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Impedance Spectroscopy of Teeth with and without Approximal Caries Lesions— an in vitro Study

M.-C.D.N.J.M. Huysmans1*, C. Longbottom2, N.B. Pitts2, P. Los3, and P.G. Bruce3

1TRIKON: Institute for Dental Clinical Research, Department of Cariology and Endodontontology, University of Nijmegen, PO Box 9101, NL-6500 HB Nijmegen, The Netherlands; *Department of Dental Health, University of Dundee, Dundee, Scotland, UK; and 3School of Chemistry, University of St Andrews, St Andrews, Scotland, UK; *to whom correspondence should be addressed

Abstract. Caries diagnosis by the measurement of electrical resistance is hampered by polarization effects when dc or single-low-frequency ac currents are used. Electrical impedance spectroscopy, measuring impedance over a large range of frequencies, will provide more detailed information about the electrical characteristics of teeth. It was the aim of this study (a) to characterize the complex impedance behavior of whole extracted teeth, measured at the approximal surface, and (b) to identify parameters of the complex impedance behavior of the teeth which would be useful in distinguishing between degrees of carious involvement. Thirty-nine extracted premolar teeth with 59 unrestored and undamaged (excepting caries) approximal surfaces were selected. The tooth surfaces were divided into three groups according to their macroscopic appearance: sound (group S, n = 16), white- or brown-spot lesion present (group L, n = 33), or cavitated (group C, n = 10). The teeth were inserted into a jig which allowed for counter-electrode contact via a conducting gel. The working electrode consisted of a carbonated fiber material. Electrical impedance measurements were performed over a maximum range of about 1 MHz to 0.1 Hz. We analyzed electrical impedance data by fitting equivalent circuits. Fit was evaluated numerically and visually. The complex impedance spectra divided naturally into three groups which corresponded almost perfectly with the classifications of S, L, and C. The groups differed most in the dc resistance (Rdc), as calculated from the impedance parameters. Mean Rdc for groups S, L, and C were 68 MΩ, 5.9 MΩ, and 321 kΩ, respectively. These means were significantly different from each other (log-transformed data, ANOVA, p < 0.001; Tukey multiple comparisons, p < 0.001). It is concluded that the in vitro performance of electrical impedance spectroscopy in differentiating among sound, non-cavitated carious, and cavitated approximal tooth surfaces is excellent.

Key words: dental caries, caries diagnosis, electrical impedance spectroscopy, diagnostic systems.

Received September 14, 1995; Accepted July 16, 1996

Introduction

Caries diagnosis by electrical measurement was introduced into dentistry about 40 years ago. The method is based on the phenomenon that dental enamel, consisting largely of hydroxyapatite, has a high electrical resistivity. This resistivity is reduced after demineralization, because this increases the size of the pores, which are filled with more conductive fluids. Once the enamel has been lost locally and a cavity exists, the resistance of a tooth is determined by the dentin, which contains many fluid-filled tubules, and thereby has a relatively low resistivity.

In the first investigations into the caries-diagnostic use of electrical resistance measurements, dc currents were used, giving rise to polarization artefacts where the electrodes came into contact with the teeth (Pincus, 1951; Mumford, 1956; Nomura et al., 1971). To avoid this problem, investigators later used ac currents but at certain fixed frequencies, selected for experimental convenience rather than diagnostic significance (White et al., 1978; Sawada et al., 1986). A few commercial instruments were marketed, of which only the most recently developed is now available. The frequencies chosen for these instruments lie between 20 and 400 Hz, and because of polarization effects, these may be too low. The performance of these instruments for occlusal caries diagnosis has, in several in vitro and in vivo studies, been shown to be comparable with or better than that of existing methods of caries diagnosis (Rock and Kidd, 1988; Pieper et al., 1990; Verdonschot et al., 1992; Ricketts et al., 1995). However, the method has several major limitations. In vivo, only occlusal surfaces have been measured until now, since the instruments used by themselves are not suited for use on other surfaces. [There is only one in vitro report relating to approximal surfaces (Longbottom et al., 1993).] Moreover, the diagnostic performance was generally best for the detection of dentinal caries (associated with advanced decay), and no correlation of measurements with lesion depth in enamel could be shown (Matsumoto and Fearnhead, 1980). Many improvements in the method itself and its practical use are necessary before it will be ready for wide and reliable use.

application as a diagnostic tool.

In most fields of research where ac impedance has been studied, fixed frequency methods were quickly replaced by measurements over wide frequency ranges. Measurements at a fixed frequency can contain contributions from many different electrical processes, both within the sample being studied and at the point of contact with the electrodes; hence very few conclusions can be drawn from them concerning specific physical phenomena (Macdonald, 1987). Kumasaki first performed ac impedance measurements on human dentin sections (Kumasaki, 1975). Later studies were concerned with the ac impedance behavior of enamel membranes (Scholberg et al., 1982; 1984), of tooth hemi-sections (Hoppenbrouwers et al., 1986), and of enamel and dentin sections (Levinkind et al., 1990, 1992). Some authors suggested equivalent electrical circuits to describe the results they found, and proposed physical models that would explain these results (Scholberg et al., 1987; Levinkind et al., 1990). The measurements were targeted at fundamental studies of the electrical behavior of dental material. The experimental conditions in these studies would be impossible to reproduce even partially in a clinical setting. In some studies, it did not prove possible to cover the entire impedance spectrum. With the exception of a brief preliminary report of part of this study (Longbottom et al., 1996), the effect of the presence of a caries lesion in enamel or dentin on the impedance characteristics of a whole tooth has not yet been reported.

It was the aim of our study (a) to characterize the complex impedance behavior of whole extracted teeth, measured at one or both of their smooth approximal surfaces, in a set-up which could be reproduced in its basic form in a clinical situation, and (b) to identify parameters of the complex impedance behavior of the teeth, which would be useful in distinguishing between degrees of carious involvement.

Materials and methods

Study material

For the study, we selected unrestored extracted premolar teeth, which showed no signs of non-carious damage, such as fractures or abrasions, on their approximal surfaces. The teeth were obtained with written parental/patient consent, and the protocol complied with the conditions set out by the Tayside Committee on Medical Research Ethics. A total of 59 tooth surfaces from 39 teeth was included. The teeth had been stored in thymolized physiological saline for a minimum of 3 months before being tested. Approximal surfaces were assigned to one of three groups according to their microscopic condition viewed with the naked eye: S (sound), if no sign of carious involvement was detected (n = 16); L (lesion), if a brown- or white-spot lesion was present with no or superficial enamel loss (n = 33); and C (cavity), if all enamel was lost locally (n = 10). In the following, the word 'surface' is used to indicate the experimental unit, where the impedance of a tooth is measured at a particular site (approximal surface). It does not imply that only surface characteristics were measured.

After all measurements were made, the teeth were serially sectioned in the mesio-distal plane to give 120-μm-thick sections. The sections were viewed dry under a stereo-microscope, so that the presence and extent of caries could be determined. Surfaces which showed microscopic evidence of non-cariologic damage were removed from the study. Caries extent was scored histologically as: 0 (caries not present); 1 (caries present in enamel up to and including the amelo-dentinal junction); or 2 (caries present in dentin).

Experiment

A perspex chamber was constructed in which the teeth could be secured by locating the root tip in a layer of wax placed on the bottom of the chamber, the crown being secured with perspex screws. A layer of conducting gel (KY Jelly, Johnson & Johnson) was applied over the wax, in contact with the tooth root. A tooth was positioned in such a way that one of its approximal surfaces was facing a hole in the chamber, through which an electrode could be inserted to touch the surface (Fig. 1). This working electrode consisted of a stainless steel rod, 4 mm in diameter, covered at the tip by a carbonated fiber (patent pending, University of Dundee, 1995), which made direct contact with the tooth. The counter-electrode consisted of a platinum foil strip suspended from one side of the chamber and dipped in the conducting gel at the bottom. The other end emerged from beneath the lid of the chamber, to be gripped by an electric clip. The enclosed perspex chamber served to minimize evaporation, thus delaying drying of the tooth. All measurements were carried out at room temperature.

Measurements and analysis

The electrical measurements were performed with a Solartron 1255 Frequency Response Analyzer (Solartron Instruments Ltd., Hampshire, UK) under the control of an ESCOM 486DX40 microcomputer. Connection to the two-electrode cell was made...
Figure 2. Schematic representation of measuring set-up with amplifier. GE = generator, V = voltage, I = current.

Figure 3. Examples of complex impedance plots for surfaces from each of the three groups. Low-impedance section of graph is shown in detail in the upper right corner. Low frequencies on the right, high frequencies on the left. A = example from S; □ = example from L; ○ = example from C. Z' = real impedance; Z'' = imaginary impedance.

Prior to carrying out measurements on the teeth, we determined the electrical response of the experimental apparatus. First, the capacitance of the leads was measured and found to be less than 0.5 pF. A cell consisting of the counter-electrode and the fiber-tipped electrode immersed in the gel was constructed. The impedance of this cell increased with decreasing frequency but did not exceed 4 kΩ at 1 Hz. These measurements confirm that the set-up makes a negligible contribution to the measured tooth impedance, since the teeth were found to exhibit impedances which were usually considerably in excess of 65 kΩ.

The teeth were kept in closed vials with thymolized physiological saline before and between measurements. Immediately before a measurement, they were taken from the saline and blotted dry with tissue paper. They were then positioned in the chamber and the measuring electrode inserted. This procedure took less than 1 min. The complex impedance spectrum was then measured, from 1 MHz to 0.1 Hz, or from the frequency of the start of the complex impedance spectrum to the frequency where electrical noise impeded accurate measurements.

To determine reproducibility of the data obtained for each tooth, where variation would include the placement of the tooth and the contact of the measuring electrode, the same operator repeated placement and measurement six times for two surfaces (from L). Five surfaces (1S, 2L, 2C) were measured by a second operator using the same chamber in an identical measurement apparatus, and the proportional difference of the parameter values was calculated as (|operator 1 - operator 2|)/operator 1.

Results

Histologic validation showed that 5 surfaces, 3 from group S and 2 from group L, should be excluded because of cracks. None of the remaining 13 surfaces in group S showed microscopic signs of caries. In group L, enamel caries only was present in 16 surfaces, and dentin caries in 15 surfaces.
Localized loss of all enamel and presence of dentin caries were confirmed for all 10 surfaces in group C. The complex impedance plots (plots with the imaginary \(Z''\), conventionally plotted as \(-Z''\) and real \(Z'\)) components of the impedance plotted on the Y- and X-axis, respectively) of the tooth surfaces divided naturally into three groups according to the magnitude of the impedance, which corresponded almost perfectly with the main visual classification of the surface (S, L, or C). An example from each group is shown in Fig. 3. For all three groups, the equivalent circuit with the smallest number of components which best fit the experimental data is shown in Fig. 4. It consists of a series of two parallel elements: a resistor \((R_b)\) in parallel with a capacitor \((C_g)\), and a resistor \((R)\) in parallel with a constant-phase element \((CPE)\). The two parallel elements in the circuit give rise to two (overlapping) semi-circles in the complex plane which model the half-oval shape of the complex impedance plots. At very low frequencies, i.e., less than 100 Hz, the complex impedance plots showed the start of a third semi-circle which was considered to represent interface properties and was not analyzed fully.

The quality of the fit of the proposed equivalent circuit was evaluated for each surface by statistics including standard deviations of the parameter estimates, which were always less than 10% of the parameter values. Fit was also evaluated visually from graphs of the experimental data and fitted curves. For two of the study groups, examples are shown in Figs. 5 and 6 for the complex impedance plots (a) as well as for the spectroscopic plots of real (b) and imaginary (c) impedance as a function of frequency. Fig. 5 shows these results for an approximal surface with a brown-spot lesion, and Fig. 6 for an approximal surface with a cavity. The graphs show that the fit of the calculated data is very good.

The values for the components of the equivalent circuit as found by the fitting procedures are summarized in Table 1, showing mean, standard deviation, and coefficient of variation for each component. A CPE is characterized by two parameters: a capacitative factor \(C_{CPE}\) and the phase angle \(\alpha\). Parameter \(C_{CPE}\) varies markedly with \(\alpha\), and it would be inappropriate to give mean values for it. Instead, the range of results is given. DC resistance, \(R_{dc}\), which cannot be measured with dc current because of polarization, is calculated from the results by adding \(R_b\) and \(R\). Table 2 shows the results for the repeated measurements of the two teeth by the same operator, and the comparison of results from two operators.

Spearman rank correlation calculation showed that the experimental parameter with the highest correlation with caries status was \(R_{dc}\) \((r = 0.69)\). Mean \(R_{dc}\) for macroscopic groups S, L, and C was 68 M\(\Omega\), 5.9 M\(\Omega\), and 321 k\(\Omega\), respectively. Mean \(R_{dc}\) for histologic groups 0, 1, and 2 was 68 M\(\Omega\), 7.2 M\(\Omega\), and 2.8 M\(\Omega\), respectively. Fig. 7 shows the ranges of \(\log(R_{dc})\) for groups S, L, and C, and also for histologic groups 0, 1, and 2. Logarithmic transformation of \(R_{dc}\) served to let the parameter value distribution approach normal distribution. Mean values of \(R_{dc}\) of groups S, L, and C were significantly different from each other (log-transformed data, ANOVA, \(p < 0.001\); Tukey multiple comparisons, \(p < 0.001\)). For caries diagnosis at the level of the enamel (histology score 0 vs. 1 and 2 or group S vs. L and C, with a diagnostic cut-off of 30 M\(\Omega\)), sensitivity and specificity of the method were 1.00 and 1.00. For diagnosis of dentin caries (histology score 0 and 1 vs. 2, with a diagnostic cut-off of 4 M\(\Omega\)), sensitivity and specificity of the

![Figure 4. Proposed equivalent circuit. \(R_b = \) bulk resistance; \(C_g = \) geometric capacitance; \(CPE = \) constant-phase element; \(R = \) resistance.](image)

![Figure 5. Example of fit of values calculated from the equivalent circuit of Fig. 4 (drawn line) to experimental data (O) from a surface from group L: (a) complex impedance plot, (b) real \((Z')\), and (c) imaginary \((Z'')\) impedance as a function of frequency.](image)
Table 1. Parameter values obtained by curve fitting for the components of the equivalent circuit shown in Fig. 4

<table>
<thead>
<tr>
<th></th>
<th>( R_b / \Omega^a )</th>
<th>( C_g / \text{pF} )</th>
<th>( R / \Omega )</th>
<th>( C_{\text{CPE}} / \text{pF}^{-1} )</th>
<th>( \alpha )</th>
<th>( R_{\text{dc}} / \Omega )</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (n = 13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean(^b)</td>
<td>13</td>
<td>11.5</td>
<td>55</td>
<td>10 - 421</td>
<td>0.77</td>
<td>68</td>
</tr>
<tr>
<td>SD</td>
<td>6</td>
<td>4.2</td>
<td>29</td>
<td></td>
<td>0.08</td>
<td>29</td>
</tr>
<tr>
<td>CoV</td>
<td>45%</td>
<td>36%</td>
<td>52%</td>
<td></td>
<td>11%</td>
<td>43%</td>
</tr>
<tr>
<td>L (n = 31)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>1.2</td>
<td>20.3</td>
<td>4.9</td>
<td>48 - 9017</td>
<td>0.67</td>
<td>5.9</td>
</tr>
<tr>
<td>SD</td>
<td>1.2</td>
<td>13.6</td>
<td>4.6</td>
<td></td>
<td>0.09</td>
<td>4.8</td>
</tr>
<tr>
<td>CoV</td>
<td>96%</td>
<td>67%</td>
<td>98%</td>
<td></td>
<td>13%</td>
<td>80%</td>
</tr>
<tr>
<td>C (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>66</td>
<td>45.4</td>
<td>255</td>
<td>1732 - 46830</td>
<td>0.57</td>
<td>321</td>
</tr>
<tr>
<td>SD</td>
<td>65</td>
<td>45.3</td>
<td>150</td>
<td></td>
<td>0.06</td>
<td>195</td>
</tr>
<tr>
<td>CoV</td>
<td>98%</td>
<td>100%</td>
<td>59%</td>
<td></td>
<td>11%</td>
<td>61%</td>
</tr>
</tbody>
</table>

\( a \) Resistors \( R_b \) and \( R \), capacitor \( C_g \), and constant-phase element (CPE, two parameters \( C_{\text{CPE}} \) and \( \alpha \)). Total estimated dc resistance \( (R_{\text{dc}}) \) is calculated by adding \( R_b \) and \( R \).

\( b \) Mean, standard deviation (SD), and coefficient of variation (CoV) or range of results is given.

Discussion

Previous research on the ac impedance of tooth materials has been performed almost exclusively on sections of non-carious tooth material. This study aimed at relating the complex impedance spectra of whole teeth to the presence and extent of caries in the measured surface of the tooth as determined by histologic validation. Approximal surfaces were investigated because these smooth surfaces can be visually assessed in extracted teeth. Clinically, these surfaces are not readily accessible visually. Bite-wing radiography is the accepted standard method for caries diagnosis, but alternative methods are being investigated to reduce exposure to ionizing radiation and improve diagnostic performance (Pitts, 1991).

This study used complex impedance spectroscopy in a set-up which could, in its basic form, be reproduced clinically. The gel in which the tooth rested represents the part of the body between the tooth and the counter-electrode, which may be hand-held or lying against the oral mucosa. The total impedance of the counter-electrode and gel was shown to be negligible compared with that of the teeth, unless cavitation had taken place; this is also likely to be the case clinically. The carbonated fiber was introduced because it is a material which may prove useful for making contact with the teeth in in vivo measurements. The proximal surfaces of teeth in a clinical situation are in contact with neighboring teeth, and the area of interest lies close to the gingivae. The fiber material can be placed proximally as a thin strip, unlike the probe-type electrodes currently used, and will adapt to the curved surface better than a metal-coated plastic strip-wedge that has been previously used (Longbottom et al., 1993). It is also hydrophobic, which aids both in preventing the measured surface from drying and in preventing contamination of the surface with saliva, which would cause current leakage.

Table 2 shows the variation in the parameters for the same teeth removed and remounted between each

Table 2. Results of the reproducibility measurements

<table>
<thead>
<tr>
<th></th>
<th>( R_b )</th>
<th>( C_g )</th>
<th>( R )</th>
<th>( \alpha )</th>
<th>( R_{\text{dc}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>One operator(^a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>surface a</td>
<td>60%</td>
<td>92%</td>
<td>103%</td>
<td>9%</td>
<td>97%</td>
</tr>
<tr>
<td>surface b</td>
<td>18%</td>
<td>22%</td>
<td>15%</td>
<td>8%</td>
<td>16%</td>
</tr>
<tr>
<td>Two operators(^b)</td>
<td>50-89%</td>
<td>4-81%</td>
<td>22-74%</td>
<td>2-26%</td>
<td>27-77%</td>
</tr>
</tbody>
</table>

\( a \) One operator: coefficient of variation of repeated measurements by one operator on two tooth surfaces (number of repeats is 6).

\( b \) Two operators: range of proportional difference of results of operator 2 as compared with results of operator 1 (number of surfaces is 5).

\( c \) Components of equivalent circuit: resistors \( R_b \) and \( R \), capacitor \( C_g \) and phase angle \( \alpha \) of constant-phase element. Total estimated dc resistance \( (R_{\text{dc}}) \) is calculated by adding \( R_b \) and \( R \).
Figure 6. Example of fit of values calculated from the equivalent circuit of Fig. 4 (drawn line) to experimental data (●) from a surface from group C: (a) complex impedance plot, (b) real ($Z'$), and (c) imaginary ($Z''$) impedance as a function of frequency.

measurement. There are several sources of variation in the measured parameters for each experiment. First, the variation of the accuracy of the electrical measurements themselves may be estimated at less than 1% in the magnitude and phase angle, based on the specifications provided by Solartron Instruments. In general, fitting of the equivalent circuit described here to the data yielded an error of < 1% in all parameter values. The major source of the variation probably arises from the repositioning of the tooth between each experiment, which results in a change in the area of contact between the tooth and the carbonated fiber tip of the working electrode. The surface with the highest variation had a caries lesion which was relatively large and could therefore be contacted at different parts. The lesion of the second tooth was small, and repositioning the electrode contact was therefore easier. The level of variation of this second surface therefore reflects the reproducibility of the measuring technique more accurately. Slight variations in temperature may also have contributed to the overall variation of the parameters. The variations, however, are small compared with the differences in resistance, particularly $R_{dc}$, among the three categories. Duplicate measurements by a second operator using an identical experimental arrangement showed results in the same range. This indicates that the technique will be reproducible in its in vitro diagnostic capabilities. It can be concluded that the method of impedance spectroscopy holds promise for application in a clinical situation.

The shape of the complex impedance plots suggests suitable components for the equivalent circuit to be used. For instance, a pure resistor would yield a point on the x-axis, and a resistor and capacitor in series would yield a line parallel to the y-axis. The least number of components to model a semi-circular complex impedance plot would be a resistor and a capacitor in parallel. Most complex impedance plots in this study appeared to be stretched horizontally (see Fig. 5). This was modeled most simply by assuming 2 more or less overlapping semi-circles, that is, two such parallel elements in series. The presence of a CPE is related to the fact that teeth are not a homogeneous material. Any heterogeneous solid will exhibit a distribution in the electrical processes. These distributions are commonly represented by a CPE, i.e., an impedance with a power-law dependence on the frequency.

It is interesting to consider whether the values extracted for the electrical properties of the teeth correspond to those expected for the bulk properties of tooth material. Teeth are heterogeneous, consisting of two principal mineralized materials, dentin covered with a layer of enamel, together enclosing the soft pulp tissue. Theoretical calculations based on extreme values reported relatively recently for the intrinsic electrical parameters, i.e., dielectric constant and resistivity (Friedman and Grayson, 1970; Hoppenbrouwers et al., 1986), and approximate values for the dimensions of enamel and dentin showed that the expected dc resistance of sound teeth in our set-up would lie between 1 and 70 MΩ. The major part of this resistance, around 95%, is attributable to the enamel. Our experimental results for the sound group are in the expected order of magnitude.

Similar calculations, with hydroxyapatite as the main constituent of a tooth, yielded an estimated 1 to 5 pF for the

Figure 7. Graphic presentation of the calculated $R_{dc}$ (after logarithmic transformation), in relation to the macroscopic grouping (bottom) and in relation to the histologic score (top). Histology score: 0 = sound, 1 = enamel caries, 2 = dentin caries.
capacitance of a sound tooth. From the results in Table 1, it is obvious that, for the sound surfaces, the capacitance values $C_s$ which we have obtained relate to the bulk properties of the sound teeth. Replacing the hydroxyapatite with an increasing volume of air or fluid electrolyte, as would be the case in a caries lesion, should have only a small effect on the capacitance. This is exactly what can be observed in Table 1.

Identification of the specific electrical properties of different regions of the teeth with the ac response is outside the scope of this study. However, we may speculate that the much lower impedance of the teeth with caries lesions arises from the presence of many fluid-filled pores in the enamel which effectively 'short-circuit' the resistive hydroxyapatite material, resulting in conductive pathways to the dentin. The contribution of the dentin to the overall spectrum is negligible for the sound teeth but becomes increasingly important as the caries process progresses.

It is evident from Fig. 7 that the surfaces may be classified according to their macroscopic caries status by the dc resistance obtained from ac impedance spectroscopy. There was an order of magnitude difference between each of the groups, with values of the average $R_{dc}$ of sound surfaces equal to 68 ± 29 MΩ, of surfaces with spot lesions 5.9 ± 4.8 MΩ, and of macroscopic cavities 321 ± 195 kΩ. This explains the partial success of the fixed frequency measuring instruments, since they essentially try to measure $R_{dc}$. The histology score grouping was used to determine the sensitivity and specificity of the electrical impedance measurements when the calculated $R_{dc}$ was used as a diagnostic criterion. For early caries diagnosis including enamel caries, sensitivity and specificity are perfect. If only diagnosis of dentin caries is considered, they are 0.64 and 0.83, respectively. These values are higher for enamel caries and slightly lower for dentin caries diagnosis than previously reported for electrical methods (Rock and Kidd, 1988; Piiper et al., 1990; Verdonchot et al., 1992; Ricketts et al., 1995). Additional research should be aimed at verifying these results and improving the differentiation between enamel and dentin lesions.

Although we have shown that the dc resistance of the tooth as found by fitting the ac impedance data is a reliable measure of the level of caries decay, other parameters of the equivalent circuit may also prove valuable. However, this will require further work which must first be directed to explaining in more detail the equivalent circuit and its physical origin.

Acknowledgments

Dr. Huysmans acknowledges financial support from the Niels Stensen Foundation, Amsterdam, The Netherlands. Prof. Pitts acknowledges financial support from the Chief Scientist Office of the Scottish Office Home and Health Department. The views expressed above are those of the authors and not necessarily those of the Scottish Office. Prof. Bruce is indebted to the Royal Society for the award of a Pickering research fellowship and to the EPSRC for financial support. The assistance of B. Manson, BSc, is gratefully acknowledged.

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