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Electrochemical Determination of Effects of Difference in Concentration between Enamel Crown's Buccal Side and Tooth Root and the Role of Fluoride Ion

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Abstract It has been studied previously that there exist surface potential in a whole tooth. This work is carried out to investigate the cause of human teeth decay in the oral cavity by electrochemical method. The enamel wafer preferentially allows the passage of monovalent cations and it restricts the passage of monovalent anions so that developing an electric potential. The developed surface potentials were measured between enamel crown's buccal side and tooth root. Surface potentials examined in all the teeth in the presence of KCl. Then the electric potentials developed this way provide an electric driving force for possible degradation and caries formation of enamel by electric means. This difference is the result of the difference in ionic strength of blood and saliva and types of food intake. It is found that potential increased with increasing KCl concentration and after acid corroded, however the addition of fluoride caused the electric current drop significantly. These results suggest that drop in current indicates a significant increase in the electric resistance of the enamel as a result of fluoride and it is also found that potentials can be affected by the surrounding electrolytes.

Keywords: *enamel, enamel wafer, tooth caries, KCl, fluoride ion*

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1. Introduction

Teeth are composed of a thin layer (1-2 mm) of dental enamel which forms the hard protective coating over the tooth. This consists mainly of calcium, phosphate and other ions in a structure known as "hydroxyapatite" [1]. Dental enamel is porous and is susceptible to acid dissolution during the process of demineralization. This demineralization process is offset by the repair process known as remineralization. Tooth susceptibility varies among individuals. The reasons are not fully known, but influences include: shape, size and order of the teeth which affect the "washing" effects of saliva.

This is largely determined by hereditary factors. Salivary components play a significant role in controlling dental caries, because they affect bacteria, immune status, plaque formation, and enamel structure and can neutralize acids. Saliva has a vital role in the balance between demineralization and remineralization. Enamel structure can be altered by a selection of mineral ions and fluoride, as well as by acid. The balance between demineralization and remineralization of the enamel determines whether caries occurs or not. Availability of fluoride favors remineralization [2].

Dental disease remains a significant problem in the world so that the vast majority of the population suffering with the consequences of this disease at some stage in their lives [4]. It can result in acute pain, aesthetic problems and can increase the risk of tooth loss, which may have long-term effects on food intake resulting in impaired nutritional status and subsequent overall well being [5]. Damage or loss of teeth may result from: - Dental caries, Acid erosion, Periodontal disease. Caries is caused by bacterial acid production in tooth plaque, which can cause deep localized lesions if it remains too near the tooth for any length of time. If left the bacteria then may penetrate the tooth further and progress into the soft pulp tissue. Untreated dental caries can lead to incapacitating pain, potential tooth loss and loss of dental function. The development and progression of dental caries is due to a number of factors, specifically bacteria in the dental plaque (particularly *Streptococcus mutans* and *lactobacilli*) on susceptible tooth surfaces and the availability of fermentable carbohydrates [6,7,8,9]. The damage to teeth from acid erosion is now thought to be a significant contributor to dental disease. Erosion arises from acid derived from foods and drinks, or regurgitations from the stomach, which repeatedly wash over the teeth and result in shallower but more widespread lesions [10]. Periodontal disease results from inflammation of the gum (gingivitis) that gradually causes destruction of the bone supporting the teeth [11]. Gingivitis usually results from infection from debris that has accumulated at crevices at the base of the teeth. Although the main reason for tooth extraction is a result of dental caries, there appears to be an increasing trend for tooth loss in adulthood resulting from periodontal disease [12,13]. Dental caries is a chemical and electrochemical dynamical process of de- and re- mineralization. It occurs at the interfaces of dental-saliva and dental-plaque. So, studying the interface character of tooth is of great importance to discover the caries' secrets and to find effective protection methods of tooth [14]. The dental hard tissue, of enamel, dentin or cementum, is formed mostly by the mineral, hydroxyapatite. It is soaked in the electrolyte surroundings of saliva, blood and enchyma, thus, composes an electrochemical system. Except for Miller using chemico-parasitic theory to study the caries in 1890, many scholars later focused on electrochemical fields to study the dental characters and caries [15,16,17,18]. A systematic study of the dental electrochemical property made by Klein and Amberson, 1932, had suggested that the dental enamel was an electrostatic ion screen and it had permselectivity. Then studies mainly about the dental permselectivity were made, which concluded that the ion surroundings developed the surface potentials of tooth [19,20,21,22].

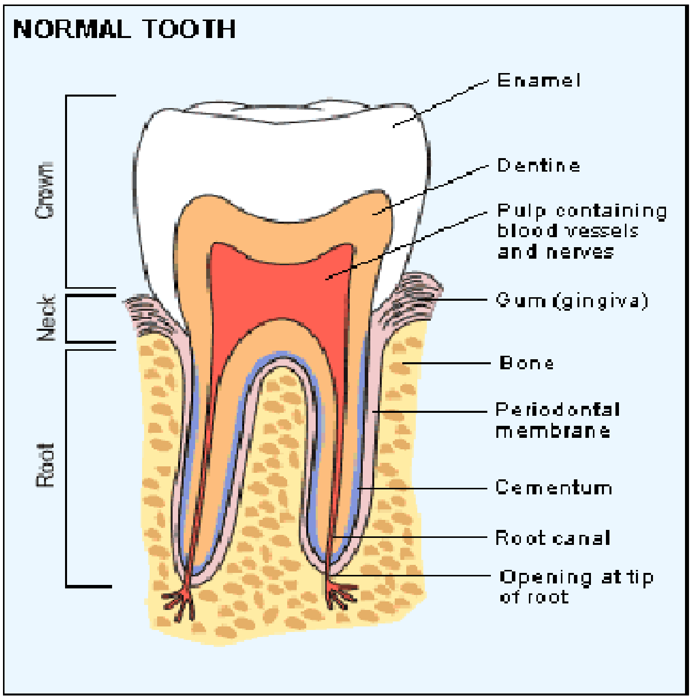

Figure 1. Cross section of human molar tooth [3]

Hence a trend toward explaining the physical and biological properties of dental enamel electrochemically has developed; on the basis of the permselectivity and chargeability of the enamel, attempts have been made to elucidate the ionic exchange, adsorption, and permeability of several substrates to the enamel in relation to incipient caries. Some early studies applying an electrochemical approach to enamel were made by Klein et al' and Klein [23,24]. They demonstrated experimentally that enamel had permselective properties and that in the KC1 solution the isoelectric point of dog's enamel laid between pH 3.6 and pH 4.9. They further demonstrated that the enamel pore wall was positively charged in solutions on the acid side of the isoelectric point, negatively on the alkaline side [24].

D. Birkhed, B. Sundin, and S.I. Westin [25], also showed that, when enamel is used as a diffusion barrier between the concentration cells, enamel has permselectivity. More recently, J.D.B. Featherstone, M.M. O'reilly [28], found that synthetic hydroxyapatite membranes have cation selective properties after exposure to the intraoral environment. S.R. Grobler and J. B. Blignaut [31], has experimentally made a dynamic study on the permeability of dental enamel in reference to tooth surfaces, tooth age and surface subsurface enamel; he reported that enamel has amphoteric properties and that a marked change of permeable voltage was shown at pH 5.0 in deciduous teeth, at pH 4.0 in impacted teeth and at pH 3.5 in permanent teeth.

It was suggested that isoelectric points might correspond to the above pH. Since a layer of medium is formed at the surface of the hydroxyapatite crystal and a hydration shell of 1.9 times of its own weight L. M. Kerebel, M. T. Le Caballec [35], goes with the crystal, an electrical double layer (Helmholtz's double layer) on Zeta potential is formed upon the surface of the crystal. The Zeta potential is thought to be related to plaque formation, the uptake mechanism of fluoride and mineralization of teeth [36]. The present study is an attempt to investigate the cause of human teeth decay in the oral cavity and to determine the role of fluoride in the prevention of caries by electrochemical method.

2. Materials and Methods

2.1. Apparatus and Reagent

A pair of conducting wires and a high impedance electrometer was used. The artificial saliva (1000 ml) was made according to Birkland procedure [22]. 0.46803g $Na_2HPO_4.2H_2O$ (MW = 156.01, analytical grade reagent), 1.68020 g $NaHCO_3$ (MW = 84.01 A.R.), 0.14703 g $CaCl_2.2H_2O$ (MW = 147.03, analytical grade reagent) was dissolved with distilled water; then the pH value was adjusted to 6.8 with KOH and acetic acid, in equilibrium with air. The KCl solutions that were prepared from reagent grade salts (MW = 74.55, analytical grade reagent), and distilled water had the concentrations of 0.01, 0.05, 0.4, 0.6, 0.8 and 1.0mol/L.

Figure 2. Illustration of apparatus for measuring electric current and the dental surface potentials

2.2. Electrical Circuit Linkage

The sample was dried under high pressure vacuum before being used. It was hold tightly in a horizontal position in a self-made two compartment paraffin wax holder. Two conducting wires were linked with the electrometer, and each working hand of these wires was connected with one hand of the other two conducting wires with crocodile teeth at the other end respectively. The other hand of the wire with crocodile teeth was connected with the tooth surface (positive hand to the mid-spot of tooth root, negative one to the mid-spot of enamel crown's buccal side) respectively, as shown in Figure 1.

2.3. Sample Preparation

Twenty four human permanent teeth 8 incisors 3 canons 11 premolars 2 molars were selected when newly extracted. Patients from whom the sample collected were aged from 13 years to 50 years, no matter what sexes are. The teeth were made clean carefully, then rinsed extensively with distilled water and immersed into fresh distilled water. The enamel wafer was also made from the enamel of non-carious human permanent tooth with a thickness of 200 - 300µm.

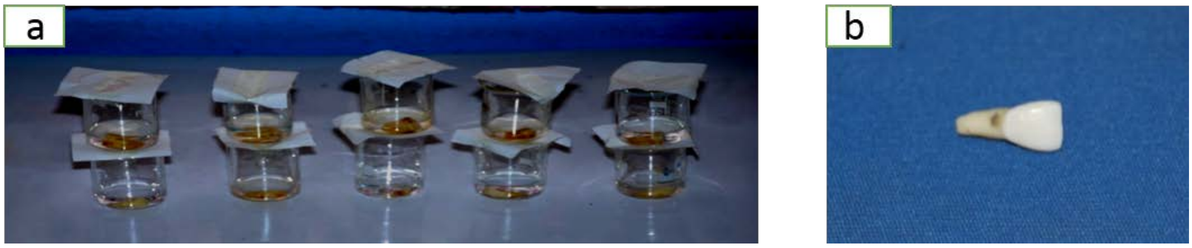

Figure 3. (a) Cleaned human permanent teeth ready to conduct an experiment. (b) A non-carious human sound incisor from which wafer was made

The surfaces of the wafers were parallel to the external surfaces of the teeth from which they were taken. This was done so that the pores that normally passed through the tooth surfaces also would pass through these wafers [12]. To ensure that the wafers were composed of pure enamel, they were taken from enamel that was at least 0.4 mm away from the external surfaces of the tooth.

3. Results and Discussion

The surface potential is developed due to difference in concentration between enamel crown and tooth roots. Surface potentials gradient of all the teeth were measured between the two sections of a tooth. The comparative measurement was also done to imitate the physiological condition of tooth by socking class X (enamel crown to the artificial saliva and root to normal saline, 20.03 mV) and class Y (enamel crown and root both to 0.1mol/L KCl, 19.10 mV) respectively. The result shows no statistical significance between both groups of measurement. Among these the 0.4 mol/L KCl series had an average potential of 19 mV, so closed to the result of class Y (19.10 mV), which had the same experimental condition. They have no statistical significance ($p>0.05$), which suggest the experiment is perfectly consistent.

Table 1 shows the surface potential at different KCl concentrations, decreasing concentration of KCl results in decreasing potential from 15 to 39 mV even if the declination is not uniform.

Table 1. Effects of KCl concentration (mol/L) on dental surface potential

Concentration of KCl (mol/L) in both side of the compartment	1.0	0.8	0.6	0.4	0.05	0.01
Surface potential (mV)	39	30	25	19	17	15

The effects of acid corrosion on dental enamel were also determined. During clinical bracing and/or from food staff, it is usual that teeth are always susceptible to acids. After enamel being corroded by the acid, the surface potentials rose by 40% comparing to the initial values got before acid treatment (35 mV to 27 mV). The change has statistical significance ($p < 0.05$).

It is preferable to use KCl solution of different concentration in the paraffin wax compartment for the electrochemical investigation, as the ions K^+ and Cl^- having equal diffusion rates and activity. So the use of 0.1 mol/L KCl solution as electrolyte had similar surface potentials with the imitated physiological condition.

These entire results indicate that the artificial saliva and normal saline can be replaced by 0.1 mol/L KCl solution as affective electrolyte solution in studying the tooth electrochemical characters. The surface potentials in the extracted teeth as a whole tissue were observed. They are complex potentials between enamel, dentin and cementum. When enamel crown and the tooth root were connected to the 0.1 mol/L KCl solution, the average potential was 19 mV. Tooth in the oral environment has the similar surroundings as it was imitated, so the acquired data in the extracted teeth can be useful references as the dental biological potentials. Also there is a stern layer, or a hydration layer of 5nm, in the enamel crystal surface, rich in attracted opposite charges. Where there's an electrical double layer, there are two layers of equivalent, opposite charges, thus, a potential must be established between the layers [2]. The surface potentials observed are all positive; which indicates that positive charges follow with the external circuit from the surface of dental root to the enamel surface.

The dental surface potentials increased after acid corrosion using the same electrolyte, confirms the fact that the dental crystal structure develops the potentials. When corroded, more enamel crystals are exposed to the surrounding ions. The interaction area of crystals and surrounding ions increased and the absorbing became more tightly, which led to an increase of the potential. The ratio of concentrations on the two sides of the enamel wafer is responsible for electric ionic membrane potential differences [17].

The ionic membrane property of dental enamel Wafer that was used in the apparatus was 200 to 300 micrometer thick and it was made from the enamel of non-carious human tooth. The conducting wires immersed in the solution were coated with a conducting aluminum foil. Variation in the concentrations of salt solutions that bathe different sides of the tooth enamel caused significant voltages to develop. These voltages depend on concentration differences. However the thin wafer allowed equilibrium potentials to be established rapidly (45 seconds).

To determine the extent of enamel deterioration, electric currents were passed through the dental enamel wafer. The conducting wires immersed in the solution were coated with a conducting aluminum foil. With this experimental arrangement, a constant voltage that was applied to the enamel wafer gave a variation of current that was dependent on time, as is seen in Figure 5.

A constant potential difference of 10 V was applied. The electric current increased with time in an exponential manner; this indicates a continual degradation of the enamel wafer that is proportional in rate to the current at any given time. When the enamel samples were removed from the apparatus illustrated in Figure 2, physical deterioration was noted. The region of the wafer that was exposed to electric current had the same white, opaque appearance that is associated usually with carious lesions. Control wafers that were subjected to identical circumstances, except for the flow of electric current had no physical deteriorations [19].

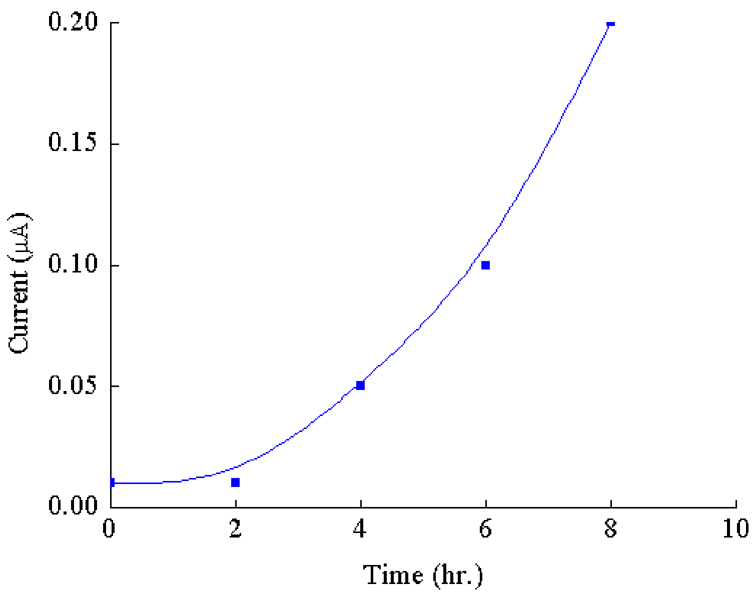

Figure 4. Time dependence of electric current through enamel wafer for 10 hours exposure

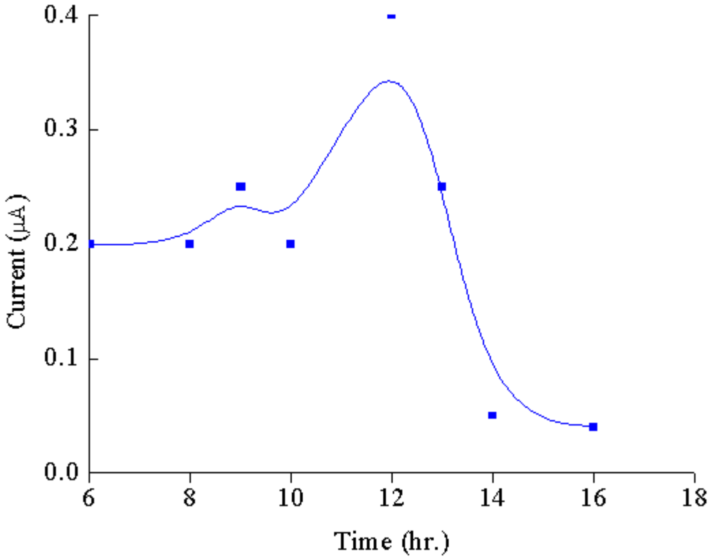

Figure 5. Effect of fluoride addition on electric current through enamel wafer

The right hand side solution of the compartment of Figure 2 was diluted from 0.1 N KCl to 0.05 N KCl plus 0.05 N KF under the applied potential of 10 V. This is used to test the effect of fluoride ion on electric current. The result of this experiment is shown in Figure 5.

After an electric current flowed through an enamel wafer for several hours, the 0.1 N KCl solution in the electrically positive compartment of the cell was diluted with an equal volume of 0.1 N KF. The polarity of this compartment of the cell was such that the fluoride ion was driven electrically into the enamel wafer. As is shown in Figure 5, the addition of fluoride ultimately caused the electric current to drop by a factor of about four. This drop in current indicates a significant increase in the electric resistance of the enamel as a result of contact with fluoride. An increase in electric resistance should be beneficial if caries are formed by an electric mechanism.

Hence the present endeavor offers some experimental data and pattern to determine the electrochemical properties and ions permselectivity of tooth. The ion transition across the interphase is greatly modified by the surplus charges and the electrical double layer in the tooth surface [20].

4. Conclusions

The experimental evidence suggests that electrochemical phenomena play an important role in the mechanism of tooth deterioration. The effect of fluoride could help eliminate these short circuits of the oral electric potentials that are caused by local regions of low quality enamel. The elimination of local short circuits would minimize the incidence of tooth deterioration if the electric current that is associated with the short circuits causes their formation. The experimental evidence suggests that electrochemical phenomena play an important role in the mechanism of tooth deterioration. All teeth have surface potentials when measured between the enamel crown and tooth root because of the difference in concentration they subjected to. There is relatively stable direct current, along the electrochemical circuit. The existence of oral electric potentials, their possible short circuiting by regions of low pH or poor enamel quality, and the degradation of enamel by an electric current indicates that an electrochemical mechanism for caries formation is a possibility. The effect of fluoride could help to eliminate these short circuits of the oral electric potentials that are caused by local regions of low quality enamel. The elimination of local short circuits would minimize the incidence of tooth deterioration if the electric current that is associated with the short circuits causes their formation.

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