Age-related changes in cyclic voltammetry and potentiodynamic studies of normal human dentine

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Impedance spectroscopy is one of the non-destructive techniques used by researchers to measure electrical resistance of biological tissues and ceramics. The purpose of this study is to investigate the voltage–current (*V–I*) characteristics of sound human dentine from young and old teeth, using cyclic voltammetry and potentiodynamic techniques. Dentine samples were prepared from freshly extracted sound third molars. After electrical measurements, dentine samples were characterized using scanning electron microscopy (SEM). Cyclic voltammetric measurements showed that variation of current through sample as a function of applied voltage is linear for dry samples of both age groups. However, for wet samples *V–I* characteristic were found to be different. The resistivity of dry young dentine is greater than that of old dentine in dry environment, whereas, it was found to be opposite for wet dentine samples. Using the same voltage sweep in potentiodynamic measurements dry samples display similar traces to controls suggesting that the dry dentine acts as an insulator. The number of dentinal tubules and their diameter has been found to decrease with increasing age. We propose that these changes determine the changes in electrical characteristics of sound human dentine.

In spite of increasing use of electrical techniques to understand electrical properties of teeth, it is clear from this study that local structural variations and environment have a marked influence. Therefore, this baseline data needs to be considered in any future study or clinical application.

1. Introduction

Direct current (d.c.) potential sweep methods such as cyclic voltammetry (CV) and potentiodynamic (POT) investigations have been used for characterizing liquids and solids. The principle is based on the scanning of potential at a selected rate and monitoring current between the electrodes produced as a result of changing potential.

Linear sweep voltammetry (LSV) is the simplest technique that uses a linear waveform. The potential range is scanned in one direction, starting at the initial potential *E1* and ending at the final potential *E2*, at sweep rate *R*. A more useful and commonly used technique is cyclic voltammetry in which the direction of the potential is reversed at the end of the first scan rather than terminated. This has the advantage that the product of any electron transfer reaction occurring in the forward scan can be investigated again in the reverse scan. In addition to this it is a powerful tool for the determination of formal redox potentials, detection of chemical reactions that follow the electrochemical reaction and evaluation of electron transfer kinetics [1].

Non-destructive methods like the alternating current (a.c.) impedance technique has been recently applied to detect cracks and caries in enamel and dentine [2–5] and also to identify microleakage between tooth structure and filling materials [6]. An *in vitro* study showed a good correlation between the measured impedance and the depth of tooth caries [5]. Furthermore, Verdonshot *et al* [7] found that the electrical method was superior to radiography and visual inspection for the detection of caries. It seems that a.c. impedance technique may be used as a clinical diagnostic tool for early detection of dental caries in future. However, to the best of our knowledge there have been no CV or POT investigations reported on sound human dentine either in dry or wet conditions. Such studies would provide baseline information for future investigations. Dentine forms the bulk of the hard tissue of a tooth and acts as a protective layer for the pulp. In order to allow development of useful

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clinical tests and appropriate diagnostic tools, it is important to conduct systematic d.c. and a.c. electrical measurements in dry and wet conditions on sound human dentine samples. As a large part of such an investigation, the aim of the present study is to investigate the response of pure human dentine samples *in vitro* to the applied d.c. potential and to determine the limit of potential when the current shows a linear behavior as a function of applied potential

2. Materials and methods

Twelve sound third molars were collected and stored immediately in hermetically sealed plastic vials containing thymolised physiological saline in order to prevent any bacterial growth during storage period.

2.1. Sample preparation

Dentine samples were cut from the teeth using a computerized water-cooled cutting machine equipped with a diamond wheel (Struers Ltd., Glasgow, UK). Following a radiographic assessment, the upper surface of the disc was cut just under the dentino-enamel junction and lower surface was sectioned just above the pulp horns as shown in Fig. 1(a). A rectangular dentine sample (Fig. 1(c)) having smooth flat surfaces and parallel sides was then prepared from the dentine disc (Fig. 1(b)) using a water-cooled cylindrical diamond bur. The dimensions of dentine samples were measured using a micrometer screwgauge at three different locations to determine their mean thickness, width and length. Each dentine sample was 2 mm thick, 5 mm wide and 7 mm long ($\pm$ 0.1 mm). Following this, the dentine samples were examined under a stereomicroscope to confirm absence of cracks or surface irregularities before conducting the electrical measurements. Samples for dry measurements were stored at 20 °C in a glass dessicator containing silica gel crystals. Samples used for wet measurement were kept in physiological saline solution. Tooth samples of two age groups were selected in this investigation; 20 ($\pm$ 1) years and 50 ($\pm$ 1) years old. Six tooth samples of each age group were used for the electrical measurements in dry and wet conditions. The experiments were repeated under the same conditions using another set of samples.

Prior to the electrical measurements, the occlusal and the pulpal surfaces of dentine samples were painted with quick dry silver paint in order to minimize the interfacial resistance between sample and connecting lead. The length of measuring leads between sample holder and the instrument was approximately 6 inches. This is mainly done to minimize the distortion of the signal from the sample during the measurements.

2.2. Sample preparation for scanning electron microscopy

Dentine samples, one from each group, were polished on wet P 1200 silicon carbide paper to remove grinding marks and washed with double distilled water (ddH_2O). Samples were etched with 35% phosphoric acid for 15 s to remove smear layers and again washed with ddH_2O. The pulpal surface was marked for each sample in order to distinguish the occlusal surface from the pulpal surface during the microscopic examination of dentine samples. The samples were dehydrated through graded alcohol containing 50% v/v, 70% v/v and 90% v/v ethyl alcohol for 30 min each followed by two changes in absolute ethyl alcohol for 30 min each in order to avoid any tissue shrinkage due to the direct exposure to absolute alcohol. After drying the samples in graded alcohol, the samples were further desiccated under vacuum (approximately 10^{-3} atm.) overnight at 20 °C. Each sample was mounted on carbon disc and then securely placed on an aluminum SEM stub. The entire dentine sample was sputter coated with gold.

2.3. Sample holder

A special sample holder, as shown in Fig. 2, for electrical measurements of dentine samples was fabricated from transparent perspex. This was designed to: (1) standardize measurements, (2) protect wet samples from drying during measurements and (3) provide good visibility of the sample throughout the measurements. The sample

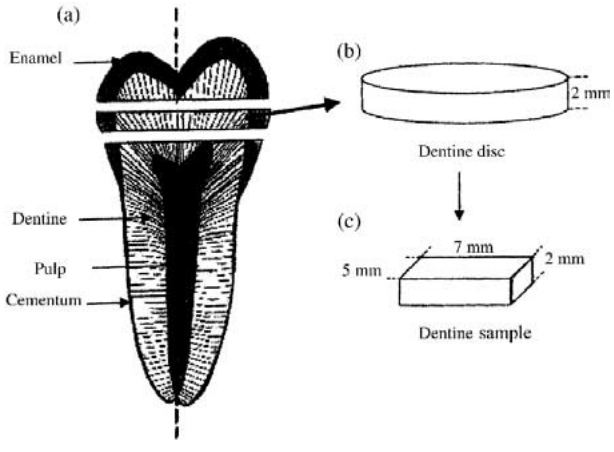

Figure 1 Schematic diagram of steps involved in dentine sample preparation from molar tooth, (a) molar tooth, (b) dentine disc and (c) dentine sample.

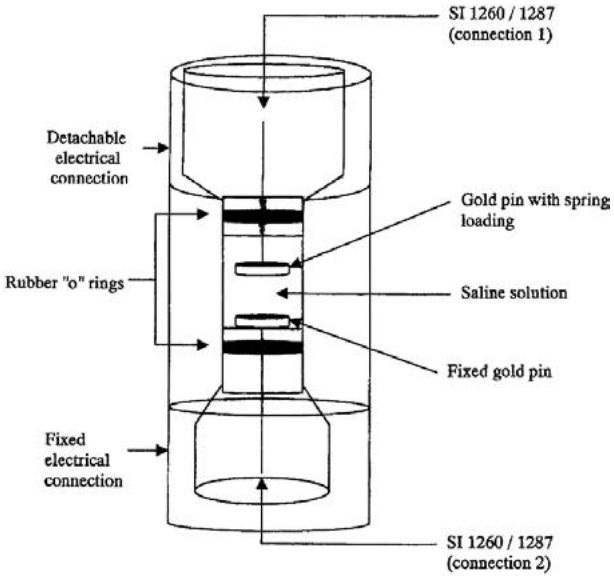

Figure 2 Schematic diagram of sample holder.

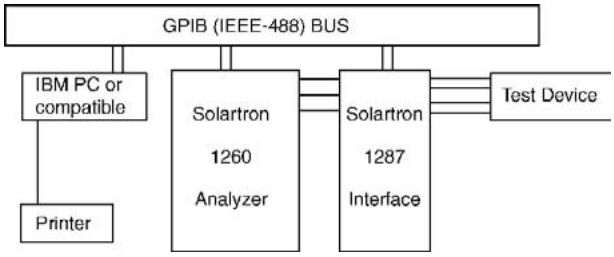

Figure 3 Schematic representation of the electrical connection between SI 1287, SI 1260, sample under test and PC.

holder consists of upper and lower parts housing two electrical contacts in the form of two gold pins and two rubber ''O'' rings. The upper part is detachable to allow sample insertion and it is attached to a silver tipped gold pin with concealed spring approximately 1 mm in diameter. The spring was used to avoid excessive pressure on the sample surfaces or the gold pins during clamping. The rubber rings were used to make an airtight seal and to prevent solution leakage from the sample holder during measurement.

2.4. Measurements

The electrical measurements were carried out using a computer controled SI 1287 electrochemical interface (Solartron Analytical, Hampshire, UK). The SI 1287 employs Corrware software (Scribner Inc., USA) to plot and analyze the data. Fig. 3 shows a schematic diagram of the electrical connections between SI 1287, SI 1260 Analyzer, the sample under test and a personal computer (PC) through the IEEE-488 GPIB bus.

In order to test a control circuit, the response of a pure resistor and a parallel combination of a resistor and a capacitor was tested. A CV and POT measurements using a 10 kΩ resistor and a 5 μF capacitor connected in parallel with a 10 kΩ resistor were performed. The CV was performed by sweeping the voltage from -1 to $+1$ V at the rate of 10 mV s^{-1} and recording the current through the circuit shown in Fig. 4.

Scanning electron microscopy of dentine samples was carried out using a JEOL JSM 35 scanning electron microscope equipped with Deben, UK, ''Genie'' computer control.

3. Results and discussion

The results of CV studies of the control circuit shown in Fig. 4 indicated that the response of the control circuit is

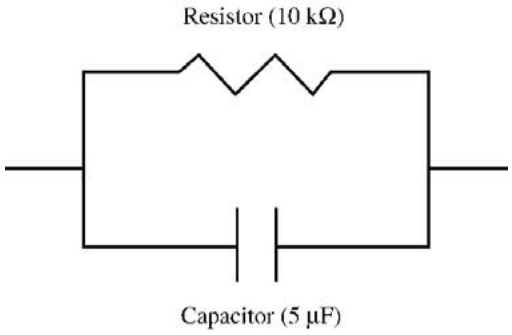

Figure 4 Schematic of resistor and a capacitor in parallel.

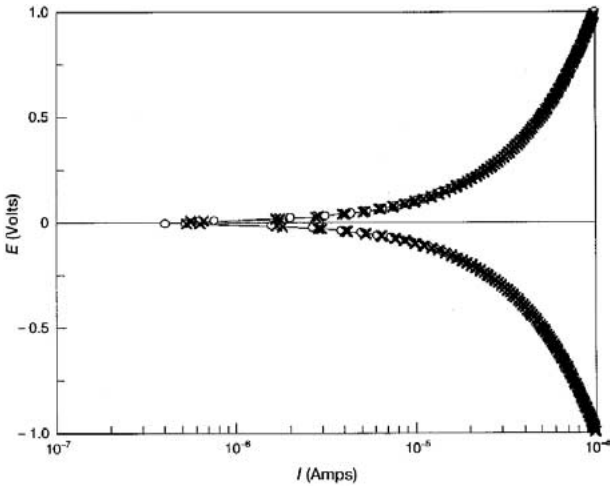

Figure 5 Potentiodynamic measurement of a resistor (×) and a resistor and a capacitor in parallel (○).

linear as a function of applied voltage. This suggests that the circuit obeys Ohm's law, $V = IR$ where V is the applied voltage, I is the recorded current and R is the resistance. Identical measurements carried on a 10 kΩ resistor alone also gave similar results.

POT measurements were also carried out on a 10 kΩ resistor as well as resistor and capacitor connected in parallel, shown in Fig. 4. The results of POT measurements on control circuit are shown in Fig. 5. These results are similar to an ideal V–I response of a nonpolarizable electrochemical cell. The results of CV and POT measurements of control circuit indicate that the equipment is connected correctly.

CV results of young and old dry human dentine samples are shown in Fig. 6. The voltage was swept between -0.5 to $+0.5$ V at rate of 10 mV s^{-1}, with respect to the open circuit potential. The range of voltage was selected such that the applied potential between the two electrodes of the cell is lower than the standard reduction potential of Ag^+, which is 0.8 V [8]. It can be seen from Fig. 6 that the variation of the current through the sample as a function of applied voltage is almost linear for the dry human dentine samples of both age groups. However, the difference between the V–I characteristics with increasing and decreasing voltage

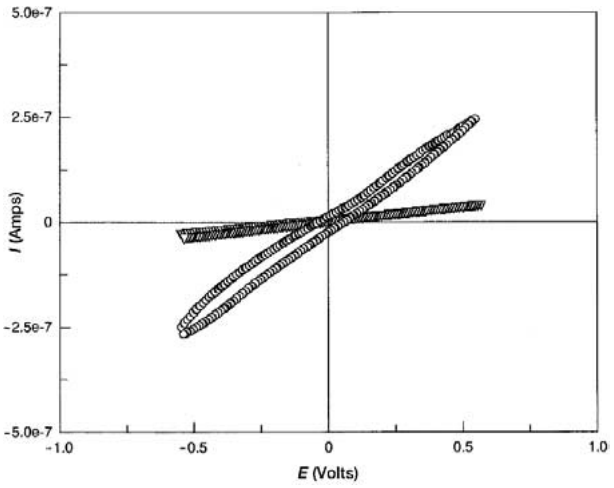

Figure 6 Cyclic voltammogram of dry dentine samples ((△) young dentine and (○) old dentine).

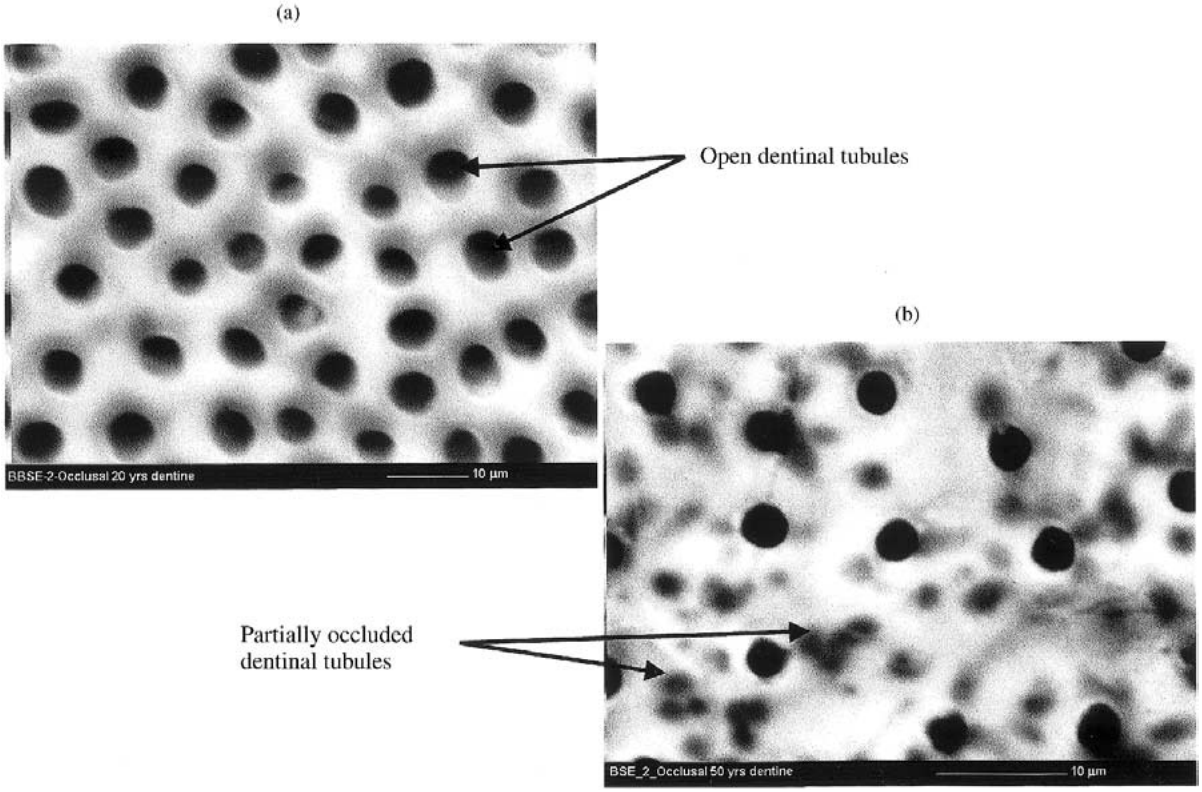

Figure 7 Scanning electron micrograph (BSE) of (a) young dentine sample (× 3464) and (b) old dentine sample (× 4192).

between -0.5 to $+0.5$ V is greater for an older dentine compared to the younger dentine. It is well known [9 and 10] that as the age of dentine increases, the dentine tubules get occluded with peritubular dentine (secondary dentine minerals) resulting into partial or complete blocking of dentine tubules. This does not seem to occur uniformly across the bulk of dentine as seen in Fig. 7(b) relative to Fig. 7(a). Therefore, we believe that the variation in the *V–I* characteristics of old dentine may be due to the greater variability of the relatively complex structure of old dentine as a result of ''secondary'' mineralization within dentinal tubules with increasing age. The variable nature of dentine tubules is clearly evident in the scanning electron micrographs of young and old dentine samples shown in Figs. 7(a) and (b), respectively.

The mean slope of the *V–I* characteristics of young dentine is significantly lower than the mean slope of old dentine as seen in Fig. 6. The resistance of dentine samples under investigation can be easily calculated using Ohm's law. From the knowledge of the sample dimensions mentioned earlier, the resistivity of young and old dentine under dry condition has been calculated. The resistivity of young dry dentine has been found to be higher $26.25\,\mathrm{M\Omega\,cm} \pm 6.4\,\mathrm{k\Omega\,cm}$ compared to $3.85\,\mathrm{M\Omega\,cm} \pm 9.6\,\mathrm{k\Omega\,cm}$ of old dentine (see Table I). Similar results were obtained for *V–I* characteristics of young and old dentine samples under dry condition by employing the POT technique.

TABLE I Summary of the cyclic voltammetry measurements of ''dry'' dentine

Sample	Young dentine	Old dentine
Forward cycle	-0.549–0.570 V	-0.547–0.549 V
Reverse cycle	-0.537–0.570 V	-0.538–0.549 V
Resistivity	$26.25\,\mathrm{M\Omega\,cm} \pm 6.4\,\mathrm{k\Omega\,cm}$	$3.85\,\mathrm{M\Omega\,cm} \pm 9.6\,\mathrm{k\Omega cm}$

SEM images of young and old dentine are shown in Fig. 7(a) and (b) respectively. It can be clearly seen in Fig. 7(a) that the mean diameter of the open dentinal tubules of young dentine is 3.3 µm ($\pm$ 0.2) whereas that of old dentine is from 1.5 to 3.1 µm ($\pm$ 0.2). The mean number of open tubules on the occlusal surface of young dentine was found to be 27 361 ($\pm$ 410) tubules mm^{-2} compared to 8379 ($\pm$ 232) ''fully open'' tubules mm^{-2}, with an estimation of 17 037 ($\pm$ 262) partially occluded tubules for old dentine. Several investigators have reported large variation in tubules diameter and surface density. Garberoglio and Bränström [11] examined human coronal dentine from tooth pulp upwards to the occlusal surface. They reported variations in tubule diameter and number at various distance from pulp. The diameter and number of tubules near the pulp was 2.5 µm and 45 000 tubules mm^{-2}, in the middle dentine was 1.2 µm and 29 500 tubules mm^{-2} and near the enamel was 0.9 µm and 20 000 tubules mm^{-2}, respectively. Also Fosse *et al*. [12] found 18 000 ($\pm$ 4000) tubules mm^{-2} at occlusal surface of young dentine compared to 54 000 ($\pm$ 1500) tubules mm^{-2} at the pulpal surface. This suggested that the number of tubules at occlusal surface of our samples is in reasonable agreement with the results of Garberoglio and Bränström [11] and Fosse *et al*. [12]. Further, although Carrigan *et al*. [13] reported much higher number of dentinal tubules (324 900 tubules mm^{-2}) for young dentine, they found that the number of tubules in coronal dentine decreased with increasing age which is in agreement with our findings.

CV and POT studies of young and old dentine samples have also been conducted in 0.9% w/v NaCl (physiological saline) solution whose chloride concentration is

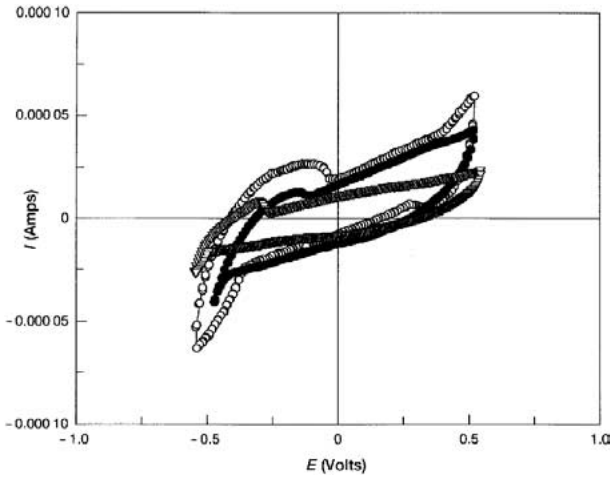

Figure 8 Cyclic voltammogram of wet dentine samples [(○) young dentine, (●) old dentine and (△) saline solution without sample].

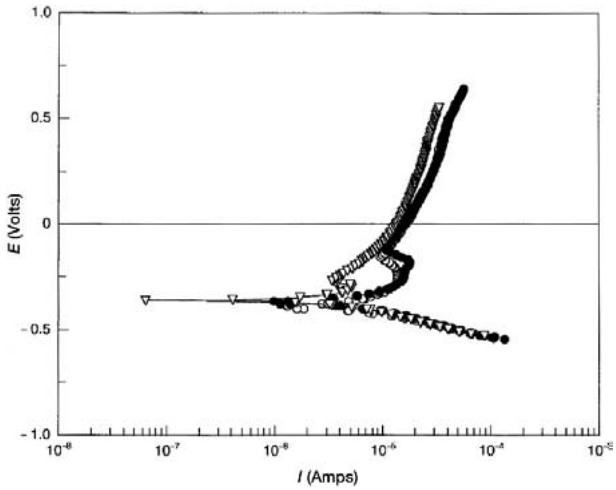

Figure 9 Potentiodynamic measurement of wet dentine samples [(○) young dentine, (●) old dentine and (△) saline solution without sample].

similar to that of body fluids [14]. The results of cyclic voltammetric studies of the two different dentine samples in physiological saline and saline alone are shown in Fig. 8. It can be seen in Fig. 8 that the cyclic voltammetric trace between -0.5 and $+0.5$ V in saline solution is linear for the young dentine between -0.02 and $+0.4$ V, for the old dentine between -0.1 and $+0.5$ V and for the saline between -0.2 and $+0.5$ V. In the reverse cycle, the linear segments are observed for young dentine between -0.3 and $+0.2$ V, for old dentine between -0.4 and $+0.2$ V and for saline between -0.4 and $+0.2$ V. It is clear that a peak is seen on all the traces at about -0.25 ± 0.1 V, which probably corresponds to the AgCl–Ag reduction reaction:

$$AgCl\ (aq) + e^- \rightarrow Ag + Cl^-\ (aq)$$

The sharp peaks at the two ends, anodic and cathodic, are probably due to the following reactions occurring at the silver electrode.

$$OH^-\ (aq) \rightarrow O_2\ (g) + 2H_2O\ (aq) + 4e^-$$
$$2H^+\ (aq) + 2e^- \rightarrow H_2\ (g)$$

However, these peak values for above reactions need to be confirmed by performing three-electrode CV and POT studies using a standard reference electrode. From the slope of the linear segments in Fig. 8 it can be concluded that the resistivity of old dentine is higher than young dentine, but both are lower than saline. Further, from the results summarised in Table II, it can be seen that the resistivity of wet samples is about three orders of magnitude lower than that of dry samples (Table I). The decreased resistivity of wet samples is probably due to the current flowing through the saline present in dentinal tubules rather than just dentine as in the case of dry samples.

Fig. 9 shows the results of potentiodynamic studies of young and old dentine along with the measured data for physiological saline. It can be seen that the three traces are similar. The most distinct feature is that at a potential of approximately -0.25 ± 0.1 V there is a drop in current between the electrodes (from 1.22E-5 to 9.97E-6) for young dentine, (from 1.37E-5 to 1.11E-5) for old dentine and (from 5.14E-6 to 3.47E-6) for saline for a short time which again starts increasing as the potential goes past the critical limit. This may be due to the formation of AgCl on the surface of the electrode, which then detaches from the electrode and allows the current to increase as a function of increased potential.

4. Conclusion

CV and POT studies of young and old human dentine samples have been carried out and clear differences have been found between dry and wet samples.

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

There are a number of companies interested in producing ''surgery-based'' machines for both caries diagnosis 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. It is apparent from this study that results are heavily dependant on moisture content and it is known that this can vary widely during dental restoration procedure.

More research is therefore required before these basic findings can be considered for any clinical application. Factors such as the effect of moisture, age of dentine and

TABLE II Summary of the cyclic voltammetry measurements of ''wet'' dentine

Sample	Young dentine	Old dentine	Saline concentration (0.154 mol/L)
Forward cycle	-0.020–0.405 V	-0.105–0.520 V	-0.265–0.544 V
Reverse cycle	-0.353–0.275 V	-0.401–0.245 V	-0.497–0.281 V
Resistivity	29.4 ± 0.07 kΩ cm	40.2 ± 0.99 kΩcm	50.3 ± 0.21 kΩcm

smear layer needs to be carefully considered during laboratory experiments.

We feel that the present investigation provides useful further information on the bulk electrical properties of dentine in dry and wet conditions. In future, a larger number of samples will need to be analyzed include enamel, before accurate analysis and diagnostic claims can be made for any on the basis of electrical measurements.

The results of this investigation and some other investigation using impedance spectroscopy of dentine [15] leads us to believe that the technique has the potential for detecting age-related changes of dentine, dental decay, integrity of dental restoration and assessment of the integrity of dento-alveolar implants, where its non-destructive *modus operandi* would be of great benefit.

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