Early report

Relation between tissue structure and imposed electrical current flow in cervical neoplasia

Brian H Brown, John A Tidy, Karen Boston, Anthony D Blackett, Rod H Smallwood, Frank Sharp

Summary

Background When an electrical potential is applied to human tissue, the pattern of the resulting current flow is determined by the shapes, arrangements, and internal structure of the tissue cells. By measurement of the electrical current patterns over a range of frequencies, and use of an inverse modelling procedure, electrical variables describing the tissue structure can be calculated. We used this method to develop a screening technique for the detection of cervical precancers.

Methods We used a pencil probe (diameter 5 mm) to measure electrical impedance spectra from eight points on the cervix in 124 women with abnormal cervical smears. Variables that should be sensitive to the expected tissue changes were calculated. These were compared with the colposcopic results.

Findings The measured electrical impedance changes were those predicted on the basis of the expected tissue structures. Measurements made on normal squamous tissues were well separated from those made on precancerous tissues. We constructed receiver-operating-characteristic curves, comparing measurements made on normal tissue and that showing cervical intraepithelial neoplasia grade 2/3; the area under the curve was 0·951. These groups of women could be separated with a sensitivity of 0·92 and a specificity of 0·92.

Interpretation Characteristics of the electrical impedance spectra of tissues can be explained by changes in cell arrangements (layering) and in the size of the nuclei. This relation opens the way to deriving tissue structure from electrical impedance spectral measurements. We show that this approach can be used to give good separation of normal and precancerous cervical tissues.

Introduction

Biological tissues have complex electrical impedance, which is a function of frequency, because tissues contain components that have both resistive and charge storage (capacitive) properties. The magnitude of the impedance and its dependence on frequency are a function of tissue composition. There have been both practical[1,2] and theoretical[3] 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 β dispersion region, cell structures are the main determinant of tissue impedance.

Within the β dispersion region, low-frequency current can be thought of as passing through the extracellular space. Because the current has to pass around the cells, the resistance to flow depends on the cell spacings and how they are arranged. However, at higher frequencies, current can penetrate the cell membranes and hence passes through both intracellular and extracellular spaces. The current will thus be determined by intracellular volume and, we propose, the size of the nucleus.

This study aimed to assess the agreement between practical measurements of electrical impedance spectra and the predictions made by taking into account the known cell arrangements in cervical tissue. The major changes in cervical tissue in the precancerous stages are the breaking down of superficial cell layering and increases in the size of cell nuclei.[4] The large amount of published material on the histology of cervical tissue in both the normal and pathological states forms the basis for predicting the tissue impedance spectra to be associated with these states.

Cancer of the cervix is the second commonest cancer affecting women in the world and the commonest cause of death from cancer. However, in more developed countries, the disorder is potentially preventable[5] by screening and treatment of the precancerous phase, cervical intraepithelial neoplasia (CIN). All screening programmes to date have used exfoliative cervical cytology. However, this technique is associated with low sensitivity and specificity, and the result of the test routinely takes many weeks, thereby creating undue anxiety.[6] The cost burden to health-care programmes of supporting the infrastructure of a cytology screening programme is immense. A test based on the measurement of an electrical impedance spectrum would give a result to the operator (most likely a primary-care physician or nurse) in the same time as it takes for a smear-test sample to be collected. A negative result could be relayed immediately to the patient. A positive result, on the other hand, would enable more rapid referral to a colposcopy clinic.

Methods

Impedance measurements were made with a pencil probe of 5·5 mm in diameter, with four gold electrodes (1 mm diameter) mounted flush with the face of the probe and spaced equally on a circle of radius 1·65 mm (figure 1). A current of 10 μA peak-to-peak was passed between an adjacent pair of electrodes, and the real part of the resulting potential was measured between the two remaining electrodes. The ratio of the measured potential to the amplitude of the imposed current determines a transfer impedance. Measurements were made at eight frequencies by doubling the frequency in steps between 4·8 kHz and 614 kHz. Measurements were made serially at 67 frames per s, and entered onto a computer. In nearly all cases two separate sets of data (each of 100 measurements recorded over 1·5 s) were recorded in succession to check reproducibility. Only the first sets of measurements in each woman are used for the results presented here.

Lancet 2000; **355:** 892–95

Department of Medical Physics, University of Sheffield, Royal Hallamshire Hospital, Sheffield S10 2JF, UK (Prof B H Brown PhD, K Boston MSc, Prof R H Smallwood PhD) **and Department of Obstetrics and Gynaecology, University of Sheffield, Clinical Sciences Centre, Northern General Hospital Trust, Sheffield** (J A Tidy MD, A D Blackett PhD, Prof F Sharp MD)

Correspondence to: Prof Brian H Brown
(e-mail: b.h.brown@sheffield.ac.uk)

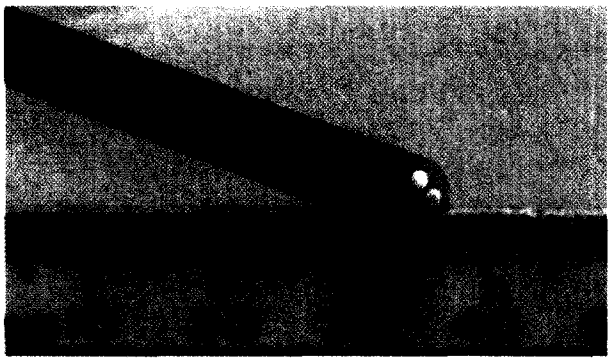

Figure 1: **Cervical measurements probe**
Scale is in mm.

The probe was calibrated in saline of known electrical conductivity. A four-electrode measurement of the transfer impedance spectrum is essentially independent of the contact impedance between electrode and tissue (which is of the order of 1 kΩ compared with the transfer impedance of 0·1 kΩ).

Measurements were made in a colposcopy clinic after the study had been approved by an ethics committee and informed consent obtained from the patients. Consecutive women with high-grade smears (ie, moderate or severe dyskaryosis) were recruited. Three women with borderline changes and two with mild dyskaryosis were also studied. Impedance measurements were made before acetic acid was applied for the purposes of colposcopy. The probe was placed in eight positions on the cervix. These points were as for the cardinal points of the compass with four positions close to the border with the endocervical canal and the remaining four well into the normal squamous epithelial surface of the cervix. Colposcopy examinations, including probe positioning, were recorded by video to allow for correlation between results obtained from colposcopic impression, histopathology of colposcopically directed multiple punch biopsy samples, and impedance measurements.

To qualify as normal, squamous epithelium had to lie outside the transformation zone, show no evidence of change with acetic acid, and stain positively with Lugol's iodine. The whole of the transformation zone was visible in all cases.

For each patient, information was obtained on the stage of her menstrual cycle at the time of the measurements and on whether she was premenopausal or postmenopausal.

The 100 measurements forming the first dataset recorded at each measurement position were averaged to give mean values of impedance at each of the eight frequencies. These data, forming an impedance spectrum, were then fitted by a least-square deviation method[7] to a Cole equation[8] of the form:

$$Z=R_\infty+\frac{(R_0-R_\infty)}{(1+[jF/F_c])^{1-\alpha}}$$

to give estimates of R_0, R_∞, and F_c. R_0 and R_∞ are the impedances (real part) at very low and very high frequencies, respectively, and F_c is a frequency. α is a constant that increases with the heterogeneity of tissue. We assumed a value of zero for α, since this value was found to improve accuracy in the estimation of F_c. In this case, an equivalent electrical circuit consisting of a resistor R placed in parallel with a resistor S and capacitor C in series will have an impedance Z, given by the above equation, where:

$$R_0=R$$

$$R_\infty=\frac{RS}{R+S}$$

$$F_c=\frac{1}{2\pi C(R+S)}$$

Variables R, S, and C can thus be calculated from the fitted Cole equation. Because the probe was calibrated in saline of known conductivity, R and S are inversely proportional to conductivity and have the units Ω m. R is related to the extracellular space (because it is the impedance at very low frequency) and S is related to the intracellular space (because it is derived from the impedance at very high frequency). C is related to the cell membrane capacitance and is given in units of μF/m.

Results

A clear colposcopy result and good impedance data were available for 756 measurements made on 124 women (maximum possible number of measurements eight for each woman; total=992). In 221 cases the tissue at the point where the probe had been placed was not clearly identified either by biopsy or by colposcopy. A further 15 measurements were rejected on technical grounds, in nearly all cases because the probe moved during data collection.

From comparison of colposcopic and histological results, we found that there were 370 measurements from normal squamous epithelium, one from an invasive cancer, 126 from CIN2/3 (high grade), 63 from CIN1 (low grade), 64 classified as mature metaplasia, 98 classified as immature metaplasia, and 34 classified as columnar tissue.

Analysis of the data showed no correlation between the impedance characteristics and the stage of the menstrual cycle. Postmenopausal data were not included in this study because initial measurements suggested that these data were distinguishable as a separate group.

The derived Cole equation variables for the four tissue groups are given in table 1. 95% CI for the means are given for guidance but these assume the distributions to be Gaussian. Non-parametric Mann-Whitney two-tailed tests were also done; they showed several significant separations of the groups. The values for R and S separated normal squamous epithelium tissue from the CIN2/3 tissues ($p<0·0001$ for each variable). R and S also separated normal squamous epithelium from CIN1 tissues ($p<0·0001$ for each variable). S separated CIN1 from CIN2/3 tissues ($p=0·0009$). The values for C did not show any significant differences. There was only one tissue sample from an invasive cancer. The measurements in this case were 8·0 Ω m, 5·1 Ω m, and 0·28 μF/m for R, S, and C respectively.

	Normal squamous epithelium (n=370)			CIN 1 (n=63)			CIN 2/3 (n=126)		
	R (Ω m)	S (Ω m)	C (μF/m)	R (Ω m)	S (Ω m)	C (μF/m)	R (Ω m)	S (Ω m)	C (μF/m)
Median and range									
Minimum	1·45	0·03	0·06	0·69	0·08	0·12	0·89	0·77	0·05
25th percentile	12·8	1·15	0·37	2·69	2·49	0·33	2·36	4·39	0·36
Median	20·1	1·91	0·65	3·27	4·53	0·66	2·98	6·08	0·64
75th percentile	26·8	2·78	1·20	5·52	6·31	1·46	4·22	7·63	1·09
Maximum	28·8	73·8	27·4	28·8	12·5	6·02	21·7	13·0	19·3
Mean									
Mean	19·0	2·31	1·12	5·36	4·79	1·01	3·85	6·10	1·01
SD	7·77	4·04	1·96	5·84	3·09	1·01	2·89	2·57	1·93
SE	0·40	0·21	0·10	0·73	0·38	0·12	0·25	0·22	0·17
95% CI									
Lower	18·2	1·90	0·92	3·88	4·02	0·76	3·34	5·64	0·67
Upper	19·8	2·72	1·32	6·83	5·57	1·27	4·36	6·55	1·35

Table 1: **Tissue measurements**

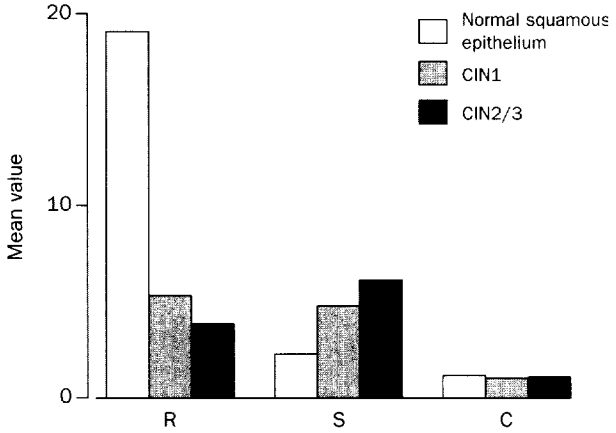

Figure 2: **Mean values of R, S, and C in normal squamous epithelium, CIN1 tissue, and CIN2/3 tissue**

The two repeated blocks of 100 measurements were used to check the reproducibility of the measurements. The coefficient of variation in the measurements was 0·108 for R, 0·263 for S, and 0·253 for C.

To assess the statistical independence of the estimated values for R and S, we calculated a Pearson correlation using the pooled data for normal squamous epithelium, CIN1, and CIN2/3 tissues. The result (r^2=0·086) can be interpreted as showing that only 8·6% of the variation in R can be attributed to variations in S and vice versa.

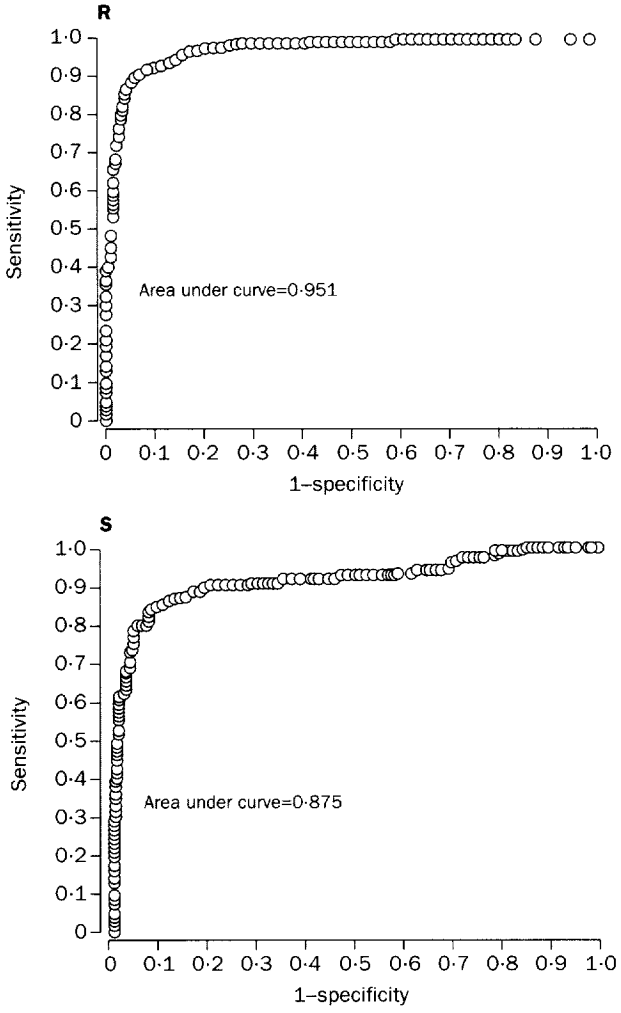

Figure 3: **ROC curves derived from data for normal squamous epithelium and CIN2/3 tissues**

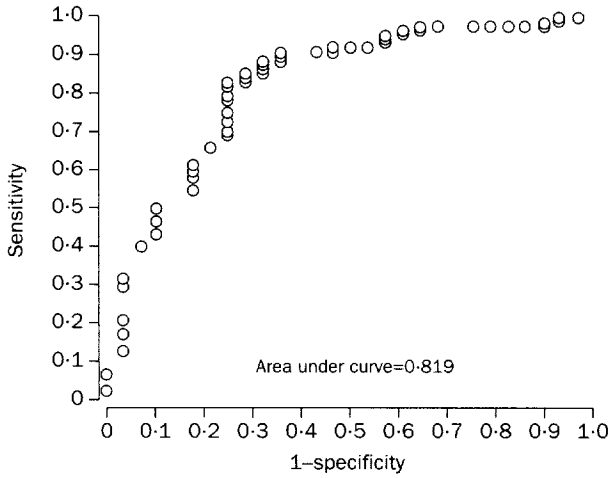

Figure 4: **Analysis per woman: ROC curve comparing groups identified by colposcopy as showing either CIN or normal epithelium with the electrical impedance indicator R/S minimum**

From normal squamous epithelium through CIN1 to CIN2/3 (figure 2) R decreased by a factor of about 5·0; S increased by a factor of about 2·5; and C did not change.

To assess the usefulness of the technique as a screening test, we derived receiver-operating-characteristic (ROC) curves[9] for the normal squamous epithelium and CIN2/3 tissue groups (figure 3). ROC curves show the sensitivities (1 minus the fraction of false-negative results) and specificities (1 minus the fraction of false-positive results) obtained with these variables used as discriminants between the normal squamous epithelium and CIN2/3 tissue groups. If the measurements give no discrimination between the two groups, a single line at 45° is obtained. If there is a discrimination, the curve is displaced upwards and to the left. The area under the curve indicates the degree of separation: an area of 0·5 corresponds to no discrimination between the groups and an area of 1·0 to perfect separation.

The data were also grouped for each woman so that we could make comparisons between the electrical impedance measurements and the results of both the referral smear test and the outcome of the colposcopy examination.

To provide a single indicator for each woman R/S was first calculated for each of the eight measurement sites. This approach was an attempt to take into account the fact that R decreases and S increases from normal squamous epithelium through CIN1 to CIN2/3. Other combinations of R and S could be used, and we made no attempt to optimise separation of the tissues on this basis. The lowest value of R/S (R/S minimum) was then taken as the outcome for each woman on the basis that this should identify the greatest abnormality. However, we found that this method of identification included several tissue sites identified by colposcopy as columnar or immature metaplasia tissues. To limit this confusion, we excluded sites where R was less than or equal to 2·36 Ω m (the 25th percentile for the CIN2/3 group) when taking the minimum value of R/S in each woman.

The colposcopy and biopsy results were used to classify women into a CIN group or a normal group. All of the normal group had had at least two colposcopic examinations, 6 months apart, with repeat cervical cytology and biopsy. If any tissue of CIN1 or CIN2/3 was identified, the woman was classified as having CIN. There were 88 women in the CIN group and 28 in the normal group. Eight women were excluded because we obtained fewer than six of

	R/S minimum	
	CIN (n=88)	Normal (n=28)
Median and range		
Minimum	0·25	0·29
25th percentile	0·35	0·8050
Median	0·49	1·885
75th percentile	0·815	5·73
Maximum	8·20	12·10
Mean		
Mean	0·86	3·46
SD	1·24	3·25
SE	0·13	0·62
95% CI		
Lower	0·59	2·20
Upper	1·12	4·72

Table 2: **Analysis on a per-woman basis**

the possible eight measurements or the outcome of the colposcopy investigation was ambiguous.

The R/S minimum results are compared with the CIN/normal classification by means of the ROC curve in figure 4. The area under this curve is 0·819. Table 2 gives a range of statistical variables derived from these data.

If we categorise the 116 women on the basis of the impedance results and use the 75th percentile point (0·81) for R/S minimum as the borderline, impedance produces the following performance measures: sensitivity 75% (66/88), specificity 71% (20/28), positive predictive value 89% (66/74), and negative predictive value 45% (20/44). In this study, cervical cytology had a positive predictive value of 76% (88/116). No other measures could be calculated because all the women had positive smear results.

Discussion

The Cole equation that has been fitted to the measured impedance spectra provides the variables R, S, and C. R is determined by the conduction pathways through the extracellular space and is therefore sensitive to the packing of cells into layers. In normal squamous epithelium R would be expected to have a high value, because current has to track around the cell layers and therefore takes a long resistive path. In tissue graded as CIN1 and CIN2/3 the superficial cell layering of normal squamous epithelium is absent and R is much lower. The observed changes in R fit well with this model.

S is determined by the conduction path through the intracellular space. If the nuclear membrane is assumed to have similar electrical properties to those of the cell membrane, we would expect this pathway to be affected by the cell nuclei. If the cells do not change in volume but the nuclei are enlarged, the conduction pathways through the intracellular space will be smaller and hence S will increase in value. There is a well-documented large increase in mean nuclear size in CIN2/3 tissue,[10,11] and we would therefore expect to see S increase in these tissues. Again, the observed changes in S fit well with this model.

C is determined by the structure of the cell membrane. There is no evidence to enable a prediction as to the changes expected in CIN2/3 tissue.

The secondary objective of this study was to assess the potential of the technique as a method of detecting precancerous changes in the cervix. Our results show good separation of the measurements made on normal squamous epithelium and on CIN1 and CIN2/3 tissues. Sensitivities and specificities of 0·9 can be obtained in detecting the changes associated with CIN2/3. However, the only way to assess the role of the impedance test as a screening tool is to carry out a large trial with both the smear test and the impedance test.

Coppleson and colleagues showed[12] that electrical measurements can be used to detect precancerous changes in cervical tissue. Their probe (Polarprobe), makes bipolar electrical measurements of the response to a 1·2 V pulse applied for 100 μs. This response is indirectly related to the low-frequency impedance of the electrodes and the underlying tissue. Current clinical trials with this probe have not yet been fully reported.

Mitchell and colleagues[13] reviewed several methods for the diagnosis of squamous intraepithelial lesions and derived ROC curves. They gave areas under the ROC curves of 0·76 for Papanicolaou smear testing, 0·84 for diagnostic colposcopy, and 0·71–0·75 for their fluorescence spectroscopy technique. In most cases these investigators grouped CIN1 and CIN2/3 tissues together in deriving the ROC curves. The comparable figures for our data with CIN1 and CIN2/3 tissues grouped together are 0·934 for R and 0·834 for S as the separating variable. However, the comparison may be misleading because our figures relate to measurements made at individual sites. A better comparison is with the results of the analysis per woman. The area under the curve for that analysis is 0·819 (figure 4).

Thus, our findings suggest that some characteristics of the electrical impedance spectrum of tissue can be explained by changes in cell arrangements (layering) and in the size of the cell nuclei. This relation opens the way to assessment of tissue structure from electrical impedance spectral measurements. For example, measurements might be made from the gastro-oesophageal junction and the bladder, where screening for precancerous changes is important.

Contributors

B H Brown conceived the idea of the probe, coordinated the project, and drafted the paper. J A Tidy had clinical responsibility for the patients, constructed the measurement protocols, led the clinical parts of the project, and collected the in-vivo data. K Boston calibrated the measurement system and archived and analysed the data. A D Blackett contributed to the pathological interpretation of the data. R H Smallwood contributed to the measurement system and to the modelling of the tissues. F Sharp was involved in the clinical aspects of the initiation of the project.

References

1 Duck FA. Physical properties of tissue. London: Academic Press, 1990.
2 Foster KR, Schwan HP. Dielectric properties of tissues and biological materials: a critical review. *Crit Rev Biomed Eng* 1989; **17:** 25–104.
3 Schwan HP. Electrical properties of tissue and cell suspensions. *Adv Biol Med Phys* 1957: 147–224.
4 Anderson MC, Jordan JA, Morse AR, Sharp F. A text and atlas of integrated colposcopy. London: Chapman & Hall Medical, 1992.
5 Sasieni P, Cuzick J, Farmery E. Accelerated decline in cervical cancer mortality in England and Wales. *Lancet* 1995; **346:** 1566–67.
6 Pritchard DA, ed. Quality assurance guidelines for the cervical screening programme. London: NHS Cervical Screening Programme Publication No 3 Jan, 1996.
7 Brown BH, Leathard AD, Lu L, Wang W, Hampshire A. Measured and expected Cole parameters from electrical impedance tomographic spectroscopy images of the human thorax. *Physiol Meas* 1995; **16:** 57–68.
8 Cole KS, Cole RH. Dispersion and absorption in dielectrics. *J Chem Phys* 1941; **9:** 341–51.
9 Metz CE. Basic principles of ROC analysis. *Semin Nucl Med* 1978; **8:** 283–98.
10 Boon ME, Kok LP. Koilocytotic lesions of the cervix: the interrelation of morphometric features, the presence of papilloma-virus antigens, and the degree of koilocytosis. *Histopathology* 1985; **9:** 751–63.
11 Sorensen FB, Bichel P, Jakobsen A. Stereological estimates of nuclear volume in squamous cell carcinoma of the uterine cervix and its precursors. *Virchows Archiv A Pathol Anat* 1991; **418:** 225–33.
12 Coppleson M, Reid BL, Skladnev VN, Dalrymple JC. An electronic approach to the detection of pre-cancer and cancer of the uterine cervix: a preliminary evaluation of Polarprobe. *Int J Gynaecol Cancer* 1994; **4:** 79–83.
13 Mitchell MF, Cantor SB, Ramanujam N, Tortolero-Luna G, Richards-Kortum R. Fluorescence spectroscopy for diagnosis of squamous intraepithelial lesions of the cervix. *Obstet Gynecol* 1999; **93:** 462–70.