Technical Paper

Electrophoretic Efficiency of an Ionic Toothbrush

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Abstract

The eventual electrophoretic effects during application of an ionic toothbrush are examined. Firstly, the electrical conditions to which the teeth are exposed during application of the ionic tootbrush are determined. Secondly, a method for monitoring the change of bacteria number density on the surface of extracted teeth under the influence of external electric field is presented. After a regular application of the ionic toothbrush this change is detectable, but - from the practical point - negligibly small. The study does not preclude other possible effects of the ionic toothbrush (e.g. electrolysis on teeth surface).

Keywords: ionic toothbrush, electrophoresis, iontophoresis, *Staphylococcus aureus*

1. Introduction

According to the producer, the Hukuba Dental Corporation (Chiba, 270-01 Japan), the advantage of the "ionic toothbrush" over conventional toothbrush types is that it removes plaque not only mechanically, but also with assistance of an electric field. The device claims to take advantage of the phenomenon of iontophoresis (also known as ionophoresis and electrophoresis) which, since early 1950's, has been successfully used in development and application of an alternative technique for sterilisation in endodontic treatment,[1-6] as well as in desensitisation of hypersensitive teeth.[7-14] Besides in dentistry, iontophoresis has been widely used in other research areas, especially in transdermal diagnosis and therapy.[15-18]

Electrophoresis can be defined as movement of electrically charged particles through a liquid (or gas) as a consequence of an externally applied electric field.[19] The construction of the ionic toothbrush is such that the teeth act as a negative electrode, while a metal rod mounted in the toothbrush holder acts as a positive electrode. This means that upon application of the ionic toothbrush all negatively charged particles, including negatively charged bacteria, will in principle move towards the toothbrush holder, i.e., away from the teeth surface. However, at this point several fundamental questions arise which have to be answered before the efficiency of an ionic toothbrush with respect to removal of bacteria can be judged:

1. What is the magnitude of the electrical current flowing during brushing of teeth using the ionic toothbrush?

2. What is the contribution of bacterial flow to the total current flow?

3. Are bacteria indeed negatively charged at conditions existing in mouth during brushing of teeth?

The purpose of the present work is to find answers to these questions. The answers should serve for an estimation of "electrophoretic efficiency" of the ionic toothbrush. In pursuing the goal, two types of experiments were carried out: (i) direct measurements of electrical properties of the ionic toothbrush and ii) indirect measurements of displacement of bacteria (*Staphylococcus aureus*) from (or to) the surface of extracted molars under conditions resembling as close as possible those in mouth during brushing of teeth.

Staphylococcus aureus are a major human pathogen that can infect almost every tissue in the body and is, therefore, all the time present in the oral flora.[20] Although Staphylococcus aureus are not the main culprit for demineralisation of teeth, we have chosen them because their application is well established in microbiology and, in particular, in microscopy. They seldom form long chains and, hence, are easy to observe and count under a microscope. This feature has been found to be essential for a quantitative study of micrographs – only if bacteria are well separated, their number can be determined unambiguously. Here it should be stressed that in the present experimental setup we were not able to distinguish between dead and alive bacteria. Nevertheless, we believe that the specific properties of the selected bacteria do not affect the generality of the findings with respect to electrophoretic properties because the only relevant parameters in this case are the net charge density and mobility of bacteria. Both parameters are within a narrow range for all kinds of bacteria usually found in oral flora, also for those responsible for enamel demineralisation, such as *Streptococcus Mutans*, *Lactobacillus* and *Streptococcus sobrinus*.[21,22]

2. Experimental

2.1. Preparation of the samples

The ionic toothbrush investigated was "THE hyG IONIC TOOTHBRUSH" (Hukuba Dental Corporation) and is schematically shown in Figure 1.

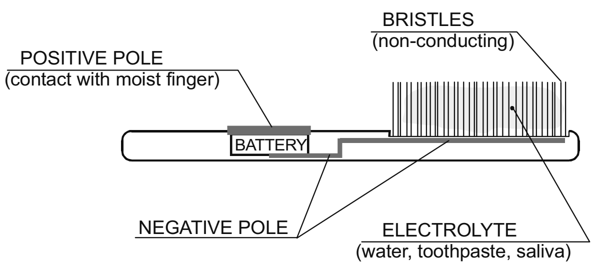

Figure 1. Schematic presentation of the construction of ionic toothbrush.

Agar-cultured *Staphylococcus aureus* were used. The suspensions were made using a physiological solution packed in 2 ml ampules ("BIO MERIEUX" IN VITRO, 70600, France). The concentration was determined using a standard densitometer ("BIO MERIEUX", France). In all experiments, the bacteria concentration was 1.5 Mc Farland ($\approx 4.5 \times 10^8$ bacteria/cm^3).

The teeth used in the experiments were extracted lower molars from adult persons, after being cleaned and stored in a physiological solution at *ca* 0 °C. The root of each molar was cut away and a copper electrode was inserted into the pulp cavity. The rest of the cut area was sealed using a two-component epoxy-based glue. The part of the electrode coming out of the glued area was electrically insulated to prevent a direct contact of the copper wire with the solution (Fig. 2b, upper schematics). The electrode-containing molars were then used either as positive or as negative electrodes in an experimental setup as shown in Figure 2b. A total of 9 teeth, exposed to selected electrical conditions, were investigated.

2.2. Electron microscopy

Prior to microscopy, the molars were coated with gold using a sputter coater (BALZERS, SCD 050). The scanning electron microscopy (JEOL JSM-T220) was carried out with a typical magnification of x3500. Occasionally, other magnifications were used. The quantitative determination of bacterial surface concentration consisted of counting the bacteria at given magnification (× 3500) on 15-25 different surface sites and taking the average value. In total, about 150 surface sites on 9 different teeth were investigated.

2.3. Electrical and pH measurements

Constant electrical current or constant potential was generated using a high voltage source measure unit (KEITHLEY 237). Additionally, voltage, resistance and current were measured using a standard multimeter (FLUKE 79, series II). pH of physiological solutions and suspensions was measured using a laboratory pH-meter (PHM 92 pH meter, Radiometer Copenhagen).

To estimate the electrophoretic efficiency of the ionic toothbrush, the following electric Circuits were constructed:

- Circuit 1 (Fig. 2a) consisted of the following elements: *positive battery pole - ammeter - moist finger - blood vessels - teeth - electrolyte around teeth - negative battery pole*. In the experiment the usual amount of toothpaste was used (*ca* 0.8 g).
- Circuit 2 (Fig. 2b) consisted of: *positive pole of galvanostat - first molar - physiological solution - second molar - negative pole of galvanostat*.

2.3. Statistical methods

The purpose of electron microscopy was to find if the two ranges of observation had equal means. For this purpose, we performed two-sample student's t-tests assuming unequal variances of both ranges (Analysis ToolPak, Microsoft® Excel 97 SR-1).

3. Results

3.1. Electrical properties the ionic toothbrush and other circuit components during brushing of teeth

The open circuit voltage of the battery implemented in the ionic toothbrush was 3.2 V. During brushing of teeth the voltage between the two battery poles dropped to about 2.8-2.9 V. The current flowing through Circuit 1 was 40-60 μA. It was found that the electrical contact between toothbrush and teeth was provided by the electrolyte solution (a mixture from saliva, toothpaste and water) captured among the bristles, while the bristles themselves were found to have very high electrical resistance ($> 10^8\ \Omega$ per 1 cm length and 1 cm^2 toothbrush area). The average circuit resistance R_1' during brushing of teeth was determined by the ratio $R_1' = 2.85\ \text{V} / 0.00005\text{A} = 57\ 000\ \Omega$. Normalising to 1 cm^2 of the contact area between the finger and the contact plate ($A = 1.3$ cm^2) one gets a value of $R_1 = 74000\ \Omega\text{cm}^2$.

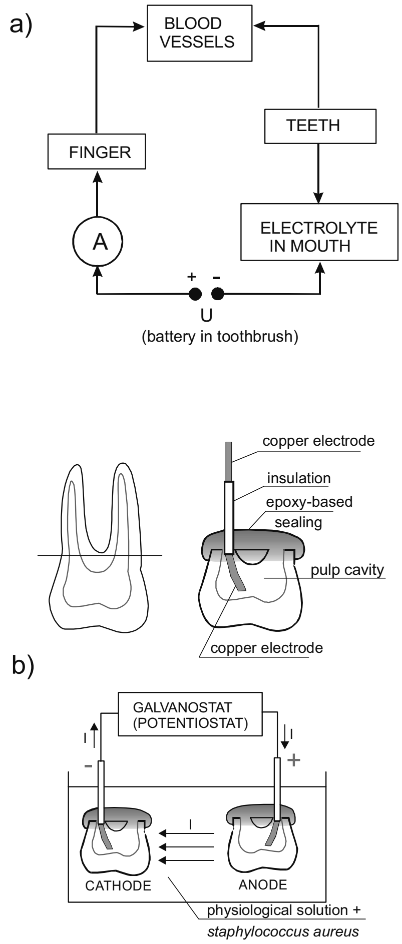

Figure 2. a) Circuit 1 used to test the magnitude of current flow during regular brushing of teeth using ionic toothbrush. b) Preparation of a lower molar to serve as an electrode in electrophoretic experiments (upper schematics) and a typical electrode arrangement in electrophoretic experiments (lower schematics).

The galvanostat in Circuit 2 was set to a constant current of 50 μA. This caused a potential difference between both molars of 2.4 - 2.7 V, depending on the cell geometry (*i.e.* mutual position of the molars, their size, the distance between them etc.). Due to the complex geometry, the actual (*i.e.* effective) surface area of molars through which the current flowed was difficult to estimate, but the value should not be less than 0.5 cm^2 and not more than 1.5 cm^2. If so, the resistance of 1 cm^2 of a molar's surface, R_2, should lie between 14000 and 42000 Ωcm^2.

3.2. Influence of external electric field on bacteria surface concentration

Figure 3 shows three typical electron micrographs of molar surfaces that had been exposed to a 1.5 Mc Farland dispersion of *Staphylococcus aureus* at various electrical conditions. It was found that the bacteria surface concentration (*i.e.* the number density) as well as their surface distribution depended on the following factors: the magnitude, the direction and the time of the current flowing through the circuit and on the total time of exposure of molars to the dispersion. For example, at a current density of 50 μA, or lower, uniform distributions such as that shown in Fig. 3b

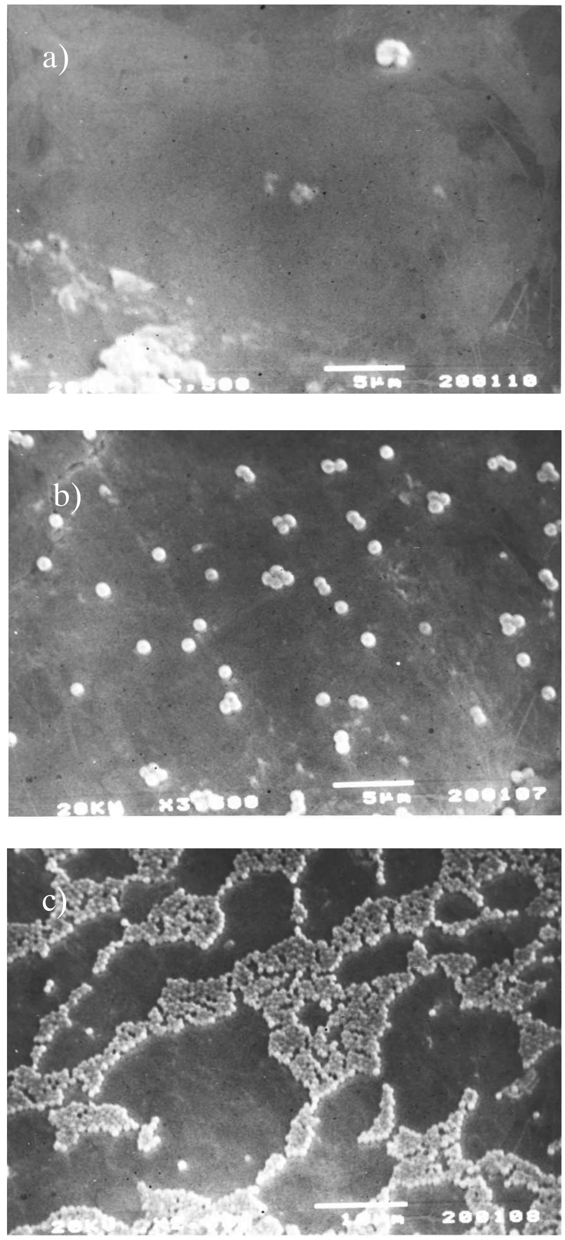

Figure 3. Typical scanning electrode micrographs of lower molar surfaces after being electrophoretically treated in a 1.5 Mc Farland dispersion of *Staphylococcus aureus*. a) Cathode (negative electrode) exposed to the bacteria dispersion for about 1h; b) anode (positive electrode) after 30 minutes of exposure, and c) anode after 2 h of exposure to the bacteria dispersion. In all cases, a constant current of 50 μA flowed through the electrodes.

could be obtained at exposure times from several minutes to about 1 h. By contrast, current densities of the order of magnitude of 1 mA/cm^2, and higher, always resulted in non-uniform distributions of the type presented in Figure 3c. Figure 3a, which shows a molar surface almost free of bacteria, was typical of negative electrodes (cathodes) exposed to the bacteria solution at prolonged times – the higher the current the shorter was the time leading to such results.

Figure 4 shows 21 surface concentrations determined on a positive electrode (anode), a negative electrode (cathode) and on a reference molar, all exposed to the same *Staphylococcus aureus* dispersion for 30 minutes. While the current through the anode and the cathode was fixed at 50 μA, the reference molar was not exposed to electric field. The concentrations for the anode (upper horizontal line) are significantly different from the concentrations for the reference molar (middle horizontal line) (P = 0.00051 for the two-tail t-Test). Similarly, the concentrations on the cathode are significantly different from those on the reference molar (P = 5.6×10^{-10}). The difference between the anode and the cathode bacteria concentration is even more pronounced (P = 9.2×10^{-11}).

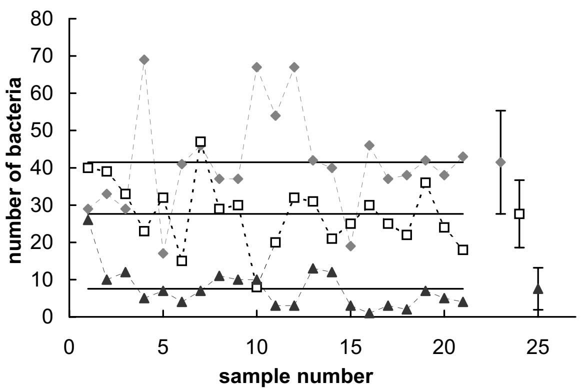

Figure 4. Surface concentrations (number densities) of *Staphylococcus aureus* determined on 21 anode (diamonds), 21 cathode (triangles) and 21 reference (open squares) electrode surfaces. All electrodes were exposed to a dispersion of *Staphylococcus aureus* for a period of 30 minutes. Additionally, a constant current of 50 μA flowed through the anode and the cathode. The horizontal lines represent the means. The vertical lines on the right correspond to the standard deviations.

In a second experiment, the time of anode and cathode exposure was reduced from 30 to 2 minutes (a typical time of brushing of teeth). The average bacteria concentration on the anode was by 32% higher than that on the cathode (see Table 1). Again, the difference was statistically significant (P = 0.01249).

In a third experiment the time of anode and cathode exposure was 1.5 h at a constant current of 50 μA. On the anode, the surface distribution of

Table 1. Rows 1-15: Surface concentrations (number densities) of *Staphylococcus aureus* determined on 15 anode and 15 cathode surfaces (magnification: × 3500). Both electrodes were exposed to a dispersion of *Staphylococcus aureus* for a period of 2 minutes at a constant current of 50 μA. Rows 16-19: statistical analysis of the observations.

Observation No.	Anode	Cathode
1	10	6
2	7	4
3	11	4
4	8	6
5	8	7
6	6	5
7	7	7
8	4	9
9	9	6
10	9	6
11	6	5
12	8	4
13	11	4
14	6	5
15	6	6
Mean:	7.733	5.600
Variance:	4.066	1.971
P(T<=t) two-tail	0.01249	
t (critical) two-tail	2.060	

bacteria was no longer uniform – colonies such as those in Figure 3c were observed on most of the molar surface. In several cases three-dimensional clusters of bacteria were observed. On the other hand, most of the cathode surface was free from bacteria. The absence of any regular bacteria pattern on both electrodes prevented us from performing a quantitative analysis as in previous two cases. Nevertheless, this experiment clearly demonstrated that the external electric field caused a pronounced shift of bacteria from the cathode towards the anode.

4. Discussion

4.1. Electrical properties of the ionic toothbrush

Circuit 1 studied in the present investigation is actually the circuit that, according to the producer, should be formed during the everyday application of the ionic toothbrush. The results show that the average current flowing through such a circuit is about 50 μA. However, from the classical iontophoresis it is well-known that currents of the order of 1 mA have to be generated in order to perform a successful sterilisation in endodontic treatment.[1-6] As mentioned before, relatively high currents are essential to devices based on electrophoresis because the electrophoretic movement of particles is directly proportional to the magnitude of current flow.

Because during brushing of teeth the current is spread over the entire teeth surface (*e.g.* over about 30 teeth) and, indeed, even over gingiva etc., this further reduces the electrophoretic efficiency of the ionic toothbrush by a factor of 30 or more. So, the higher limit for the average current density (i.e. current per unit surface area) produced by the ionic toothbrush is estimated to be about $10 \times 30 = 300$ times smaller than in the case of the classical iontophoresis (assuming equal time of both treatments).

4.2. Influence of external electric field on bacteria surface concentration

Although it is well-known that bacteria do move in electrolyte solutions under the influence of external electric field, this does not necessarily imply that electric field can also remove bacteria from surfaces to which they are attached, usually with van der Waals forces.[22, 23] All results obtained in the electron microscopy, however, show clearly that a constant current of 50 μA (which coincides with the average value generated by the ionic toothbrush) is able to cause a measurable displacement of bacteria away from the surface of the tooth acting as cathode and onto the surface of the tooth serving as anode. The same results also indicate that at the given current density the number of displaced bacteria increases with duration of the current flow. Comparing the results in Figure 4 and in Table 1, it is possible to calculate the number of displaced bacteria under conditions given during normal application of the ionic toothbrush. As mentioned before, these conditions are similar to those corresponding to Table 1 (2 minutes and 50 μA), except that the current is now spread over about 30 times larger surface area. Dividing the results of Table 1 with 30, one gets 0.031 displaced bacteria. The number relates to a tooth surface such as seen under microscope at a magnification of ×3500, in our case about 800 μm^2. It is known from literature[22] that plaque consists from a dense network of bacteria and on an area of 800 μm^2 their number can easily reach several thousands. Obviously, under such circumstances, the displacement of 0.031 bacteria represents a negligible effect.

The results shown in this study point out at another problem related to the concept of use of electrophoresis in designing an “ionic” toothbrush. As mentioned several times, the bacteria number density was reduced on the cathode and increased on the anode surface. This means that under given conditions the bacteria were charged negatively. Upon application of the ionic toothbrush under the same conditions, the bacteria would flow in the expected way, that is, from the teeth surface towards toothbrush. However, it is well-known that the net charge of many colloidal particles depends on pH of the surrounding solution.[19,23] At pH values below the isoelectric point the net charge is positive, while at pH values higher than the isoelectric point the net charge is negative. The isoelectric point of bacteria usually lies between 4 and 6.[1] Knowing that in our experiments the value of pH was about 7, the negative charge of bacteria is not surprising. On the other hand, the pH of plaque may vary from 7 to less than 5.[24] It is probable that at the lower margin many types of bacteria in the plaque will be positively charged, although some will still be charged negatively. In practice, the situation is probably even more complex, since one can expect considerable spatial variation in pH, and hence in bacteria charge, along the inhomogeneously colonised teeth surface.

In a conclusion, one can realize that due to the presence of oppositely charged bacteria on the teeth surface, already the basic idea of their removal using electrophoresis may be questionable. Potentially, such an idea could be realised under relatively harsh pH conditions, i.e. at pH values below 4 or above 8. Of course, an iontophoretically effective device should be able to transport much more charge (the product between time and current) than the existing toothbrush. This study has shown that in the present configuration, a 30 hour-cleaning would be required in order to remove most of bacteria from teeth surface.

The results of this investigation are seemingly in contradiction with several clinical studies[26] which report on statistically significant improvements in Loe Gingival Index scores and the Quigley-Hein Plaque Index scores after a several-month use of the ionic toothbrush. The present study does not preclude such possibilities. Electrophoresis is only one of the several phenomena that can occur in electrochemical systems upon application of external electric field. Electrolysis might also be important. During electrolysis, new species, such as H_3O^+ or OH^- ions, are formed. These species can have a pronounced bactericidal effect, but now due to the phenomenon of electrolysis, a fundamentally different process than the iontophoresis.

5. Conclusions

1. The current generated during application of the “so-called” ionic toothbrush is 40-60 μA, a value much lower than used in the conventional iontophoresis (ca. 1 mA).

2. The average current generated by the ionic toothbrush (50 μA) is able to shift the model bacteria

(*Staphylococcus aureus*) from negatively to positively charged tooth surface. However, during a typical 2-minute brushing of teeth, the number of displaced bacteria on a given surface area was found to be negligibly small compared to the typical number of bacteria in plaque.

3. An important point in designing an ionic toothbrush seems to be the fact that the surface charge of bacteria is critically pH-dependent. Non-uniform plaque distribution on teeth surface may cause that on different areas bacteria will be oppositely charged - even if they have similar isoelectric points (which, generally, is not the case). This fact makes a future realisation of the idea of electrophoretical removal of dental plaque very difficult - unless extreme pH conditions were used.

4. Although the ionic toothbrush could not efficiently remove the model bacteria (*Staphylococcus aureus*) used in the present study, the effect should not be generalised to all kinds of bacteria and to all possible conditions in the oral cavity.

5. Ionic species that are the products of electrolysis may have bactericidal properties. This possibility remains a challenge for the future studies.

6. Acknowledgements

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Povzetek

Preverili smo, ali ionska ščetka zaradi svojega elektroforetskega delovanja bolj učinkovito odstranjuje bakterije od navadne zobne ščetke. Najprej smo podrobno preučili pogoje, ki so jim izpostavljeni zobje pri uporabi ionske ščetke. Podobne električne pogoje smo nato uporabili na izdrtih zobnih kočnikih, v katere smo predhodno vstavili bakrene elektrode ter jih izpostavili raztopinam z izbranimi koncentracijami bakterije *Staphylococcus aureus*. Spremembe v površinski koncentraciji bakterij zaradi delovanja zunanjega električnega polja smo opazovali z *ex-situ* vrstično elektronsko mikroskopijo. Pri dolgotrajnem delovanju zunanjega električnega polja na posamezen zob se je površinska koncentracija bakterij sicer statistično signifikantno zmanjšala, vendar je učinek premajhen, da bi pomembno vplival na zmanjšanje površinske koncentracije bakterij pri predvideni uporabi ionske ščetke. Študija ne izključuje drugih možnih električnih učinkov pri uporabi ionske zobne ščetke, denimo elektrolize na zobnih površinah.