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Age-related changes in ac-impedance spectroscopy studies of normal human dentine

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Abstract Non-destructive methods, such as the ac-impedance technique, have recently been applied to early caries detection and to identify micro-leakage between tooth structure and filling materials. However, in vitro impedance measurements are affected by a number of external factors. The purpose of present study was to investigate the effect of the age of teeth on impedance measurements of human dentine by employing electrical impedance spectroscopy (EIS). Fully hydrated dentine samples were prepared from extracted third molars of 20 and 50 year old patients. Ac-impedance measurements were carried out over a wide frequency range. Impedance measurements showed that there were differences in impedance between young and older dentine. In their circuit models, both resistance and capacitance were found to be significantly different ($p < 0.05$) for the two age groups. One of the age-related changes in dentine is the formation of peritubular dentine on the inner walls of dentinal tubules and we propose that this is responsible for the differences in impedance. Sample or patient age therefore must be considered when making impedance measurements on any tooth.

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Introduction

Dentine compromises the bulk of most human teeth and may be classified as intertubular or peritubular. Intertubular dentine consists of a fibrous network of collagen with deposited mineral crystals. Peritubular dentine is a more highly mineralized tissue with fewer collagen fibers than intertubular dentine. In effect, it forms a highly mineralized sheath lining each dentinal tubule. Dentinal tubules contain odontoblast processes and extend from the pulp to the tooth's outer edge, terminating at the dentino-enamel or dentino-cemental junctions. They assume a 'lazy' sinusoidal pattern and taper from pulp outwards. Their number and diameter are commonly taken to be about 45,000 mm^{-2} and 2.5 μm at the pulpal aspect of dentine to 20,000 mm^{-2} and 0.9 μm at the dentino-enamel junction (DEJ) [1].

The true number and diameter of dentinal tubules is, however, determined by deposited peritubular dentine, which can over time entirely occlude a tubule [2]. Carrigan et al. [3], using only maxillary central incisors, showed that the number of tubules significantly decreased with increasing age from 242,775 for ages 20–34 to 149,025 for ages 80 and above. Tagami et al. [4] found that the permeability of coronal dentine samples prepared from teeth of old patients was much lower (80% reduction) than that prepared from young teeth.

Contrary to this finding, Garberoglio and Brännström [1] found age had an insignificant effect on the number of tubules in teeth collected from 8 to 60 year old patients. Furthermore, Mendis and Darling [5, 6] believed that age had little effect on the closure of tubules by formation of peritubular dentine. Rather, they believed that the formation of peritubular dentine was accelerated by attrition.

Electrical impedance is a measure of resistance to current flow through a given material. For an alternating current (ac) circuit, impedance is affected by the inductance and capacitance in a circuit, which build up voltages that act in opposition to the flow of current. This opposition is called reactance, and must be combined with resistance to determine the total impedance of the electrical circuit. If a measurement of impedance over a suitable frequency range is made, it is possible to relate results to physical and chemical properties of the material under test [7].

In ac-impedance spectroscopy, the impedance of a material under test, which is represented by a parallel combination of resistor capacitor (RC) equivalent circuit containing an inductor, could be expressed mathematically by the equation:

$$Z(\omega) = R_1 - \frac{\mathrm{i}l}{C\omega} \qquad (1)$$

where Z is the impedance, R_1 is the resistance of the sample under test, l is the inductance, C is the capacitance and ω is the angular frequency of the ac signal. According to Eq. (1) the impedance of the sample can be split into real and imaginary parts given by the equation:

$$Z(\omega) = |Z|\mathrm{e}^{\mathrm{i}\theta} = Z_{\mathrm{real}} + \mathrm{i}Z_{\mathrm{imaginary}} = Z' + Z'' \qquad (2)$$

As can be seen from Eq. (2), impedance of a sample can be represented graphically on a complex plane with real abscissa (Z') and imaginary ordinate (Z''). The results of the complex plane can also be represented using a Bode plane with logarithm of frequency as abscissa and either logarithm of $|Z|$ or theta (phase angle, θ) as ordinate.

The impedance of a resistor and a capacitor that are electrically connected in parallel gives rise to a semicircle on a complex plane according to Eq. (1). Translating the semicircle from a complex plane to a Bode plane gives rise to a horizontal line (corresponding to a resistor) and an inclined line having a slope of –1 (for the capacitor).

Equation (1) is strictly only valid for homogenous materials which are acting as pure capacitors. For non-homogeneous materials, such as dentine, a constant-phase element (CPE) is used in the circuit to compensate for this heterogeneity [8].

The value of the CPE has two parts: $\mathrm{CPE_T}$ and $\mathrm{CPE_P}$. If the impedance of a normal capacitor is given by the expression:

$$Z_C = \frac{1}{\mathrm{i}\omega' C} \qquad (3)$$

the impedance of a CPE is given by the relationship

$$Z_{\mathrm{CPE}} = \frac{1}{\mathrm{CPE_T}(\mathrm{i}\omega')^{\mathrm{CPE_P}}} \qquad (4)$$

where $\mathrm{CPE_P}$ has a value between –1 and 1, and $\mathrm{CPE_T}$ is the effective capacitance only if $\mathrm{CPE_P}$ is equal to 1 [8]. Different constant-phase-elements can only be compared if their $\mathrm{CPE_P}$ values are similar.

Ac-impedance spectroscopy has been used to detect cracks and caries in enamel and dentine. Indeed, the performance of impedance-based instruments has been shown, in several in vitro and in vivo studies to be better than existing methods of caries diagnosis [9–14].

Impedance techniques have been used to investigate the effect of smear layer [15] and dentine conditioners [16] on dentine impedance, also to identify microleakage between tooth and filling materials in vitro [17]. However, in vitro impedance measurements were affected by a number of external factors, such as size of the electrode area, repositioning of the electrode contact and change of temperature [18–20] and changing concentrations of saline in storage solutions [21]. Lussi et al. [22] showed a 23% false-positive resistance reading rate which may lead to unnecessary restoration of sound teeth. They postulated that this may be due to tooth cracks and irregularities, uneven distribution of mineralization, post eruptive mineralization or variable age of tooth. There have been no electrical impedance spectroscopy (EIS) investigations reported on the effect of dentine age on impedance. Such studies would provide better understanding of the structure of dentine and could form the basis of meaningful clinical impedance measurements in vitro and in vivo. Therefore, the aim of the present study is to investigate effects of age on impedance of dentine using EIS.

Materials and methods

Tooth collection and dentine samples preparation

Freshly extracted un-erupted human third molars were used for this investigation. Un-erupted third molars were used to avoid the effect of attrition due to age. Immediately after extraction soft tissue debris and bone fragments were removed and the teeth stored in hermetically sealed vials containing physiological saline with a few Thymol crystals. Written patient consent was obtained prior to extraction. Two age groups were selected in this investigation; 20 (±1) and 50 (±1) years old and 10 dentine samples were prepared, five samples for each age group.

The preparation of dentine samples was standard throughout the project. Details of the sample preparation and the sample holder have been given previously [23]. The samples were prepared from the extracted molars using a computerized cutting machine equipped with a diamond wheel under water spray. Each sample was 5[±0.1] mm wide, 7[±0.1] mm long and 2[±0.1] mm thick. For successful measurement, it was found to be essential that the dentine samples were fully hydrated. This displaced any air from the dentinal tubules, which would have had high impedance, and therefore better modelled the in vivo situation.

In order to minimize the interfacial impedance between the sample and electrical leads used for ac-impedance measurement, the upper and lower surfaces of each dentine sample were painted with quick-dry silver paint (Agar scientific Ltd, Essex, U.K.) that produced a dry metallic film in less than 10 s. The sample was subsequently inserted into a special holder filled with physiological saline solution.

Sample holder

The sample holder was fabricated from transparent Perspex. This was designed to: (1) standardize measurements, (2) protect wet samples from drying and (3) provide good visibility of the sample throughout the measurements. The geometrical configuration of the two electrodes was selected such that it could be easily adopted for fabricating a commercial prototype and the results compared with laboratory data.

Impedance measurements

Electrical measurements were carried out at 20°C using a computer controlled SI 1260 Impedance Gain-Phase Analyzer (Solartron Analytical, Hampshire, UK) over a frequency range 0.01 Hz to 10 MHz. The applied amplitude of the ac potential was 100 mV rms under open circuit conditions. In order to minimize stray capacitance, coaxial leads were used to connect the sample to the impedance measuring device and these leads were kept as short as practically possible (<15 cm). The SI 1260 frequency response analyzer employs Z-plot software (Scribner Inc, USA) to control and run the desired experiment and Z-view software (Scribner Inc, USA) that includes a complex non-linear, least-square fitting, program to model and analyse the measured impedance data. The fitted data can thus be modelled such that it represents the real material under test by selecting an appropriate equivalent circuit and numerical values can be assigned to each component of that circuit.

For each sample, a 5 min delay was introduced prior to measurement to allow the sample to equilibrate in the holder. Minitab 12.1 (Minitab Inc, USA) was used to perform appropriate paired *t*-tests at a confidence level of 95%.

To examine the short and long term reproducibility of the technique, including the fit to the proposed equivalent circuit, five repetitions of impedance measurements were made on a sample from each age group on one day. In addition, a measurement was made for a sample from each group on each of five different days.

Prior to conducting impedance measurements on dentine samples in physiological saline (0.9% w/v), the impedance of a control electrical circuit consisting of a resistor (10 kΩ) connected in parallel with a capacitor (5 μF) was measured between 100 μHz and 32 MHz and electrodes in saline without sample (blank electrolyte cell) were also measured. These measurements were carried out to confirm that the impedance analyzer was functioning accurately and the sample holder had negligible effect on measurements.

Scanning electron microscopy examination (SEM)

After performing all impedance measurements, dentine samples were fractured and examined under high resolution field emission gun SEM-LEO 1530 (FEG-SEM) in order to correlate impedance measurements with structure of dentine samples.

Results and discussion

Previous studies on electrical properties of teeth have ignored the age of the samples, apart from Levinkind et al. [24] who looked at enamel only. Our paper is the first study to investigate the effect of tooth age on the impedance of dentine.

Electrochemical impedance spectroscopy has been shown to be a powerful tool for investigating electrical properties of dentine, but requires exacting standards of sample preparation and experimental set-up to get meaningful results.

Having confirmed the validity of the experimental set-up with a standard circuit, measurements were made on the blank electrolyte cell filled with saline only. It was found that there was only nominal impedance (0.3–0.6 Ω) over the whole frequency range. Values for impedance were thus assumed to arise from sample only.

Plotting complex and Bode plane graphs for young and older dentine revealed similar appearances for both age groups. An example of these measurements is shown in Fig. 1(a, b, c). Fig. 1a, and the magnified view shown in Fig. 1b, for young and older dentine clearly show that there are two semicircles in the complex plane due to the presence of (1) a smear layer over the dentine surface and (2) the dentine itself. The presence of two semicircles can also be seen clearly in the Bode plane, represented by twin peaks, as shown in Fig. 1c. The first semicircle appears between 0.1 Hz and 100 Hz and the second appears between 5 kHz and 1 MHz.

Both the smear layer and dentine will have their own resistance (R) and capacitance (C). The proposed equivalent electrical circuit that accurately represents the bi-layer composite used in this investigation is a series combination of the two parallel combinations of resistor (R) and a capacitor in the form of a CPE, one each for smear layer and dentine.

All the dentine samples consist of a smear layer covering underlying dentine (seen in Fig. 2) and this is analogous to a bi-layer composite material. The

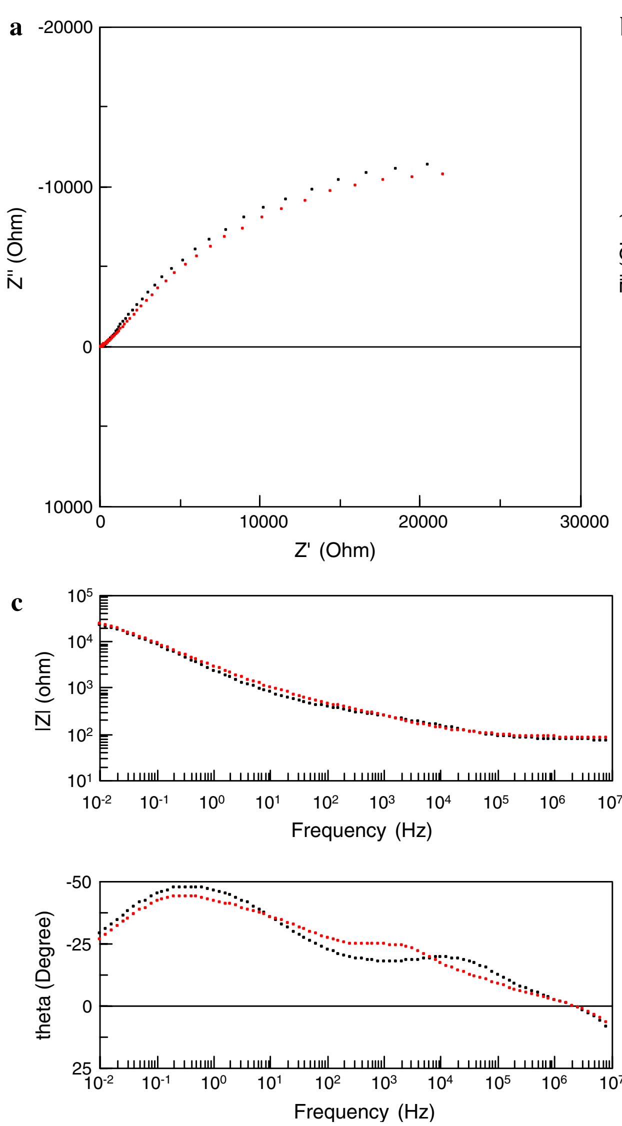

Fig. 1(a, b, c) Impedance measurements of 20 years old dentine (black dots) and 50 years old dentine (red dots)

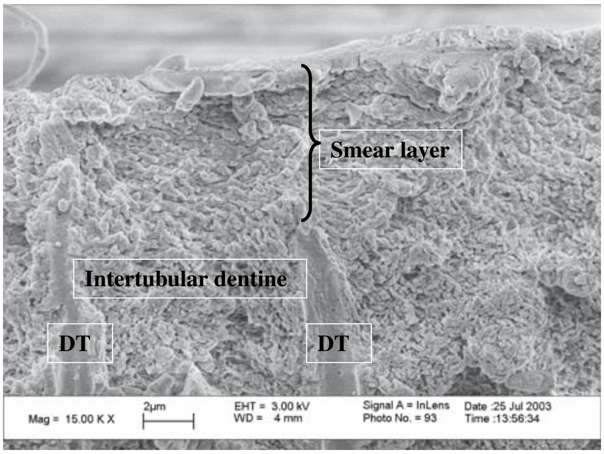

Fig. 2 Scanning electron micrograph of dentine sample showing surface smear-layer overlying dentinal tubules (DT) and inter-tubular dentine (Freeze-fracture specimen)

proposed equivalent circuit used for modelling the measured impedance of young and older dentine is shown in Fig. 3.

A typical example of proposed equivalent circuit model fitting to the measured data of dentine is shown in Fig. 4(a, b, c). It can be seen that the calculated data from the proposed model of the equivalent circuit model of the equivalent circuit fits well with the measured data (fitting error < 10%).

The proposed equivalent circuit comprises a resistor (R-ss) corresponding to the resistance of saline in the sample holder and a resistor (*R*) and CPE in parallel for each of the two semicircles. We propose that the appearance of two semicircles shows that the sample consisted of two materials having significantly different electrical properties; namely hydrated dentine (d) and smear layer (s). Given that the smear layer consists of the same constituent components as dentine [25], it is perhaps surprising that the values for both R-s and CPE_T-s are very different to those of R-d and CPE_T-d for both age groups. These differences can be explained in terms of the relative thicknesses of the smear layer and dentine. The thickness of the smear layer is generally considered to be 2–5 μm [26] compared to our sample thickness of 2 mm.

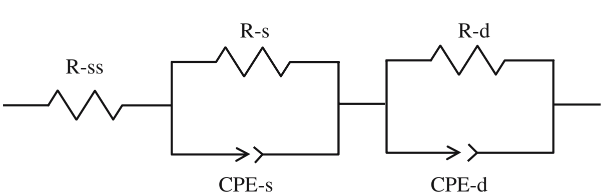

Fig. 3 Proposed equivalent circuit for dentine samples (where: R-ss is resistance of saline solution, R-s is resistance of smear layer, CPE-s is constant-phase element of smear layer, R-d is resistance of dentine and CPE-d is constant-phase element of dentine)

The assignment of the second semicircle in the complex plane at frequency range 5 kHz to 1 MHz to the smear layer has been previously reported [27]. In this, the smear layer was removed prior to measurement, resulting in disappearance of the second semicircle.

We propose that both young and older dentine can be represented by one equivalent circuit in which the values of the components are different. The CPE element for dentine can not be considered as a pure capacitor since the phase shift represented by CPE_P is not a full 90^{o} as it would be for a true capacitor (i.e. in our system $CPE_P = 0.5$, rather than 1). *T*-tests showed that values for CPE_P whether for young or old dentine or smear layer were statistically similar ($p > 0.05$ at 95% confidence interval) which is an important finding because if the values for CPE_P were different, a comparison of CPE_T values would not be valid.

Various models of equivalent circuits have been proposed by investigators to characterize the electrical properties of teeth. The most relevant to this study are those by Kumasaki [28] and Levinkind et al. [15]. Kumasaki [28] proposed an equivalent circuit for dentine comprising a single resistor and capacitor in parallel. As mentioned earlier, dentine is a non-homogenous composite structure, and cannot therefore be modelled by a simple capacitor. Levinkind et al. [15] found only one semicircle in the complex plane which resulted in an equivalent circuit model consisting of three resistors in series with a CPE element in parallel. However, their study was carried out over a much smaller frequency range (1 Hz to 65 kHz) and so the authors may have missed this semicircle due to the smear layer. In addition, their samples were considerably thinner (300 μm) than those used in the present study and the dentine samples were taken from only two teeth from one patient. We propose that our model more accurately represents the impedance properties of dentine.

The mean values and standard deviations (SD) of different electrical components generated by the equivalent circuit models of five samples for each age group are shown in Table 1.

The reproducibility of impedance measurements on dentine samples and their subsequent fitting to the model was assessed. Table 2a, b shows mean, SD and coefficient of variation (CoV) values of repeated measurements either on the same day or over 5 days for young (Table 2a) and older (Table 2b) dentine.

Comparing measurements for the different age groups (Table 1), both R-d and CPE_T-d are significantly different ($p < 0.05$). Having been very careful to fully validate the method [27], we believe that these

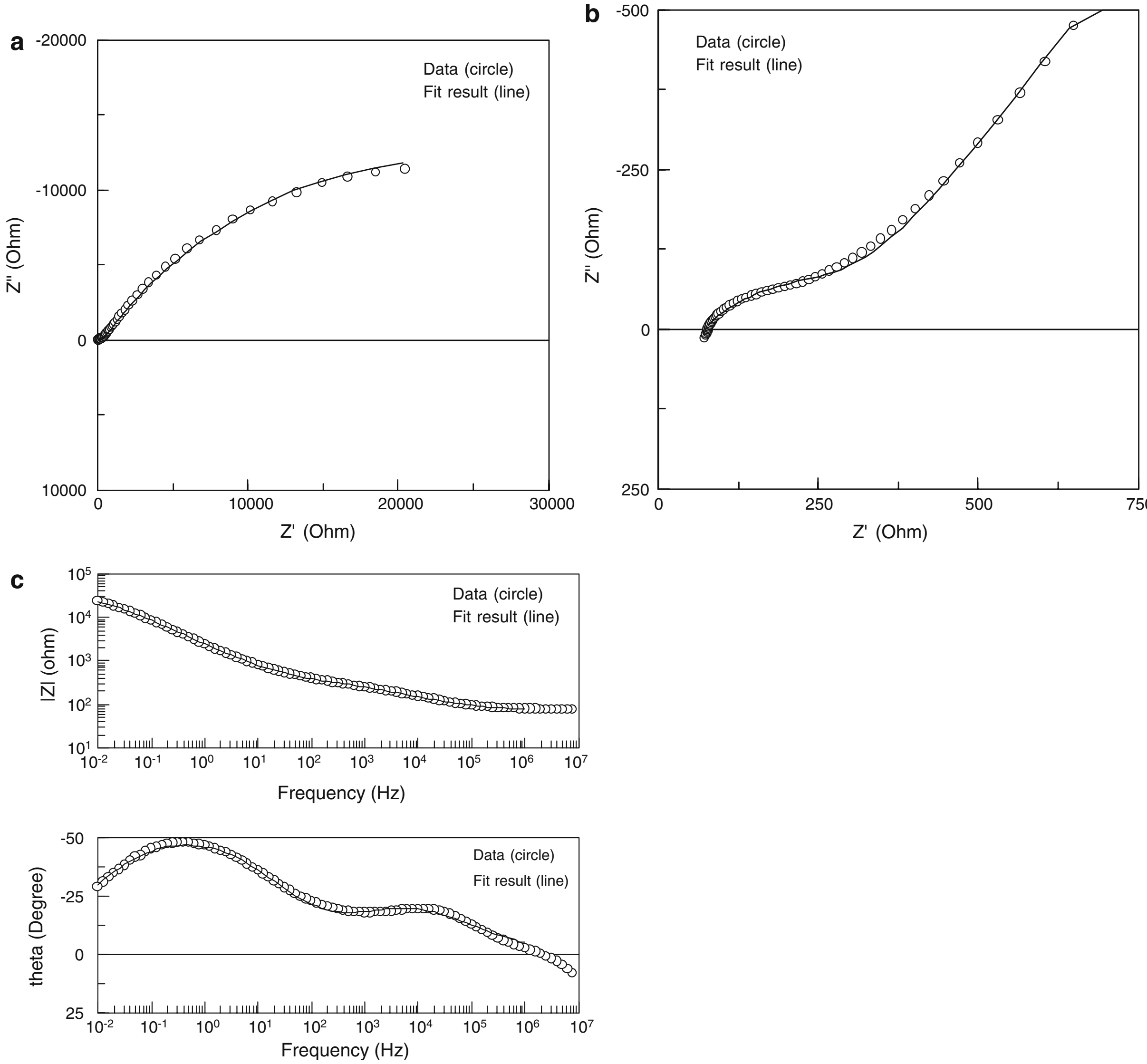

Fig. 4(a, b, c) Impedance measurements for young dentine ((○) measured data and (-) equivalent circuit model fitting)

differences are real and suggest that the charge storing ability of dentine in younger teeth is lower than that for dentine in older teeth.

Table 1 Parameter values (mean ± SD) obtained by curve fitting for the components of the equivalent circuit for each age group

Component	Elements	Young dentine	Old dentine
Saline solution	R-ss (Ω)	71.5 ± 0.6	72.1 ± 0.2
Smear layer	CPE_T-s (μF)	23.8 ± 7.8	14.6 ± 9.2
	CPE_P-s	0.5 ± 0.0	0.5 ± 0.0
	R-s (Ω)	244.0 ± 2.1	128.1 ± 5.5
Dentine	CPE_T-d (μF)	45.6 ± 4.8*	182.8 ± 14.7*
	CPE_P-d	0.5 ± 0.0	0.5 ± 0.0
	R-d (kΩ)	43.1 ± 3.2*	60.9 ± 7.3*

* Shows significant difference ($p < 0.05$ at 95% confidence level) in dentine

These differences can be explained as a consequence of continuous deposition of peritubular dentine on the inner walls of dentinal tubules, which is known to reduce the diameter of dentinal tubules, eventually leading to complete occlusion of the tubules with advancing age. In effect, young dentine can be thought of as a composite of intertubular dentine, peritubular dentine and saline in the dentinal tubules. Whilst old dentine has the same components, there is more peritubular dentine in occluded tubules and consequently less saline. In effect a good conductor of electricity has been replaced by a good insulating component, resulting in increased overall resistance and capacitance-like elements of dentine with increasing age.

The reproducibility (Table 2a, b) for both age groups was similar and was generally better for

Table 2 Results of reproducibility measurements, (five measurements on the same day and one measurement on each of 5 different days) for (a) 20 years old dentine (value of R is in Ω and CPE is in F) (b) 50 years old dentine (value of R is in Ω and CPE is in F)

Elements	Saline	Smear layer			Dentine		
	R-ss	CPE_T-s	CPE_P-s	R-s	CPE_T-d	CPE_P-d	R-d
Panel (a)							
Same day							
Mean	71.3	1.30E – 05	0.5	244.8	4.60E – 05	0.5	43,506
SD	0.5	1.00E – 06	0	3.1	8.00E – 06	0	928
CoV	0.70%	8%	0%	1%	9%	0%	2%
Different days							
Mean	71.3	1.50E – 05	0.5	242.2	3.90E – 05	0.5	45,306
SD	0.6	9.00E – 06	0	2.1	5.00E – 06	0	968
CoV	0.80%	60%	0%	0.80%	13%	0%	2%
Panel (b)							
Same day							
Mean	72.1	5.30E – 06	0.5	135.5	1.66E – 04	0.5	56,925
SD	0.5	2.40E – 06	0	1.9	2.90E – 05	0	1,926
CoV	0.60%	45%	0%	3%	17%	0%	3%
Different days							
Mean	71.5	5.90E – 06	0.5	132.9	1.77E – 04	0.5	58,525
SD	0.6	2.00E – 06	0	1.3	2.40E – 05	0	899
CoV	0.80%	34%	0%	0.90%	17%	0%	2%

resistance than for CPE_T and was better for dentine than for smear layer. However, the coefficients of variation suggested a very high level of reproducibility, both in taking repeat measurements on one day or on different days thus confirming the robust nature of this impedance-based technique.

Conclusion

Impedance measurements showed that there were significant differences in impedance between young and older dentine. Therefore, sample or patient age must be considered when making impedance measurements on any tooth.

Although we have shown that impedance spectroscopy technique can be used to detect structural changes in human dentine due to age for 20 and 50 years old groups, work is now in progress to investigate dentine of intermediate ages to further investigate these changes.

This finding may also have potential implications in designing commercial instrumentation for forensic or anthropological applications, such as age-assessment of dentine samples of isolated teeth or even partial tooth fragments.

Clearly there will need to be further work to characterize variations across the whole spectrum of ages, before results can be applied to studies in vivo on caries or failure of dental restorations due to microleakage.

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