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

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Abstract One of the age-related changes occurring in dentine structure is the formation of peritubular dentine on the inner walls of dentinal tubules leading to complete closure of tubules. Ac-impedance is safe, fast and non-invasive technique. In the last decade, the popularity of the technique has increased in dental research. Several investigators have used the technique to detect tooth cracks and caries. The results of in vitro studies showed that ac-impedance technique was more advanced for caries detection than visual and radiographic methods. However, other studies demonstrated that the accuracy of impedance measurements can be affected by many factors such as remineralization after tooth eruption. A study has been published on effect of age on impedance measurements by the authors for two age groups by employing ac-impedance spectroscopy. Therefore, the aim of this study was to demonstrate the importance of this technique by conducting further investigations on dentine samples of wider age groups. Dentine samples were prepared from extracted sound third molars of known patient age. The ac-impedance measurements were carried out over a wide range of frequency. After performing all electrical measurements, dentine samples were examined under SEM to correlate the electrical measurements with their structure. Impedance measurements showed that there were differences in impedance between young and old dentine. One-way ANOVA of the means of resistance and capacitance for all age groups (20, 25, 30, 40 and 50 years old dentine) revealed a significant difference (ANOVA, $P < 0.0001$) as a function of age. Applying Tukey's post hoc test, to the same data showed that this difference was due to the 50 years old dentine for resistance and was due to the 40 and 50 years old dentine for capacitance which were statistically different to all other groups. SEM investigation of dentine samples showed that young dentine is characterized by open dentinal tubules distributed all over the sample while in old dentine most dentinal tubules were occluded by peritubular dentine. It is believed that this peritubular deposition is responsible for differences in impedance measurements. In spite of increasing use of electrical techniques to understand electrical properties of teeth, it is clear from this study that local structural variations have a marked influence.

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1 Introduction

Dentine structure is considered as a complex hydrated composite [1], consisting of mineralized intertubular dentine, highly mineralized peritubular dentine and microtubules filled with dentinal fluid and odontoblastic process. Dentine constitutes the bulk of a human tooth and has unique ability to undergo structural changes. These changes are brought on by chronological events and environmental factors. Physico-chemical changes of dentine due to aging is characterised by continuous deposition of peritubular dentine on the inner walls of dentinal tubules and the process can progress until total obliteration of the tubule space. The effect of age on the number of dentinal tubules has been investigated by Carrigan et al. [2] who showed that the number of dentinal tubules significantly decreased with increasing age. Obliteration of several tubules in a portion of a tooth reduces its permeability and gives dentine a glass-like appearance which is known as dentine sclerosis. Dentine sclerosis is often seen in older teeth under cusp tips and at the apical third of roots. Tagami et al. [3] found that the permeability of coronal dentine samples prepared from the teeth of old patients was much lower than that prepared from young teeth. Age-related changes also involve the organic part of dentine and increases structural changes of the collagen fibrils. Ager et al. [4] in their study on the increasing fragility of human teeth with age using UV Resonance Raman technique showed that the height of the resonance-enhanced amide I feature increases with increasing age of dentin and bone, which suggests that similar changes in the collagen fibers may be occurring in both mineralized tissues as a result of aging.

Electrochemical impedance spectroscopy (EIS) technique is used to monitor the electrical properties of human teeth in terms of their ability to transfer and store charge, which are directly related to their conductance (G) and capacitance (C), respectively. The use of electrochemical impedance spectroscopy (EIS) records the response of the teeth to a small-applied perturbation of an ac-signal, over a certain frequency range.

The EIS technique is non-destructive and sensitive to any changes in the structure of sample under investigation. It is worthwhile to mention that the 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 [5–10]. Moreover, it has been used to investigate the effect of smear layer [11] and dentine conditioners [12] on dentine, and to identify microleakage between tooth structure and filling materials in vitro [13].

Electrical tests on such biomaterials (human teeth) to investigate their ability to transfer (conductance) and store (capacitance) charges can provide valuable information in terms of the teeth's physical and chemical properties including: impedance, porosity, phase changes, compositional variations and permittivity, all of which can be related to the health of the teeth.

There has been hardly any electrical impedance spectroscopy investigations reported to understand the effect of dentine age on impedance. A study has been published on effect of age on impedance measurements by the authors for two age groups by employing ac-impedance spectroscopy and also to investigate age-related changes of dentine structure using scanning electron microscopy [14]. Therefore, the aim of this study was to conduct further investigations on other age groups. 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.

2 Materials and methods

2.1 Teeth collection and dentine samples preparation

Freshly extracted un-erupted human third molars were used in 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 were stored in hermetically sealed vials containing physiological saline with a few Thymol crystals. Written patient consent was obtained prior to extraction. Six age groups were selected in this investigation; 20, 25, 30, 40, 50 and 80 years old. Five dentine samples were prepared for each age group except for 80 years old due to the difficulty of obtaining un-erupted molars for older age group.

The samples were prepared from the extracted molars using a computerized cutting machine equipped with a diamond wheel cooled by water spray. Each sample was 5[±0.1] mm wide, 7[±0.1] mm long and 2[±0.1] mm thick. The preparation of dentine samples was standard throughout the study. Details of the sample preparation have been given previously [15]. 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, UK) that produce a dry metallic film in less than 10 s. The sample was subsequently inserted into a specially designed in-house holder filled with physiological saline solution.

2.2 Sample holder

The sample holder was fabricated from transparent Perspex. This was designed to standardize measurements, keep constant sample-electrode contact, protect wet samples from drying and provide good visibility of the sample throughout the measurements. The geometrical configuration of the two electrodes has been selected such that it could be easily adopted for fabricating a commercial prototype and the results compared with laboratory data. Design details of the sample holder have been described earlier [15].

2.3 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–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 analyser 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 using SI 1260 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.

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.

2.4 Scanning electron microscopy examination (SEM)

After performing all electrical measurements, dentine samples were examined under Environmental SEM-Philips FEI XL30 (ESEM) in order to correlate impedance measurements with structure of dentine samples.

2.5 Statistical analysis

Minitab 12.1 (Minitab Inc, USA) was used to perform *t*-test, ANOVA and Tukey's post hoc tests at a confidence level of 95% to ascertain if any differences between the age groups were statistically significant. Coefficient of variation (CoV) percentage was calculated from the equation published by Bowers (1996):

$$\mathrm{CoV} = \frac{s}{\tilde{x}}(x100\%)$$

where CoV is the coefficient of variation, s is the standard deviation and $\tilde{x}$ is the mean value.

3 Results

The proposed equivalent electrical circuit consists of a lumped resistor corresponding to the resistance of saline and electrodes in the sample holder, resistor corresponding to the resistance of smear layer or dentine substance and constant phase element corresponding to the capacitance of smear layer or dentine substance in parallel for each of the two semicircles arising due to different relaxation processes in the sample having different time constants. Model of the proposed equivalent electrical circuit and a typical example for equivalent circuit fit have been given previously [14].

Impedance measurements were carried out on dentine samples of 20, 25, 30, 40, 50 and 80 years old in physiological saline 0.9% w/v. The mean values of electrical resistance and capacitance generated by fitting the raw data to the equivalent circuit model for each age group are shown in Figs. 1 and 2 respectively.

Statistical analysis of mean values for all age groups by using ANOVA and Tukey's post hoc tests, suggests that

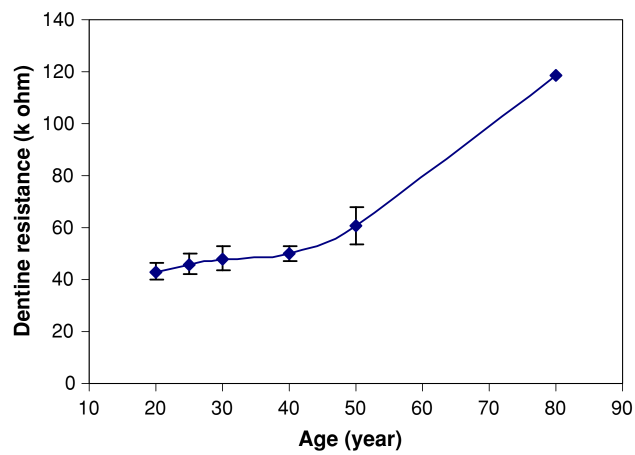

Fig. 1 Resistance of dentine for all age groups [$n = 5$ for all age groups except for 80 years old ($n = 1$)]

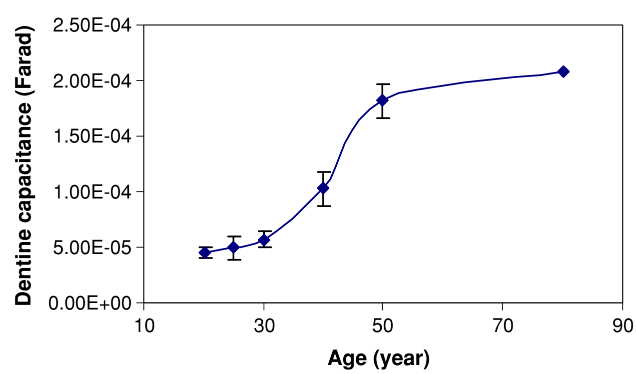

Fig. 2 Capacitance of dentine for all age groups [$n = 5$ for all age groups except for 80 years old ($n = 1$)]

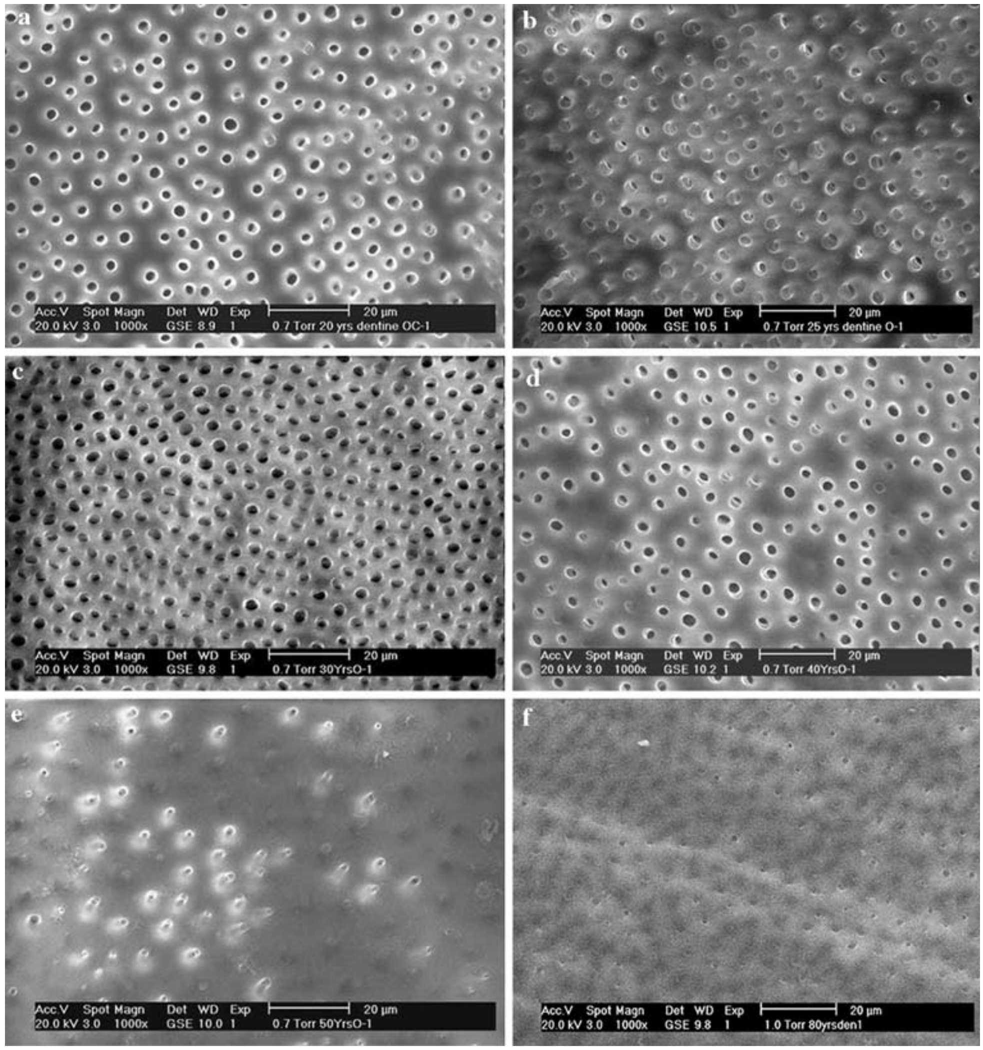

Fig. 3 **a** Scanning electron micrograph (SE) of 20 years old dentine. **b** Scanning electron micrograph (SE) of 25 years old dentine. **c** Scanning electron micrograph (SE) of 30 years old dentine. **d** Scanning electron micrograph (SE) of 40 years old dentine. **e** Scanning electron micrograph (SE) of 50 years old dentine. **f** Scanning electron micrograph (SE) of 80 years old dentine

the age groups were found differing in resistance and capacitance. Mean values of dentine resistance of age groups 20, 25, 30, 40 and 50 years old dentine samples were 43.1, 46.0, 48.2, 49.9 and 60.9 kΩ, respectively as calculated from equivalent circuit model fitted to measured impedance. One-way ANOVA revealed a significant difference (ANOVA, $P < 0.0001$) as a function of age. Applying Tukey's post hoc test, to the same data showed that this significance was due to the 50 years old dentine which was statistically different from all other groups.

Mean values of dentine capacitance of age groups 20, 25, 30, 40 and 50 years old dentine samples were 45.6, 49.2, 56.4, 101.9 and 182.8 µF, respectively. One-way ANOVA revealed a significant difference (ANOVA, $P < 0.0001$) as a function of age. Applying Tukey's post hoc test, to the same data showed that this significance was due to the 40 and 50 years old dentine which was statistically different compared to the 20, 25 and 30 years old dentine. The 40 years old dentine was also different to the 50 years old dentine.

Scanning electron micrograph (SEM) of 20, 25, 30, 40, 50 and 80 years old are shown in Fig. 3a–f.

4 Discussion

Impedance measurements and fitting the appropriate equivalent circuit model to the measured impedance of the control electrical circuit indicated that the control circuit had a resistance of 9.97 kΩ and a capacitance of 5.01 µF. These values are in good agreement with the values (10 kΩ and 5 µF) stated for the control electrical circuit. Having confirmed the experimental set-up with a control circuit, measurements were made on the 'blank' electrolyte cell. It was found that there was only nominal impedance (0.4 Ω over the whole frequency range) due to the 'blank' electrolyte cell (two electrodes immersed in saline solution only). Therefore, values of impedance are believed to arise from samples only.

Table 1 Resistivity and resistance of enamel and dentine reported in the literature

References	Enamel	Dentine	Sample thickness	Electrolyte
[16]	4.5 ± 1.5 MΩ cm	33 ± 13 kΩ cm	NA	Physiological saline
[17]	19.4 MΩ cm	2.35 MΩ cm	1.5 mm thick	Artificial saliva
[18]	21 MΩ cm–680 kΩ cm	<1 kΩ cm	NA	HEPES*
[19]	100 MΩ	8 kΩ	100 μm thick	Physiological saline
[20]	NA	1.6–1.9 kΩ	300 μm thick	KCl

* 2-(4-(2-hydroxyethyl)-piperazinyl-(1)-ethane sulfonic acid (HEPES) containing Hibitane and rubidium chloride

Under the same conditions, impedance measurements were also performed on fully hydrated dentine samples of 20, 25, 30, 40, 50 and 80 years old. We demonstrated in our earlier investigation [14] the presence of two semicircles in complex plane and a two peak pattern in theta angle graph for all age groups. However, a difference in measured impedance between the samples can be seen in both complex and Bode planes.

The appearance of two semicircles suggests that the dentine sample consisted of two materials having significantly different electrical properties; namely hydrated dentine and smear layer. The assignment of the second semicircle in the complex plane at high frequency range (1 kHz–1 MHz) to the smear layer was confirmed in the earlier experiments [11] where impedance measurements had been performed on dentine samples before and after removal of smear layer by etching the sample surfaces with phosphoric acid. These measurements were carried out to confirm that the second semicircle appearing at high frequency between 1 kHz and 1 MHz in complex plane and theta angle graph of Bode plane is indeed due to the presence of smear layer on the surfaces of dentine samples. There is also a noticeable change of dentine impedance after etching [11].

After confirming the validity of the proposed equivalent circuit for impedance measurements of young and old dentine [14], the equivalent circuit was fitted to the measured impedance of other age groups. Dentine resistance and capacitance values are the mean values of five dentine samples of each age group except for the 80 year old dentine sample which represents a value of only one sample, due to the difficulty of obtaining samples of unerupted sound teeth of advanced age. It can be clearly seen (Figs. 2 and 3) that the dentine resistance and capacitance increases steadily with increasing age. However, the sensitivity of the technique to age-related changes of dentine is measurable above 40 years old with resistance and above 30 years with capacitance. This suggests that dentine capacitance may be a more practical parameter to measure than dentine resistance for detecting age-related changes. However, an appropriately used combined resistance and capacitance parameter could widen the scope of measurements.

Different resistivity and resistance values reported in previous studies of enamel and dentine are presented in Table 1. The differences are due to variations in the technique of measuring the parameters. Therefore, it was difficult to compare these data with the data obtained in the present study.

Having been careful to fully validate the method, it is believed that these differences are real and suggest that the charge storing ability of dentine in younger teeth is lower than that for dentine in older teeth. This can be explained on the basis of the evidence from SEM images shown in Fig. 3a–f arising due to the 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 closure of 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 much more peritubular dentine in occluded tubules and consequently less saline. In effect a good conductor of electricity has been replaced by a good insulating material, resulting in an increase in the overall resistance and the overall capacitance-like element of the dentine in the older group as reflected in Figs. 1 and 2, respectively.

SEM investigations of dentine samples for all age groups were carried out after impedance measurements to correlate between the electrical measurements of dentine samples and their structural change due to age. Micrographs of 20 and 25 year old dentine shown in Fig. 3a and b clearly indicate that dentinal tubules are uniformly distributed throughout the samples and they are clear, patent tubules without significant peritubular dentine deposition.

However, it is also clear from the SEM images of 20 and 25 year old samples that the tubules do not go down straight from occlusal to pulpal surfaces. The tubules are oriented at different angles while going from occlusal to pulpal surface of dentine. In fact, the inclined arrangement of dentine tubules could be due to branching of central tubules.

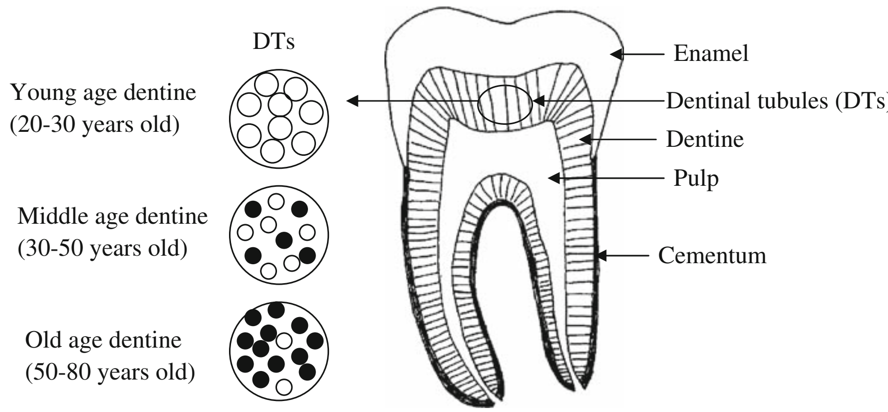

Fig. 4 A schematic diagram of human tooth shows occlusion process of dentinal tubules by peritubular dentine

It is interesting to notice in SEM micrographs of 30 year old dentine (Fig. 3c) that peritubular dentine deposition starts to appear on the inner walls of some dentinal tubules, while in SEM micrographs of 40 year old dentine show complete occlusion of few dentinal tubules (Fig. 3d). However, 50 year old dentine (Fig. 3e) indicates that most of the dentinal tubules have been either partially or fully closed. In 80 year old dentine, almost all the dentinal tubules are fully occluded (Fig. 3f). The occlusion process of dentinal tubules by peritubular dentine is presented in tooth diagram (Fig. 4).

SEM investigation of young and old dentine samples agrees well with the results for impedance measurements. SEM micrographs showed good evidence that most of dentinal tubules in old dentine samples are either partially or fully occluded with peritubular dentine. Peritubular dentine is accepted to be highly mineralized dentine. The deposition of peritubular dentine on the inner wall of tubules results in an increase of the inorganic component of old dentine and hence an increase in electrical resistance and capacitance.

5 Conclusions

Impedance technique is a non-invasive technique which can be used safely for in vivo studies. There are a number of companies interested in producing 'surgery-based' equipment for both caries diagnosis, microleakage and root length analysis that rely on the electrical properties of not only dentine, but also the covering enamel and even tooth filling materials whenever present.

The current study showed that intrinsic structural changes in young and old human dentine can be successfully investigated by employing electrical impedance spectroscopy over a wide range of frequencies, typically between 0.01 Hz to 10 MHz.

This study is limited to a small number of samples. However, these findings are important because they bring into question the reliability and accuracy of some earlier clinical tests and reports.

The results of this investigation and some other investigations using impedance spectroscopy of dentine leads to the belief that the technique has the potential for detecting age-related changes of dentine, dental decay, integrity of dental restorations and even assessment of the integrity of dento-alveolar implants.

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