Rectal electrical bio-impedance spectroscopy in the detection of colorectal anomalies associated with cancer

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Abstract. Colorectal cancer is the third most common cancer worldwide. Tests available for screening this cancer have low sensitivity or are challenging and costly to implement. Based on the concept of field cancerization, or carcinogenic field effect, the possibility of early detection of cancer by electrical bioimpedance spectroscopy measurements on the rectum was studied. For this research, seventy-seven subjects who attended the clinic for total colonoscopy were examined, and readings were taken at eight frequencies with an electrical bioimpedance spectroscopy probe. Four measurements were taken from the rectum of each subject, with the electrical probe being introduced into the rectum through an anoscope, before a total colonoscopy. An inverse model was used to obtain Cole-Cole parameters for each electrical bioimpedance spectra. Two results were the more prominent in this research; firstly, the field cancerization effect was observed and secondly, a significative correlation between rectal electrical bioimpedance and abnormalities associated with cancer was found (p-value = 0.002).

1. Introduction

According to Globocan-2012 (WHO-IARC 2012), colorectal cancer (CRC) is the third most common cancer and the fourth most common cause of cancer deaths worldwide. Mainly due to wider screening coverage, CRC incidence and mortality declined by approximately 2% and 3% per year in the United States during the 1990s and during the past decade, respectively [1]. It is estimated that at least fifty percent of these declines can be attributed to the increase in CRC screening and the use of best tests to detect advanced adenomas and cancers at an early stage [2]. Among the different tests available [3], colonoscopy, flexible sigmoidoscopy and fecal occult blood tests (FOBTs) are the most common screening tools used and the “...prime candidates for effective and cost-effective options” [4]. However, all three
tests have major disadvantages [5] that we mention briefly. Colonoscopy is considered as the gold standard, but this screening test is expensive, invasive and not well accepted by the general population. In less developed countries, with approximately 60% of the burden posed by CRC [6], costs are a very critical issue. The major disadvantage of flexible sigmoidoscopy is that it cannot detect polyps or cancers located on the right side of the colon. FOBTs, although non-invasive and not expensive, lack high sensitivity and specificity. Additionally, in many cases, when the latter two methods are used, there is a need to perform a total colonoscopy. Another criticism of CRC screening is related to the lead time bias [7], which means a perceived survival time without actually affecting the course of the disease. Therefore, an ideal screening test should be able to detect the disease at a very early stage or, much better, before it begins, i.e., to detect people who are at an increased risk of developing the disease, which would be more in line with prevention. The search for new, more cost-effective and easy to use pre-screening and screening tools which are more likely to be accepted by the public is therefore justified. Based on existing evidence that intestinal mucosal permeability increases with CRC [8-10], as well as on the theory of field carcinogenesis, we hypothesize that a technique that we would like to call rectal electrical bioimpedance spectroscopy (REBIS) could be used for CRC pre-screening or screening and could be a good candidate to fulfill the above mentioned requirements.

Among the different studies that have been dedicated to the detection of “field effect” in the colon, those by the research group lead by Roy & Backman [11] are among the most prominent. They have explored the use of different optical techniques, especially what they call low-coherence enhanced backscattering (LEBS) spectroscopy “in order to detect the presence of adenomas throughout the colon via optical interrogation of the rectal mucosa...” [12]. For the presence of advanced adenomas, they have reported an overall accuracy of 89.5% with in vivo readings taken from 619 patients.

In this paper, it is proposed that the electrical bioimpedance measurements in the rectum, REBIS, could be useful in detecting early changes in the rectal mucosa associated with field effect carcinogenesis of the colon. Changes such as increases/decreases in the microbial population, thickness, ultrastructure and composition of the mucus layer, disruption of the paracellular pathway (distortion of the tight junctions and increased epithelial permeability), and all those associated with inflammation, may well alter the passive electrical properties of the intestinal wall. EBIS is a very safe, easy to use and low-cost technique that has been used to explore early diagnosis of cancer in different organs such as the cervix [13], esophagus [14], skin [15] and bladder [16]. A couple of years ago, the British firm Zilico launched a device called ZedScan®, as an adjunct to colposcopy to help in early detection of cervical cancer [17]) EBIS is based on the injection of a small electrical current (40 µA in this study) at different frequencies to measure the real part of the electrical impedance. Although most of the studies published with this technique only consider the real part, especially at low frequencies (typically around 10 kHz), we decided to obtain the four parameters of the Cole-Cole model by inverse modelling, using the algorithm proposed by Miranda-Mercado & Lopez-Rivera [18]. These four parameters are resistivity at zero frequency ($\rho_0$), resistivity at infinite frequency ($\rho_\infty$), a time constant called Tao ($\tau$) and a heterogeneity parameter ($1 - \alpha$).

For this in vivo application, we used a minimally invasive 6.0 mm diameter metallic probe previously developed in Sheffield for research on cervical cancer, figure 1. We hypothesize that, under the umbrella concept of field carcinization [19, 20], the small ultrastructural changes reported in the literature for premalignant lesions, must change the electrical impedance properties of the rectum. REBIS should be sensitive to such changes and eventually could differentiate between rectal mucosa not presenting them from rectal mucosa with them, even if the tissue may be classified as normal, either histologically or by macroscopic appearance under direct or endoscopic visualization. The main aim of this study was to provide initial evidence to support this hypothesis, as a proof of principle.
2. Methods

2.1 Participants

This cross-sectional study was approved by the Institutional Ethical Review Board at Universidad de Caldas, Manizales, Colombia, South America. Patients were eligible for recruitment into the study if they were already scheduled for diagnostic colonoscopy screening or surveillance as recommended by their general practitioner or gastroenterologist. Volunteers were recruited among patients sent for diagnostic colonoscopy to “Unión de Cirujanos SAS” at Clínica de la Presentación in Manizales, Caldas, Colombia, South-America, between December 2014 and February 2015. All measurements were acquired through the point-of-care instrument Sheffield-Mark3- Multi-Frequency-Tissue-Impedance-Meter (created by the Department of Medical Physics at the Royal Hallamshire Hospital, in Sheffield-UK). Before a colonoscopy, the 6.0-mm diameter EIS probe, figure 1, was introduced into the rectal vault via an anoscope, figure 2.

![Figure 1](image1.png)

**Figure 1.** Tip of the 6.0 mm probe used.

![Figure 2](image2.png)

**Figure 2.** Positions where readings were taken (view from the back).

The colorectal surgeon involved in the study (EMV) then took four readings from locations identified as 00:00, 03:00, 06:00 and 09:00 hours within the rectum (figure 2), applying gentle contact to the tissue surface. Each reading recorded the impedance readings at eight different frequencies.
(from 4.8 up to 614.4 kHz) and took less than 10 seconds. The entire procedure from probe insertion to extraction typically took less than 1-2 minutes. At the time of data acquisition, the colorectal surgeon was blinded to the colonoscopy findings. After the measurements were acquired, the surgeon made way for the endoscopist to carry out the procedure. Data analysis was performed in post-processing by the researchers. For diagnostic purposes, patients were classified into five categories according to the findings during the colonoscopy: normal (no apparent lesion, \( n = 38 \)), polyps (\( n = 18 \)), diverticulosis (\( n = 8 \)), colitis (\( n = 6 \), one collagenous, one disuse and four ulcerative) and cancer (\( n = 7 \), one in the anus, three in the colon and three in the rectum).

### 2.2 Electrical Bio-Impedance measurements and parameter calculation

Using the same equipment as [13], [14] and [21], and with the same type of pencil-probe used on the cervix (figure 1), an electrical current of 40 µA peak-to-peak at 8 time different frequencies was passed between two adjacent electrodes. The probe had a diameter of 6.0 mm with four gold electrodes (1 mm diameter each) equally spaced on a circle with a radius of 1.65 mm and mounted flush with the face of the probe. The eight frequencies used went from 4.8 kHz to 614.4 kHz, each of the last seven frequencies being double the previous one. The real part of the resulting potential was measured between the two remaining electrodes, and the transfer impedance was calculated as the ratio of the measured potential to the amplitude of the imposed current. Raw data was converted into resistivity using conversion factors obtained from calibration of the equipment and probe with saline solutions of known conductivities. Electrical readings were taken before the colonoscopy by the colorectal surgeon participating in the project (EMV). With the patient in fetal position and after lubrication of the anus, a disposable anoscope was gently introduced. The surgeon then proceeded to take measurements at positions 00:00 (posterior, i.e. middle line, immediately below the coccyx), 03:00 (right lateral), 06:00 (anterior), and 09:00 (left lateral) (figure 2).

To obtain the four parameters of the Cole-Cole model (\( \rho_0, \rho_\infty, \tau \) and \( 1 - \alpha \)), the algorithm by Miranda-Mercado & Lopez-Rivera [22] was used. Parameters were individually compared to all others as well as grouped in three different ways: a) cancer compared with the remainder of the groups, b) normal compared with the remainder of the groups and c) five different diagnoses between them.

### 2.3 Data Analysis

For data analysis, the IBM SPSS Statistics 2013® was used. A one-way ANOVA with repeated measurements on one factor was used to assess the statistical significance of the differences between the parameters of the different points and between the data of the five groups of patients (cancer, colitis, diverticulosis, polyps and normal). When there was a \( p < 0.05 \), Fisher’s Least Significant Difference (LSD) test was performed as a post hoc analysis to specify where the differences were.

### 3. Results

Seventy-seven patients were recruited into the study after providing written, informed consent. Mean, and standard deviation (SD) of ages by gender are given in Table 1.

| Gender | \( n \) | Mean age (SD) |
|--------|--------|---------------|
| Female | 57     | 50.0 (15.6)   |
| Male   | 20     | 51.5 (15.8)   |
| Total  | 77     | 50.4 (15.8)   |

The parameters obtained with all readings at the four different points (Table 2) were compared among them.
Table 2. Mean (SD) of Cole-Cole parameters by site of measurement

| Site | ρ0 [Ωm] | ρ∞ [Ωm] | τ [µs] | 1−α |
|------|---------|---------|--------|------|
| 0:00 | 5.3 (1.2) | 1.2 (0.6) | 60 (60) | 0.22 (0.17) |
| 3:00 | 5.2 (1.0) | 1.3 (0.6) | 50 (50) | 0.23 (0.15) |
| 6:00 | 5.1 (1.1) | 1.1 (0.3) | 50 (50) | 0.20 (0.08) |
| 9:00 | 5.5 (1.2) | 1.3 (0.5) | 70 (50) | 0.20 (0.11) |

The ANOVA gave a p = 0.00 for the parameter τ, and the Fisher’s test showed that the mean value of the point identified in this study with 09:00 (figure 2), has a statistically different value when compared with the other three points (00:00, 03:00 and 06:00). No differences were found between the means of the other three parameters.

In addition, the means of the parameters of the Cole-Cole model (Table 3) for the five subgroups of volunteers showed statistically significant differences in ρ0 in the three different groups proposed in this study (a. cancer versus non-cancer, b. normal versus disease, c. all five subgroups compared with each other), and a difference in τ when comparing normal versus disease, as well as comparing the subgroups formed by the five different endoscopic diagnoses (Table 4). The post-hoc analysis with the Fisher’s test showed that, for ρ0 (p-value = 0.002), the group with cancer is different from those with either colitis (p = 0.007), normal (p = 0.007) or polyps (p = 0.042). For τ, the group with diverticulosis is different from those with either colitis (p = 0.029) or normal (p = 0.023).

Impedance spectra, calculated from the Cole-Cole parameters obtained in this study, for the group of patients diagnosed as “Normal” (no apparent disease) and the group diagnosed with cancer, both after colonoscopy, are shown in figure 3. It can be seen that, at very low frequencies (down from about 10 kHz), the profiles of the two groups diverge.

Table 3. Means (SD) of Cole-Cole parameters by type of diagnosis.

| Diagnosis   | ρ0 (Ω)       | ρ∞ (Ω)       | τ [µs] | 1−α |
|-------------|--------------|--------------|--------|-----|
| Normal      | 5.47 (1.01)  | 1.28 (0.50)  | 70 (50) | 0.20 (0.01) |
| Polyps      | 5.39 (1.08)  | 1.25 (0.58)  | 50 (50) | 0.22 (0.13) |
| Diverticulosis | 4.89 (1.36)  | 1.05 (0.29)  | 40 (40) | 0.23 (0.12) |
| Colitis     | 5.59 (1.07)  | 1.16 (0.25)  | 80 (50) | 0.20 (0.09) |
| Cancer      | 4.50 (1.18)  | 1.09 (0.65)  | 50 (50) | 0.24 (0.24) |
| No cancer   | 5.39 (1.09)  | 1.24 (0.49)  | 60 (50) | 0.21 (0.11) |
| No disease  | 5.16 (1.21)  | 1.17 (0.51)  | 53 (48) | 0.22 (0.15) |

Table 4. p-values for the comparison of the Cole-Cole parameters by type of diagnosis.

| Diagnosis                        | ρ0       | ρ∞       | τ         | 1−α       |
|---------------------------------|----------|----------|-----------|-----------|
| Cancer vs. no-cancer            | 0.002    | 0.258    | 0.279     | 0.491     |
| Normal vs. disease              | 0.044    | 0.086    | 0.030     | 0.372     |
| Diverticulosis, colitis, cancer, polyposis, normal | 0.005    | 0.255    | 0.009     | 0.861     |
Figure 3. Example of an impedance profile obtained with the Cole-Cole parameters

4. Discussion
To the best of our knowledge, this is the first time that in vivo measurements of the passive electrical response of rectal mucosa in humans is reported. For instance, a search in PubMed with the search strategy “Electric Impedance” [Mesh] AND “Colonic Neoplasms” [Mesh], provides only a few references, most of them done in vitro and either with cells or resected tissue. This study was carried out to explore the feasibility of what we call REBIS for its possible future use as a screening tool for CRC. This proposal was based both on published literature that shows an increased transepithelial permeability, leading to a decrease in its electrical impedance [8-10], associated with the development of cancer, as well as on the concept of field carcinogenesis [23].

CRC has a long period of incubation, during which early changes in the pathway to overt disease are not evident to the traditional screening or diagnostic tools available. These changes are in line with the concept of field carcinogenesis, as well as with what could be considered as an emerging paradigm of disease development. The concept of “field effect in cancer”, also known as “field carcinogenesis”, “cancer field effect”, “field defect” or “field cancerization” among others (see, for instance, [23]), was proposed by Slaughter and colleagues in 1953 [24]. The main idea behind this concept is that cancer arises from an area of an organ that has been preconditioned by a “process of irreversible change toward cancer...”. Moreover, therefore, it would explain the multifocal origin of many lesions and their horizontal coalescence, as well as the recurrence or persistence of cancer after treatment [24]. This pioneering work focused on cancer arising in the oral stratified squamous epithelium, but the concept has been extended practically to all organs of the human body, the colon and rectum among them [23].

It is noteworthy to point out that, as early as 1983, Traynor et al [25] had invoked the idea of “field change” in relation to CRC. At present, it is accepted that “… molecular alterations can be present even in microscopically normal tissue...” [23]. These authors have proposed an extension of the original concept to one of “etiologic field effect”, “Defined by presence of etiologic exposures and their influence on tissue microenvironment”, which is “… Not restricted by anatomic boundaries and may involve multiple anatomic sites”. They also suggest the possibility of a “whole body field effect”.

In the same way as many other chronic diseases, CRC may follow the pathophysiological pathway that is now emerging as a new paradigm in medical science, as probably first described by Cani et al [26] for metabolic diseases in experimental rats. This pathway involves the following sequence: 1) high-fat feeding, 2) change in gut flora, 3) increased intestinal permeability, 4) increased endotoxemia, 5) inflammation and, 6) metabolic disorders. To this paradigm, we would, however, have to add the disruption of the colonic mucus layer between steps 2) and 3), and bacterial translocation between steps 4) and 5), simultaneously with “increased endotoxemia”. 
For CRC, we would have (20): 1) unhealthy diet (rich in fat and red meat and low in fiber) (27), 2) dysbiosis (alteration in the ratio of “harmful” and “beneficial” commensal bacteria) (27), 3) alteration in the mucus layer (25), 4) disruption of the tight junctions [28] with increased intestinal permeability (10), 5) metabolic endotoxemia and bacterial translocation, 6) inflammation (27), CRC.

The first finding of this study seems to show that readings taken on the site identified in this study as 09:00, differ in the parameter \( r \) from the other three locations. We do not have an explanation for this, but the fact that this site is on the same side as that on which the person lies (his/her left side) could influence this result. Nevertheless, parameter \( R_0 \) does not differ among the four sites. More interesting is the finding that parameter \( R_0 \) seems to be able to separate different groups, like those established in this study. Cancer is separated, both collectively from all those participants not presenting cancer, as well as individually from colitis, polyps and normal. For parameter \( r \), it separates normal from disease, as well as diverticulosis from colitis (\( p = 0.029 \)) and from normal (\( p = 0.023 \)). In both cases, this parameter has a lower value for diverticulosis.

5. Conclusion
This work supports the hypothesis that carcinogenic field effect in the colon and rectum are detectable with electrical bioimpedance spectroscopy readings taken in the rectum. This result opens the possibility of proposing it as a possible tool for colorectal cancer screening programs.

The initial findings of this study are very encouraging and warrant further investigation in the same line of research. A more extensive study would be needed to corroborate these initial results. Another point to be considered would be to try different probe sizes, as it is known that electrode separation influences the depth of penetration of the injected electrical current which means that different probe sizes could be useful to investigate different substructures of the rectal wall.

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