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Original Article

Influence of sodium chloride content in electrolyte solution on electrochemical impedance measurements of human dentin

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ABSTRACT

Background: The aim of this study was to investigate the influence of sodium chloride (NaCl) content in electrolyte solution on electrochemical impedance measurements of human dentin by employing electrochemical impedance spectroscopy.

Materials and Methods: Dentin samples were prepared from extracted molars. Electrochemical impedance measurements were carried out over a wide frequency range (0.01Hz-10MHz). After measurements, samples were characterized using scanning electron microscopy.

Results: Electrochemical impedance measurements showed that the mean values of dentin electrical resistance were 4284, 2062, 1336, 53 and 48kΩ at different NaCl contents in electrolyte solution. One-way ANOVA test of mean values of dentin electrical resistance revealed a significant difference ($P < 0.0001$) as a function of NaCl content in electrolyte solution. Comparing electrical resistance values of dentin samples at 0.05% w/v and 0.9% w/v concentrations were found to be significantly different ($P < 0.05$ at 95% confidence level). Scanning electron microscopy revealed structure of dentin sample with intertubular dentin matrix and distribution of patent dentinal tubules.

Conclusion: This *in vitro* study indicated, through electrochemical impedance spectroscopy measurements, that electrical resistance of dentin was affected by the concentration of NaCl in electrolyte solution. It is clear from the current study that NaCl concentration in electrolyte solution has a marked influence on dentin electrical resistance. Therefore, this baseline data need to be considered in any future study on dental samples.

Key Words: Electric impedance, spectroscopy, dentin, electrical, resistance, scanning electron microscopies, sodium chloride

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INTRODUCTION

Dentin layer constitutes the major component of human teeth. Dentinal tubules are a normal feature of dentin structure. The dentinal tubules extend throughout dentin from the tooth pulp to the tooth's outer edge, terminating at dentino-enamel or dentino-cemental junctions. They assume a sinusoidal pattern and taper in shape from pulp outwards. Their density is approximately 27,000 tubules mm^{-2} at the outer dentinal surface, whilst at the inner dentinal surface the density increases to approximately 45,000 tubules mm^{-2}.[1] Their diameter is about 1 μm to 4 μm from outer to inner surfaces respectively.[1] *In-vivo*, patent dentinal tubules are occupied by an odontoblast

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process and extracellular fluid. It is known that osmotic gradients are responsible for 'sensitivity' of exposed dentine in carious or fractured teeth. Young dentin is characterized by a large number of patent dentinal tubules. These dentinal tubules often get partially or fully occluded with increasing age as a result of the deposition of highly mineralized 'intratubular' dentin on their inner walls. [2, 3]

Non-destructive methods such as permeability measurement to measure fluid flow through dentin disks,[4] and electrochemical impedance measurements for characterizing dental tissues and to measure their electrical resistance have gained popularity in recent times.[5-11] Several investigators have used the electrochemical impedance spectroscopy to investigate age-related changes of dentin,[3,6] bacterial-demineralization of human dentin[12,13] and the effect of restorative materials on dentin.[14] Recently, the technique has been utilized to study the cleaning effect with sodium hypochlorite on endodontic bacteria.[15]

The principles of these techniques rely on the use of appropriate electrodes and an electrolyte solution, to measure the electrical resistance of sample under test.

In previous studies dentin was found to have differing electrical resistivity and resistance values. Resistivity of dentin has been found to be 33 ± 13 KΩcm using physiological saline,[16] 2.35 MΩcm using artificial saliva[17] and <1 KΩcm using hydroxyethyl-piperazinyl-1-ethane sulfonic acid containing hibitane and rubidium chloride as electrolytes.[18]

Electrical dentin resistance has been reported to be 8 KΩ, using physiological saline,[19] <2 KΩ in potassium chloride solution[20] and it changes with the change of sodium chloride (NaCl) content in storage solution for dental samples.[21]

The variation in dentin electrical resistivity and resistance values could be due to several reasons including type and concentration of electrolyte solution. The concentration of electrolyte solution is significant because any change in the electrolyte concentration means an alteration in the number of charge-carrying ions, which in turn affects the electrical response of the sample under test.[18]

Since electrochemical impedance spectroscopy technique was successfully employed by the authors to investigate the effect of age and acid-etching procedure on impedance measurements of human dentin.[3,5,6] It would be beneficial to carry further investigations to clarify the effect of NaCl content in electrolyte solution on the electrical resistance of dentin.

There have been no electrochemical impedance spectroscopy investigations reported on the influence of NaCl content in electrolyte solution on the electrical resistance of human dentin. Such studies would provide a basis for understanding the interaction of filling materials such as composite resins and glass cements with dentin layer in the presence of Na+ ions, normally present in body fluids. Therefore, the aim of this study is to investigate and clarify the influence of NaCl content in an aqueous electrolyte solution on electrical resistance of human dentin samples using electrochemical impedance spectroscopy.

MATERIALS AND METHODS

Sample preparation

Extracted un-erupted human third molars were used for the current study. Un-erupted third molars were utilized to avoid the effect of caries, restorations, cracks, or attrition due to age. The age group was chosen in this study was 20 (±1) years old. The molars were stored in deionized water with a few Thymol crystals inside hermetically sealed vials. Dentin samples were prepared from the molars by cutting each molar crown perpendicular to its long axis and parallel to the occlusal plane. Then a 2mm thick disc was prepared from each molar crown with a diamond wheel in computerized water-cooled cutting machine (Struers Ltd., Glasgow, U.K.). The upper surface of cut disc was cut just under the dentino-enamel junction and the lower surface just above pulp horns. A rectangular dentin sample with smooth flat surfaces and parallel sides was then prepared from the disc using a water-cooled cylindrical diamond bur. The preparation of dentine samples was standard throughout the project. Details of the sample preparation are shown schematically in Figure 1a-c. Dentin samples were measured using a micrometer screw-gauge at three different locations to determine mean thickness, width and length. Each dentin sample was 2mm thick, 5mm wide and 7mm long (±0.1mm). Twenty-five dentin samples were used for the electrical impedance measurements. The dentin samples were divided into five groups, each group includes five samples for each electrolyte solution.

Before conducting electrical impedance measurements, the samples were examined by stereomicroscope to

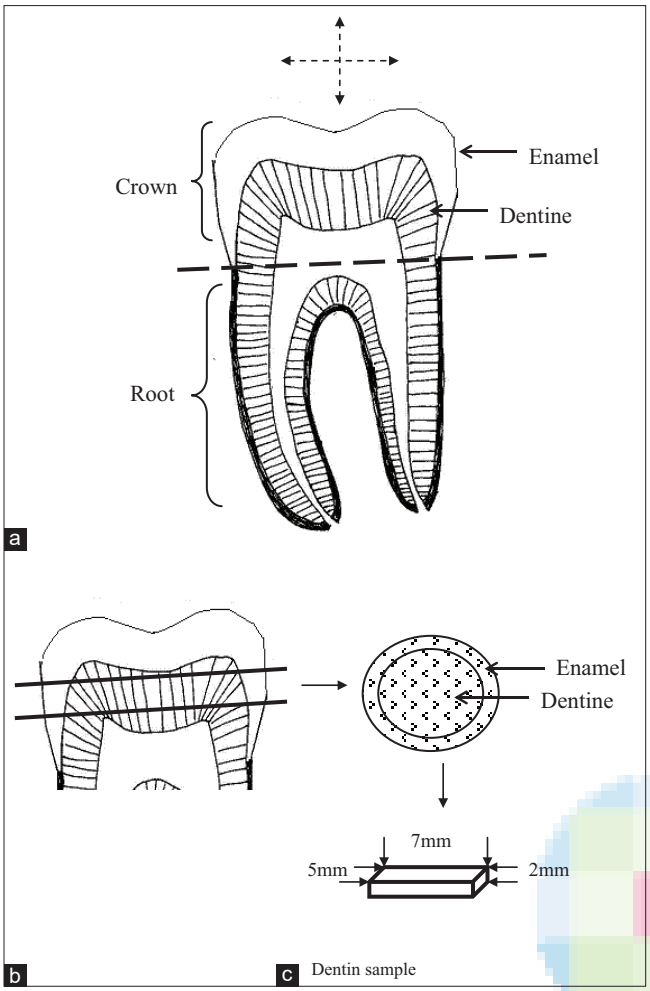

Figure 1: Schematic diagram of the longitudinal section of a molar tooth showing steps of dentin sample preparation (a-c).

confirm the absence of cracks or surface irregularities. The occlusal and pulpal surfaces were painted with quick-dry silver paint (Agar Scientific Ltd, Essex, U.K.) to minimize interfacial impedance between sample, electrodes and connecting leads. As soon as the paint had dried, samples were inserted in a special '*in-house'* designed sample holder containing freshly prepared electrolyte solution.

Electrolyte solution preparation

Fresh electrolyte solutions with different concentrations of NaCl content were prepared by diluting sterile physiological saline (0.9% w/v NaCl) with double distilled water (ddH_2O). Five electrolyte solutions were prepared (0.05% w/v, 0.1% w/v, 0.2% w/v, 0.4% w/v, and 0.9% w/v). Each concentration was stored immediately in new sterile and hermetically sealed glass containers to avoid contamination or room temperature effect. Molar concentration of electrolyte solutions was calculated using the equation: M = g/l/MW.[22]

Sample holder

A special sample holder was fabricated from transparent Perspex. This was designed to standardize measurements, protect wet samples from drying and provide good visibility of the sample throughout electrical impedance measurements. The sample holder includes 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 is attached to a gold pin with concealed spring approximately 1mm in diameter. The spring was used to avoid excessive or uneven pressure on the sample surfaces or the gold pins during clamping and measurement. The rubber rings were used to make an airtight seal and to prevent solution leakage or evaporation from the sample holder during measurement.

Electrochemical impedance measurements

The electrochemical impedance measurements of dentin samples in different NaCl contents in electrolyte solution were carried out at 20ºC using a computer controlled SI 1260 Impedance Gain-Phase Analyzer (Solartron Analytical, Hampshire, UK) over the frequency range from 0.01Hz to 10 MHz. The applied amplitude of the potential was 100 mVrms under open circuit conditions. To minimize stray capacitance, coaxial leads were used to connect the sample to the electrochemical 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 analyze the measured impedance data. The fitted data can thus be modeled 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. Before conducting any electrical impedance measurements on dentine samples, the electrical impedance measurements of a standard electrical circuit consisting of a resistor (10 KΩ) in parallel with a capacitor (5 μF) was measured between 100 μHz and 32 MHz to confirm the impedance analyzer calibration. Furthermore, electrical impedance measurements were made on the blank electrolyte cell filled with normal saline only. Connections between the sample holder and electrochemical impedance measurement device are shown schematically in Figure 2.

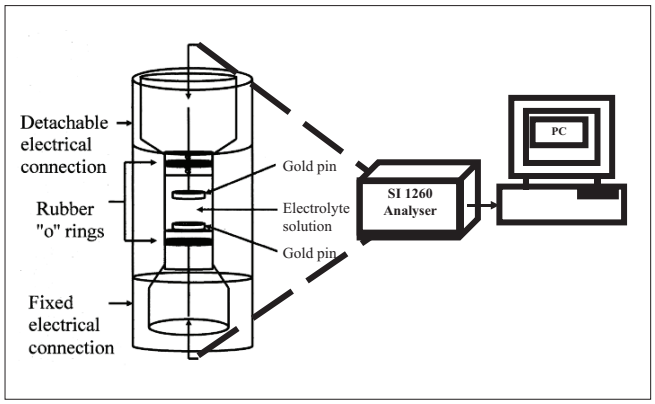

Figure 2: Schematic diagram of sample holder and the connections to the SI 1260 impedance analyser and PC.

Scanning electron microscopy

Dentin samples were examined under a scanning electron microscope (JEOL JSM 35 with Deben, UK "Genie" computer control) after performing all electrical impedance measurements. Samples for scanning electron microscopy (SEM) examination were sputter coated with gold to avoid charge build-up on sample surfaces.

Statistical analysis

Minitab 12.1 (Minitab Inc, USA) was used to perform *t*-test and ANOVA tests at a confidence level of 95% to ascertain if any differences between electrical resistance of dentin samples measured in different concentrations were statistically significant.

RESULTS

The electrochemical impedance measurements for dentine samples in varying NaCl content in electrolyte solution are shown in Figure 3a. A magnified view of the complex plane is shown in Figure 3b to highlight variations at high frequencies of the electrochemical impedance measurement. Similarly, the measured data are shown in Bode plane as shown in Figure 3c. Table 1 shows the variation of resistance of dentin samples as a function of NaCl content in electrolyte solution. Dentin samples were examined under SEM after the electrochemical impedance measurements. Scanning electron micrographs at different magnifications of dentin sample is shown in Figure 4.

DISCUSSION

In terms of the standard experiments of the control electrical circuit, 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 standard electrical circuit. Having confirmed the experimental set-up with a control circuit, measurements were made on the blank electrolyte cell filled with 0.9%w/v NaCl content in electrolyte solution only without a sample. It was found that there was only nominal impedance (0.3-0.6 Ω) over the whole frequency range. Therefore, values for electrical impedance measurements were believed to arise from the sample only.

The electrochemical impedance measurements for dentin samples in varying NaCl content in electrolyte solution are shown in Figure 3a. A magnified view of the complex plane is shown in Figure 3b to highlight variations at high frequencies of the electrochemical impedance measurement. Similarly, the measured data are shown in Bode plane as shown in Figure 3c. Figure 3c shows a variation of |Z| and theta angle as a function of ac signal frequency. The results shown in Figure 3a-c clearly demonstrate that the electrical resistance of dentin in an electrolyte solution containing varying amount of NaCl changes with the change of the content of NaCl in electrolyte solution. The magnified view of complex plane shown in Figure 3b and theta angle graph of Bode plane in Figure 3c of measured electrochemical impedance clearly show unusual behaviour at high frequency end (10MHz) for lower NaCl contents (0.05% w/v, 0.1% w/v and 0.2% w/v). This behavior disappear for 0.4%w/v and 0.9%w/v NaCl contents in electrolyte solution. More detailed analysis of the data was carried out for the measurements corresponding to 0.05% and 0.9% w/v NaCl contents in electrolyte solution. Measured data fits the best to the proposed equivalent circuit model by the impedance analyzer software after including a Warburg-like element for NaCl content (0.05%w/v). This element generally describes the diffusion of charged particles diffusing through a medium and is arising as a result of the difference in kinetics of electron exchange at the triple junction of electrode surface, electrolyte solution and dentin sample. Whereas, the measured electrochemical impedance data fits the equivalent circuit well without incorporating Warburg-like element for 0.9%w/v of NaCl content in electrolyte solution. This suggests that the kinetics of ionic diffusion and kinetics of electron exchange were comparable for concentration

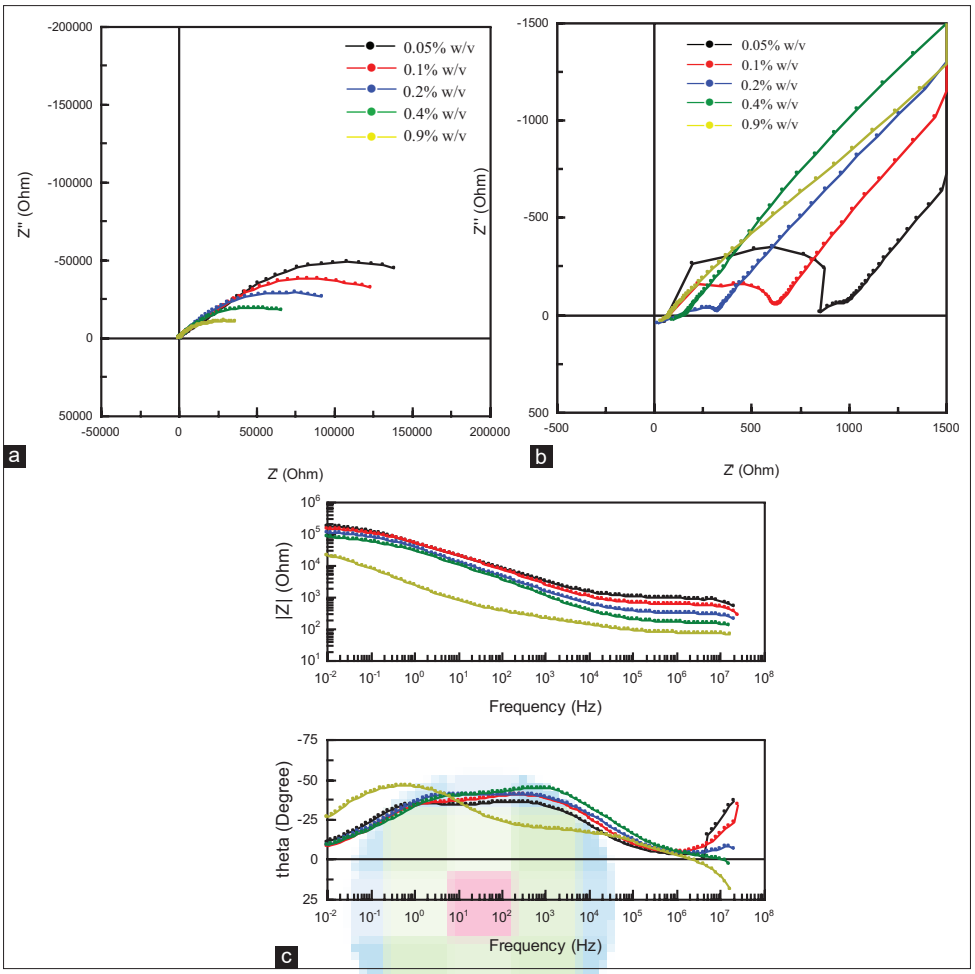

Figure 3: Impedance measurements of dentine samples in various NaCl contents in electrolyte solution 0.05% (black dotted line), 0.1% (red dotted line), 0.2% (blue dotted line), 0.4% (green dotted line) and 0.9% (yellow dotted line). (a) Complex plane, (b) Zoomed view of Complex plane and (c) Bode plane (a-c).

equal to 0.9% w/v of NaCl content in electrolyte solution. It appears that the threshold for Warburg-like element playing an active role in determining the response of dentin sample during electrochemical impedance measurements in 0.05% w/v to 0.4% w/v of NaCl content in electrolyte solution. *In-vivo* electrochemical impedance studies would not, therefore, need to include a Warburg-like element in electrochemical impedance measurements. This is because NaCl concentration in body serum is similar to that of physiological saline solution (0.9%w/v NaCl content).[22] *In-vitro* studies may need to incorporate Warburg-like element in electrochemical impedance measurements if the teeth were measured in non-physiological saline solutions.

The mean values of resistance generated by the equivalent circuit model of dentin samples for each NaCl content in electrolyte solution are shown in Table 1. Table 1 shows the variation of resistance of dentin samples as function of NaCl content in electrolyte solution. Electrochemical impedance measurements also showed that there were differences in resistance between different electrolyte solution concentrations. Statistical analysis of mean values for all concentrations (0.05%w/v, 0.1%w/v, 0.2%w/v, 0.4%w/v and 0.9%w/v) using ANOVA test, the concentrations were found differing in resistance. Mean values of electrical resistance at different NaCl content (0.05%w/v, 0.1%w/v, 0.2%w/v, 0.4%w/v and 0.9%w/v) in electrolyte solution were 830 ± 1.2 Ω, 512 ± 1.0 Ω, 291 ± 0.4 Ω, 141 ± 0.5 Ω and 74 ± 0.9 Ω, respectively and in that order for dentin samples were 4284 ± 18.7 kΩ, 2062 ± 1.7 kΩ, 1336 ± 0.6 kΩ, 53 ± 0.8 kΩ and 48 ± 0.3 kΩ. One-way ANOVA of the means of resistance for all concentrations (0.05%w/v, 0.1%w/v, 0.2%w/v, 0.4%w/v and 0.9%w/v) revealed a significant difference (ANOVA, $P < 0.0001$) as a function of concentration. Comparing resistance values

Table 1: Mean values (±standard deviation) of electrical resistance obtained for dentin samples in each sodium chloride content in electrolyte solution

Sodium chloride content in electrolyte solution (w/v)	0.05%	0.1%	0.2%	0.4%	0.9%
Electrolyte solution resistance (Ω)	830±1.2*	512±1.0	291±0.4	141±0.5	74±0.9*
Dentine resistance (kΩ)	4284±18.7*	2062±1.7	1336±0.6	53±0.8	48±0.3*

*Significant difference (P<0.05 at 95% CI). CI: Confidence level

of 0.05%w/v and 0.9% w/v NaCl content in electrolyte solution are shown in Table 1. Resistance values of dentin samples were found to be significantly different for the two NaCl content ($P < 0.05$ at 95% confidence level).

The electrical resistance of dentin samples is significantly higher compared to the resistance of the interfacial resistance of the electrolyte solution as shown in Table 1. The higher resistance of dentine clearly indicates that it is a poor electrical conductor. The poor electrical conductivity of dentine is probably because it mainly consists of calcium and phosphate hydroxyapatite crystals and collagen fibers, both of which are poor electrical conductors.[23] The electrical resistance of electrolyte solution was found to decrease with increasing NaCl content due to the increase in the number of charge carriers. Similarly, the electrical resistance of dentin decreases steadily with the increase in NaCl content in electrolyte solution.

Dentin samples were examined under SEM after the electrochemical impedance measurements. Scanning electron micrographs at different magnifications of dentin sample are shown in Figure 4. As can be seen clearly in the SEM micrographs at different magnifications dentinal tubules are uniformly distributed throughout dentin matrix of the sample, but are also known to have cross-branches.[24]

CONCLUSION

Electrical impedance measurements of young, sound dentin presented in this paper clearly indicate that the electrical resistance of dentin is affected by NaCl content in electrolyte solution. In spite of increasing use of electrochemical impedance technique to understand electrical properties of human teeth, it is clear from this study that NaCl concentration in electrolyte solution has a marked influence on sample electrical resistance. Therefore, this baseline data need to be considered in any future study on dental samples.

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Nil.

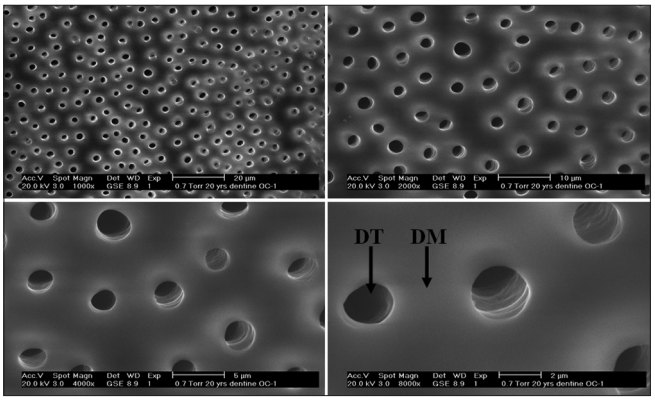

Figure 4: Scanning electron micrographs of dentin sample at different magnifications (DM = Dentin matrix, DT = Dentinal tubule).

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

REFERENCES

1. Shellis P. Dental tissues: Human tissues: Dentine. In: Osborn JW, editor. Dental Anatomy and Embryology. Vol. 1. London: Blackwell Scientific Publications; 1981. p. 155-209.
2. Eldarrat A, High A, Kale GM. Age-related changes in cyclic voltammetry and potentiodynamic studies of normal human dentine. J Mater Sci Mater Med 2003;14:979-84.
3. Eldarrat AH, Wood DJ, Kale GM, High AS. Age-related changes in ac-impedance spectroscopy studies of normal human dentine. J Mater Sci Mater Med 2007;18:1203-10.
4. Delannée M, Grégoire G, Vergnes JN, Sharrock P. Fluid flow through dentin-self-etch resin interface during long term *in vitro* aging. Mater SciEng C Mater Biol Appl. 2013;33(7):3711-5.
5. Eldarrat AH, High AS, Kale GM. *In-vitro* analysis of 'smear layer' on human dentine using ac-impedance spectroscopy. J Dent 2004;32:547-54.
6. Eldarrat AH, High AS, Kale GM. Age-related changes in ac-impedance spectroscopy studies of normal human dentine-further studies. J Mater Sci Mater Med 2010;21:45-51.
7. Levinkind M, Vandernoot TJ, Elliott JC. Electrochemical impedance characterization of human and bovine enamel. J Dent Res 1990;69:1806-11.
8. VanderNoot TJ, Levinkind M. Ac impedance characteristics of human dental enamel and dentine. J Electroanal Chem 1991;300:191-8.

9. Verdonschot EH, Bronkhorst EM, Burgersdijk RC, König KG, Schaeken MJ, Truin GJ. Performance of some diagnostic systems in examinations for small occlusal carious lesions. Caries Res 1992;26:59-64.
10. Ricketts DN, Kidd EA, Wilson RF. A re-evaluation of electrical resistance measurements for the diagnosis of occlusal caries. Br Dent J 1995;178:11-17.
11. Longbottom C, Huysmans MC, Pitts NB, Los P, Bruce PG. Detection of dental decay and its extent using a.c. impedance spectroscopy. Nat Med 1996;2:235-7.
12. Xu Z, Neoh KG, Kishen, A. Monitoring acid-demineralization of human dentine by electrochemical impedance spectroscopy (EIS). J Dent 2008;36(12):1005-12.
13. Xu Z, Neoh KG, Amaechi B, Kishen A. Monitoring bacterial-demineralization of human dentine by electrochemical impedance spectroscopy. J Dent 2010;38 (2):138-148
14. Rivas B, Botta PM, Varela P, Martín B, Fondado A, Rivas J. *In vitro* electrical impedance spectroscopy of human dentine: The effect of restorative materials. Bioelectromagnetics 2008;29(3):163-8.
15. Penta V, Vornicescu D, Keusgen M, Pirvu C. Understanding the cleaning effect with sodium hypochlorite of *Enterococcus faecalis* endodontic pathogen using electrochemical impedance spectroscopy (EIS), atomic force microscopy (AFM) and surface plasmon resonance (SPR) Digest Journal of Nanomaterials and Biostructures 2013;8:1205-14.
16. Mumford JM. Resistivity of human enamel and dentine. Arch Oral Biol 1967;12:925-7.
17. Friedman J, Grayson AS. Electrical properties of tooth structure. Proc. 23rd ACEMB, Conference on Engineering in Medicine and Biology, Washington; 1970. P. 164.
18. Hoppenbrouwers PM, Scholberg HP, Borggreven JM. Measurement of the permeability of dental enamel and its variation with depth using an electrochemical method. J Dent Res 1986;65:154-7.
19. Atkinson HF, Parker DA. Bioelectric properties of the tooth. J Dent Res 1969;48:789-94.
20. Levinkind M, Vandernoot TJ, Elliott JC. Evaluation of smear layers on serial sections of human dentin by means of electrochemical impedance measurements. J Dent Res 1992;71:426-33.
21. Schulte A, Gente M, Pieper K, Arends J. The electrical resistance of enamel-dentine cylinders. Influence of NaCl content in storage solutions. J Dent 1998;26:113-8.
22. Kenner CT, Busch KW. Methods of expressing concentrations of solutions. In: Quantitative analysis. New York: Macmillan Publishing Co. Inc. 1979. p. 24-46.
23. Sun D, Monaghan P, Brantley WA, Johnston WM. Electrochemical impedance spectroscopy study of high-palladium dental alloys. Part 1: Behaviour at open-circuit potential. J Mater Sci Mater Med 2002;13:435-42.
24. Shamos MH, Lavine LS. Physical bases for bioelectric effects in mineralized tissues. ClinOrthop 1964;35:177-88.