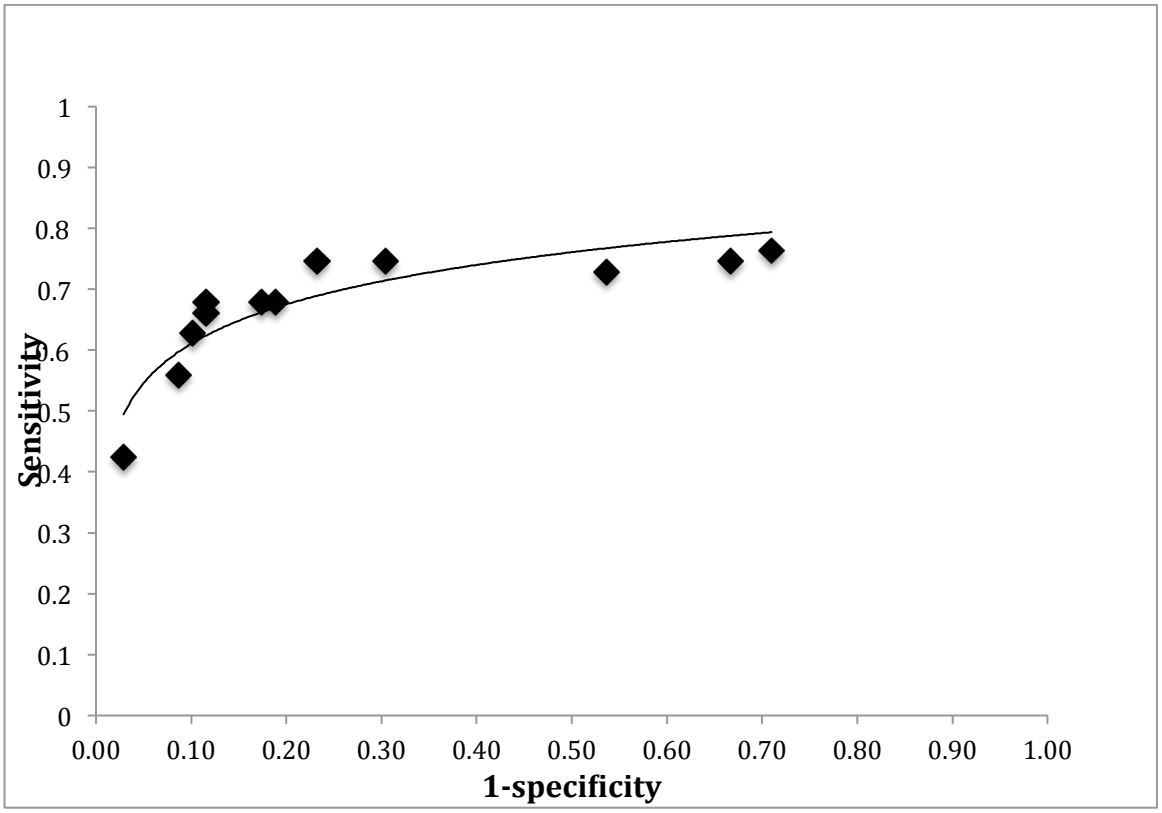

Figure 4: Receiver operating characteristic (ROC) curve comparing tissue analysed by the ZedScan probe as either malignant or normal CRC, based on the cut-off electrical impedance indicator of 350Ohms (AUROC = 0.7097).

4 Discussion:

The purpose of our prospective ex-vivo pilot study was to evaluate whether bioimpedance technology was able to discriminate between colorectal cancer tissue and normal colonic mucosa. The results presented here support other published data of larger studies, which report the effect of dysplastic changes of specific epithelial tissue on electrical impedance spectra (Brown *et al,* 2000, Keshtkar *et al,* 2012, Mahara *et al,* 2015). Similarly, smaller studies looking at oesophageal cancers showed that oesophageal squamous epithelium also had much higher impedances than the dysplastic tissue and adenocarcinomas and squamous cell carcinomas (Knabe *et al,* 2013) Interestingly, our study of normal colonic columnar epithelium against colorectal adenocarcinomatous tissue showed that cancer in general had higher impedance than the normal columnar epithelium. Further work to compare the microscopic structure of the different types of colorectal tissue would facilitate further understanding of why these bioimpedance changes occur.

Although 15 of the 19 adenocarcinoma samples showed bioimpedance spectra that were uniformly higher, 4 of the 19 adenocarcinoma samples showed bioimpedance spectra that were lower than the normal colorectal tissue bioimpedance spectra. It is unclear why this was the case. However, it may reflect the fact that 3 of these were found to be poorly or undifferentiated adenocarcinomas, whereas the majority of the adenocarcinomas that showed higher bioimpedances were moderately differentiated adenocarcinomas. It may be that the lack of differentiation in tissue structure affects the electrical resistance and subsequent bioimpedance characteristics of these samples. However, more work needs to be done to investigate this hypothesis further.

Also of interest, the specimens where a complete pathological response was observed following chemoradiotherapy, demonstrated bioimpedence spectra that appears to be normalizing to that of normal tissue. However, this was in a very small sample (n=2) and would need further investigation with more samples before any firm conclusions could be drawn.

Although our study presents promising results, there are limitations that should be addressed. It

should be noted that our results are based on a small initial sample size of 22 surgically resected colorectal samples, of which 19 were histopathologically-proven colorectal cancer specimens. Additionally, although the ROC curve is a promising representation of our initial data, the specificities observed were moderate at the lower measured frequencies.

Our specimens were collected immediately after surgical resection, there was some delay between the tissue initially losing its blood supply and the EIS measurements being obtained - an ischaemic time. It is known that once ex-vivo, tissue cellular structure begins to change with increasing ischaemic times (Spruessel *et al,* 2004). Additionally, as malignant tissue is known to be highly vascularized, increasing ischaemic times may also result in dehydration of the tissue, which may in turn result in alteration of electrical conductivity and impedance measurement. Further work needs to be done to investigate the effect of ischaemic times on EIS measurements.

Finally, it is worth noting that whilst the Zedscan tool enabled us to make reproducible measurements of bioimpedance in colorectal tissue, it has been ergonomically designed for the specific measurement of cervical tissue at colposcopy. Consequently, whilst the long probe is required for application down a colposcope, conversely it prohibits stable measurement of colorectal tissue, as pressure effects on the tip can result in altered measurements (Keshtkar and Keshtkar, 2009). The future development of bioimpedance technology that is designed to facilitate more controlled measurements of colorectal tissue may result in more sensitive readings.

5 Conclusions

Our initial pilot study is the first of its kind to show that EIS can discriminate between surgically resected colorectal cancer and normal colorectal mucosa in patients with confirmed colorectal cancer, in a real-time and non-invasive manner. Although our initial sample size was small, and various limitations of our study have been discussed, we believe that with further research and development of the biophysical software and its ergonomics, EIS technology may have the potential to provide a real-time virtual biopsy of colorectal tissue in the future. Further work is required to adapt the technology specifically for bowel cancer and to test it on a much wider pathological range of colorectal samples to validate this original study. We hope that by developed into an EIS probe that could be deployed down the working channel of an endoscope or integrated into robotic surgical tools, in order to provide a real-time virtual biopsy and diagnosis of colorectal cancer.

Acknowledgements:

We would like to thank Peter Highfield from Zilico Ltd for the continued support throughout this study. Dr Angela Pathiraja received a National Institute of Health Research Academic Clinical fellowship (2013-16), which enabled her to set up and conduct this study.

This study was undertaken with ethical approval, reference 135339.

Reference List

Cunningham D, Atkin W, Lenz HJ, Lynch HT, Minsky B, Nordlinger B, et al. Colorectal cancer. Lancet. 2010; **375**(9719): 1030-47.Andre N et al,. Gut. 2005; **54**(8): 1194-202.

Andre N, Schmiegel W. Chemoradiotherapy for colorectal cancer. Gut. 2005; **54**(8): 1194-202.

Jayne DG, Guillou PJ, Thorpe H, Quirke P, Copeland J, Smith AM, et al. Randomized trial of laparoscopic-assisted resection of colorectal carcinoma: 3-year results of the UK MRC CLASICC Trial Group. J Clin Oncol. 2007; **25**(21): 3061-8.

Chand M, Bhoday J, Brown G, Moran B, Parvaiz A. Laparoscopic surgery for rectal cancer. J R Soc Med. 2012; **105**(10): 429-35.

Woodward TA, Heckman MG, Cleveland P, De Melo S, Raimondo M, Wallace M. Predictors of complete endoscopic mucosal resection of flat and depressed gastrointestinal neoplasia of the colon. Am J Gastroenterol. 2012; **107**(5): 650-4.

Waye JD. Endoscopic mucosal resection of colon polyps. Gastrointest Endosc Clin N Am. 2001; **11**(3): 537-48, vii.

De Graaf EJ, Doornebosch PG, Stassen LP, Debets JM, Tetteroo GW, Hop WC. Transanal endoscopic microsurgery for rectal cancer. Eur J Cancer. 2002; **38**(7): 904-10.

De Graaf EJ, Doornebosch PG, Tollenaar RA, Meershoek-Klein Kranenbarg E, de Boer AC, Bekkering FC, et al. Transanal endoscopic microsurgery versus total mesorectal excision of T1 rectal adenocarcinomas with curative intention. Eur J Surg Oncol. 2009; **35**(12): 1280-5.

McGill SK, Evangelou E, Ioannidis JP et al. Narrow band imaging to differentiate neoplastic and non-neoplastic colorectal polyps in real time: a meta-analysis of diagnostic operating characteristics. Gut. 2013;62(12):1704-13.

Balog J, Sasi-Szabo L, Kinross J et al. Intraoperative tissue identification using rapid evaporative ionization mass spectrometry. Science translational medicine.
2013;5(194):194ra93.

Loughrey MB, Quirke P, Shepherd NA. Dataset for colorectal cancer histopathology reports. 3 ed. London: Royal College of Pathologists; 2014.

S. Abdul, B. H. Brown, P. Milnes, and J. A. Tidy, "A clinical study of the use of impedance spectroscopy in the detection of cervical intraepithelial neoplasia (CIN)," *Gynecologic Oncology,* vol. 99, pp. S64-S66, 2005.
S. Abdul, B. H. Brown, P. Milnes, and J. A. Tidy, "The use of electrical impedance spectroscopy in the detection of cervical intraepithelial neoplasia," *Int. J. Gynecol. Cancer,* vol. 16, pp. 1823-1832, 2006.

JA Tidy, BH Brown, TJ Healey, S Daayana, M Martin, W Prendiville, HC Kitchener. (2013) Accuracy of detection of high-grade cervical intraepithelial neoplasia using electrical impedance spectroscopy with colposcopy Br J Obstet Gynae 120; 400-411.

Duck FA. Physical properties of tissue. London: Academic Press, 1990.

Foster KR, Schwan HP. Dielectric properties of tissues and biological materials: a critical review. Crit Rev Biomed Eng1989; 17:25–104.

Gabriel S, Lau WR and Gabriel C. The dielectric properties of biological tissues: III. Parametric models for the dielectric spectrum of tissues. Physics in Medicine and Biology. 1996; 41 (11): 2271

Wilke LG, Brown JQ, Bydlon TM, Kennedy SA, Richards LM, Junker MK, et al. Rapid noninvasive optical imaging of tissue composition in breast tumor margins. Am J Surg. 2009; **198**(4): 566-74.

Allweis TM, Kaufman Z, Lelcuk S, Pappo I, Karni T, Schneebaum S, et al. A prospective, randomized, controlled, multicenter study of a real-time, intraoperative probe for positive margin detection in breast-conserving surgery. Am J Surg. 2008; **196**(4): 483-9.

Van Dam GM, Themelis G, Crane LM, Harlaar NJ, Pleijhuis RG, Kelder W, et al. Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-alpha targeting: first in-human results. Nat Med. 2011; **17**(10): 1315-9.

Brown BH, Tidy JA, Boston K et al. Relation between tissue structure and imposed electrical current flow in cervical neoplasia. Lancet. 2000;355(9207):892-5.

Keshtkar A, Salehnia Z, Keshtkar A et al. Bladder cancer detection using electrical impedance technique (tabriz mark 1). Patholog Res Int. 2012;2012:470101.

Mahara A, Khan S, Murphy E et al. 3D Microendoscopic Electrical Impedance Tomography for Margin Assessment during Robot-Assisted Laparoscopic Prostatectomy. IEEE Trans Med Imaging. 2015.

Knabe M, Kurz C, Knoll T, et al. Diagnosing early Barrett's neoplasia and oesophageal squamous cell neoplasia by bioimpedance spectroscopy in human tissue. United European Gastroenterol J. 2013;1(4):236-41.

Spruessel, Steinmann, Jung et al. Tissue ischaemia time affects gene and protein expression patterns within minutes following surgical tumour excision. BioTechnique 2004; 36: 1030-1037

Keshtkar A and Keshtkar A. Probe Pressure optimization in Bio-imedance spectroscopy. World Congress on Medical Physics and Biomedical Engineering. 2009.