Detection of dental decay and its extent using a.c. impedance spectroscopy

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Dental caries (decay), the most prevalent of diseases, represents a health problem of immense proportions1. It principally affects posterior (back) teeth on occlusal (biting) and approximal (adjacent contacting) surfaces. Caries starts as a subsurface demineralization of enamel, may progress to the underlying dentine and, eventually, to cavitation of the surface. Accurate diagnosis before cavitation would permit targeted preventive treatment, thereby significantly improving dental health and reducing the need for expensive drilling and filling. Inaccessibility of caries initiation sites and recent changes in lesion morphology contribute to the relatively poor accuracy of conventional diagnostic methods2. Among alternative techniques, measurements of electrical resistance3 have shown the most promise. Here we describe a new experimental technique that demonstrates an outstanding 100% correlation between a.c. impedance measurements of whole teeth and the actual extent of approximal caries in vitro. Only relatively minor modifications should be required to transfer the technique to in vivo applications.

Impedance spectroscopy, which involves the measurement of current in response to the application of a sinusoidally varying voltage over a wide frequency range4, has been used to study dental enamel5 and dentine6. The effects of a carious lesion on the electrical properties of enamel and dentine in whole teeth have not yet been studied systematically. The aims of this study were (1) to characterize the complex impedance behavior of whole extracted premolar teeth, measured at one or both of their approximal surfaces, by a method which could in its essential features be reproduced in a clinical situation, and (2) to identify parameters of the complex impedance behavior of the teeth that would be useful in distinguishing between degrees of carious involvement. In order to establish a method of measurement that could be reproduced in a clinical situation, it was necessary to develop a contact electrode from a material that could be used in a thickness of only a fraction of a millimeter, was flexible and hydrophobic, and could be impregnated at specific sites with electrically conducting material. Such a material was identified and a device designed for in vivo use (patent pending, University of Dundee, 1995).

The sample of teeth that were studied consisted of 26 unrestored extracted premolar teeth, with varying degrees of carious lesions in the approximal surfaces. The individual approximal surfaces were assigned to one of three groups on the basis of their direct visual appearance, sound (S) if no visible sign of caries was apparent; white or brown spot lesion (indicative of demineralization due to caries) with no detectable loss of surface enamel (L), and cavitated (C) if there was a carious lesion with an area where there was obvious loss of surface enamel. For each group (S, L and C) ten surfaces were measured. Subsequent to measurement, the teeth were hemisectioned and serially sectioned to validate the visual categorization of the teeth and to determine the true extent of any caries in enamel or dentine.

The a.c. Impedance measurements were carried out with the teeth placed in a custom-built Perspex chamber. Details of the chamber and the technique are given in the Methods section. Measurements of a.c. impedance were carried out over a wide range of frequencies, typically from 300 kHz to 1 Hz. Preliminary experiments were carried out to establish reproducibility of the technique. At least six measurements were carried out on each of these teeth. Reproducibility was excellent, representing variations of less than a factor of two, which was minimal compared with the variation of 100 in the resistances between the different categories of tooth surfaces. The results of the measurements of a representative tooth from each of the three categories S, L and C are presented in Fig. 1.

The electrical response of any material can be represented by an equivalent circuit. The equivalent circuit described in the Methods section and derived for use in this experiment was fitted to our data using a nonlinear least-squares procedure. In Fig. 1 the solid lines represent the best fit obtained, and the dots represent the data. The scale of the differences in the electrical responses of the teeth in each of the three histological categories is readily apparent.

The mean values (with standard deviations) for the total resistances in MΩ (the sum of the two resistances in the equivalent circuit) are presented in Table 1. The standard deviations are relatively small, indicating a high degree of reproducibility of the measurements. It is thus possible to use the total resistance as a diagnostic parameter. It is readily apparent from Fig. 1 that the normal resistances associated with whole teeth (S) are much lower than the resistances associated with teeth that have lost surface enamel (L). The resistances associated with teeth that have cavitated (C) are the lowest of all, clearly indicating that the presence of a cavity increases the electrical resistance of the tooth.

**Fig. 1** Examples of the complex plane plots of a representative tooth from each of the three groups: S, shown in blue; L, shown in black; and C, shown in red. The plot for the C group tooth is enlarged in the inset to facilitate visualization. The graphs plot the values for $Z'$, the imaginary impedance, against $Z''$, the real impedance, at the various frequencies applied to each tooth.
The graphs represent the magnitude of the impulse on the angle of the paper. In the context of the experiment, the impulse is defined as the change in momentum of the paper. The graphs show the impulse as a function of the angle of the paper. The impulse is calculated by integrating the force over time.

The positions of the paper are given at the origin of the coordinate system. The impulse is measured at the angles specified in the graph. The impulse is plotted against the angle of the paper. The impulse is shown to increase with the angle of the paper.

The graphs also show the impulse as a function of the time. The impulse is calculated by integrating the force over time. The impulse is measured at the specified times. The impulse is plotted against the time. The impulse is shown to increase with the time.

The graphs are useful for understanding the relationship between the impulse and the angle of the paper. The graphs can be used to predict the impulse at other angles and times.

**Figure 1:** The graphs represent the magnitude of the impulse on the angle of the paper. The impulse is defined as the change in momentum of the paper. The graphs show the impulse as a function of the angle of the paper. The graphs also show the impulse as a function of the time. The impulse is calculated by integrating the force over time. The impulse is measured at the specified angles and times. The impulse is plotted against the angle of the paper and against the time. The impulse is shown to increase with the angle of the paper and the time.
subtended with the x-axis represents the phase angle θ.

The electrical response of any material can be represented by an equivalent electrical circuit consisting only of resistors and capacitors. In this particular case the equivalent circuit consists of four components: two resistors and two capacitors. The component representing the bulk resistance is connected in parallel with the capacitor representing the bulk capacitance. The second resistor is connected in parallel with a constant phase element (the impedance of which is given by \( A\omega^n - jB\omega^n \)) where \( A, B \) and \( n \) are constants and \( \omega = 2\pi f \), where \( f \) is the frequency of the sinusoidally varying voltage.

The complex impedance data were analyzed using the program Z plot (Solartron Instruments). A series of initial experiments were carried out in order to establish the contribution of the electrodes, gel, contacts and leads. These were found to be negligible compared with the impedance of the teeth.

The approximal aspects of the teeth that were measured were photographed before hemisection of the teeth in the mesio-distal plane. The hemisections were examined under \( \times 2.5 \) and \( \times 10 \) magnification in a stereomicroscope, using reflected light, to assess the presence and extent of caries. Photographs were taken of each hemisection and the teeth subsequently serially sectioned to produce 120-μm-thick sections, which were then viewed microscopically and scored for caries (Sound, Lesion and Cavitated). Presection and hemisection photographs of one example from each of the S, L and C group teeth are shown in Fig. 2.

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Retinoic acid normalizes the increased gene transcription rate of TGF-α and EGFR in head and neck cancer cell lines

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Retinoic acid (RA) has been shown to be effective in eradicating premalignant lesions1 and preventing second primary malignancies in patients cured of squamous cell carcinoma of the head and neck (SCCHN) in clinical trials2. The basis for this effect is unclear. We have previously demonstrated that messenger RNA from tumor growth factor-α (TGF-α) and its receptor, the epidermal growth factor (EGFR), is upregulated in tumors and histologically normal mucosal samples from patients with SCCHN compared with control normal mucosa from patients without cancer, implicating this ligand-receptor pair in an autocrine growth pathway early in the molecular pathogenesis of this disease3. In this report, we examined the hypothesis that the action of RA on the mucosa of the upper aerodigestive tract is mediated via downregulation of steady-state TGF-α and/or EGFR mRNA levels. Following exposure to all-trans-RA, a series of SCCHN cell lines demonstrated a 35.4% reduction in TGF-α mRNA expression (P = 0.022) and 58.5% reduction in EGFR mRNA (P = 0.0027). Nuclear run-on analysis indicated that the RA-mediated reduction of TGF-α and EGFR steady-state mRNA levels was a result of decreased gene transcription. These results suggest that the clinical effects of RA in SCCHN patients may be due to a downmodulation of TGF-α and EGFR mRNA production.

The pathogenesis of SCCHN is incompletely understood. Although alcohol and tobacco exposure are predisposing factors, the vast majority of individuals who smoke and drink do not get head and neck cancer. It is likely that affected individuals have accumulated a series of genetic events in the mucosa of the upper aerodigestive tract resulting in malignant transformation. Genetic abnormalities that may contribute to transformation in this tumor system include alterations in specific oncogenes (for example, myc)4, and tumor suppressor genes (such as p53)5, up-regulation of growth factors and their receptors (for example, TGF-α and EGFR)6, cytogenetic changes (involving numerous chromosomes including 3p, 11q, 15p, 17p)7,8 and modifications...