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SHORT COMMUNICATION

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Evaluation of a skin reference electrode used for intraoral pH measurements in combination with a microtouch electrode

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Abstract The aim of this in vivo study was to evaluate a skin reference electrode used with the microtouch method for measurements of intraoral pH. Registrations of plaque pH were made in ten healthy subjects before and up to 30 min after a mouth rinse with 10% sucrose. A microtouch electrode (Beetrode) was used in combination with two different half-cell reference electrodes: a skin silver-silver chloride plate or a glass capillary reference electrode. The skin electrode was placed on the subject's forearm in combination with a conductive gel. The glass capillary electrode, together with one of the test subject's fingers, was placed in a beaker with a 3 M KCl solution. High agreement between the two reference systems resulted in almost identical plaque-pH curves (r=0.97). The mean differences between the two systems at the different time points varied between −0.15 and +0.01 pH units, with an overall mean pH difference of 0.03 pH units. It can be concluded that the use of the skin reference electrode may very well replace the traditional reference electrode used in combination with a microtouch electrode for intraoral use.

Keywords Dental plaque · Electrode · Plaque pH

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Introduction

Plaque pH is an important variable in relation to different oral manifestations such as the development of dental caries and dental erosion. The pH value is directly or indirectly determined by a number of external and internal factors such as fermentable carbohydrates, cariogenic microorganisms, dental plaque composition, saliva, oral motorics and tooth anatomy [15]. After a challenge, changes in pH are often followed over a certain time period and the data are presented in a pH curve, as originally described by Stephan [18]. The registration of plaque pH has become an important tool in assessing the individual risk of caries [2], determining food cariogenicity [9, 13] and evaluating sugar-free substances [5].

Since the pH process was first described, different methods for registering plaque pH have been developed [6, 10, 12]. Nowadays, three methods are primarily used: the sampling, the telemetric, and the microtouch methods [10]. For the microtouch method, which is currently the most commonly used, a hand-held microelectrode is inserted into the area of measurement [10, 16]. The method requires a reference electrode to have a closed circuit for measurement. Independent of the pH, the function of the reference electrode is to maintain a constant potential in comparison to the object. For this purpose, the reference electrode, together with one of the test subject's fingers, is placed in a beaker with a 3 M KCl solution in order to create a salt bridge between the reference electrode and the subject [10, 16]. One disadvantage of this reference system is that it has been found to be complicated for patients who are unable to cooperate fully, as the test subject needs to keep both his/her head and finger in a stable position during measurement. Within the medical field, other reference systems can be found. For the in vivo recording of esophageal pH, a reference electrode is attached to the skin of the arm [7]. An electrically conductive electrode gel is used to build the salt bridge between the electrode and the subject. This reference system is also well established in bioelectrical

recording [14] but has, to our knowledge, been used to a very limited extent within the oral field. However, a combination with electrodes mounted in a splint [11, 17] and the touch method [4] is described.

The aim of this study was to evaluate the clinical performance of a skin reference electrode in combination with a microtouch electrode for intraoral measurements.

Material and methods

Subjects

Ten healthy volunteers (three men and seven women), with a mean age of 47 years (range 32–55 years) and a mean DMFS of 41 (range 4–75), participated in the study. Their mean stimulated salivary secretion rate was 1.8±1.0 and their buffer capacity was 5.9±1.8. They all performed good oral hygiene and were known to be able to lower their plaque pH by at least 1 pH unit after exposure to a sugar challenge. The study was approved by the ethics committee at the University of Umeå. Written consent was obtained prior to study.

Experimental design

The tests were performed at the Dental School, University of Umeå. Prior to the test session, the subjects were instructed not to clean their teeth for 3 days. On day 4, the volunteers were not allowed to eat, drink or use tobacco for 2 h prior to the measurement. After measuring baseline plaque pH (0 min), the subjects rinsed for 1 min with 10 ml of a 10% sucrose solution (SIGMA, St. Louis, MO, USA). Plaque pH was then measured at 2, 5, 10, 15, 20 and 30 min after the mouth rinse.

Measurement of plaque pH

Two identical pH systems were set up. For both, plaque pH was measured using an iridium microtouch electrode with a diameter of 0.1 mm (Beetrode; NMPH-1, W.P. Instruments, Sarasota, FL, USA) [16]. The pH electrode was connected to a pH/ISE meter (Orion SA 290 A and Orion SA 720, respectively; Orion Research, Boston, MA, USA). One of the microtouch electrodes was combined with a skin reference electrode and the other with a glass capillary reference electrode, both of which were half-cell reference electrodes. Before each test session, both pH electrodes were calibrated against standard buffers at pH 7.00 and 4.01 for verification of the electrode slope and function (Thermo Orion, Beverly, MA, USA) [10, 16]. Calibrations were also made prior to each time point. Measurements were carried out at two sites in the upper jaw, in the premolar/molar and front regions. All the measurement sites were free from amalgam or other metal restorations. The pH electrodes were inserted into the proximal plaque, cervical of the contact point.

At each time point, plaque pH was measured consecutively with the two systems with only a few seconds between the two measurements. After registering pH with the first system, measurements were then repeated directly using the second system. The order of the two systems was randomly alternated from one subject to another, but they were used in the same order for each individual. Two operators (AP and PL), both experienced when it came to using the microtouch method, performed the measurements. The electrode was inserted into the proximal area by one person and the value, when stabilized, was read independently on the pH meter by the other.

Skin reference electrode

For this system, a plate of silver-silver chloride (ECG: type Syntectics Medical, Stockholm, Sweden) was applied on either the left or right forearm [4, 11, 17]. Prior to application, the skin was cleaned with 45% isopropanol (M-Ytdes, Kemetyl AB, Haninge, Sweden), after which a conductive electrode gel was applied to the electrode plate (Lectro Derm, Viroderm, Stockholm, Sweden). The plate was then placed on the forearm and attached with an adhesive plaster, after which it was connected to the pH meter. After attachment to the skin and before each measurement, a one-point calibration was performed with the subject's finger and the pH electrode in a beaker containing standard buffer pH 7.00 (Thermo Orion).

Glass capillary reference electrode

For the second system, a glass capillary reference electrode (MERE 1, W.P. Instruments) was connected to the pH meter. A salt bridge was created during measurements by having the test subject to dip one of his/her fingers into a beaker with a 3 M KCl solution into which the reference electrode was also placed [16]. Prior to the measurements, the pH electrode was calibrated against the standard buffer pH 7.00 (Thermo Orion).

Statistical analysis

The data were analyzed separately for the two sites, as well as for the mean of the two sites. The difference between the two pH systems was calculated for each time point, after which the mean pH difference for the seven sites was calculated. The total area of the pH response curve below pH 5.7 ($AUC_{5.7}$) and pH 6.2 ($AUC_{6.2}$) was calculated for each individual pH curve. To compare the two systems, the correlation coefficient (r) was calculated. Two-way analysis of variance (ANOVA) was used to test the significance of the different pH variables. When the ANOVA rejected the multisample hypothesis of equal means, multiple-comparison testing was performed with Fisher's PLSD. Testing was performed at the 5% significance level.

Results and discussion

Out of a total of 140 pH measurements, data from five registrations were excluded for technical reasons. No statistically significant differences were found between the premolar/molar and front regions and, as a result, only mean values for the two sites are presented. The present study revealed a high correlation, both when calculated as average figures and when analyzed on individual basis, between the two reference systems when used for intraoral measurements with the microtouch method. The two reference systems resulted in almost identical mean plaque pH for the ten individuals during the whole of the 30-min test period, with the lowest pH values registered at 10 min (Fig. 1, Table 1). When all the time points were taken together, the mean plaque pH was 5.53±0.72 for the system using the skin reference electrode and 5.58±0.64 when using the glass capillary reference electrode (Fig. 2). Overall, a high agreement between the two reference systems was found with a correlation coefficient (r) of 0.97 for the mean pH at the different time points (Fig. 3).

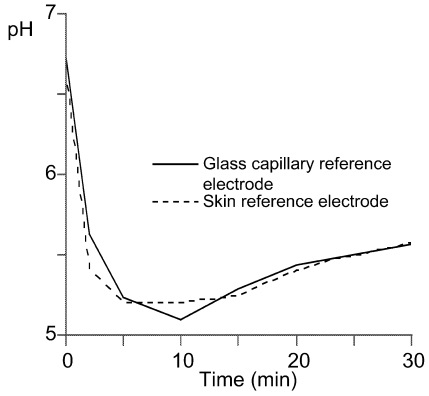

Fig. 1 Changes in pH of human dental plaque after a mouth rinse with 10% sucrose when using the skin and the glass capillary reference electrodes in combination with a microelectrode. n=10

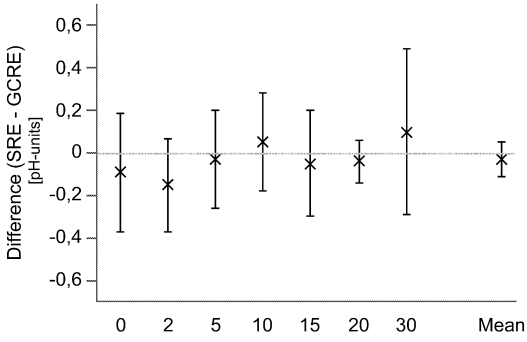

Fig. 2 Mean difference (±SD) in plaque pH between the skin reference electrode (*SRE*) and the glass capillary reference electrode (*GCRE*) at the different time points. The mean for seven time points is also shown. n=10

The small mean difference found between the two systems, 0.03±0.08 pH units all time points taken together, cannot be attributed to the new reference system. This is most probably due to biological and technical factors. The small discrepancies between the two systems varied not only from one individual to another but also from one time point to another within the same individual. This is explained by the fact that pH is a metabolic process influenced by several biological and behavioral factors and therefore continuously undergoes changes. Minor changes in saliva exposure to the area of measurement may influence the pH value as well as tongue and cheek movements. Furthermore, when measurements, like those

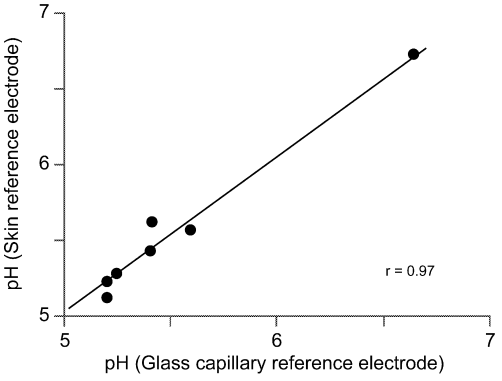

Fig. 3 Correlation between the skin and the glass capillary reference electrodes. Each *circle* indicates the mean pH value at one time point. n=10

in the present study, are carried out after a mouth rinse with sucrose, a time factor is involved. During the first phase of the pH curve (0–15 min), the metabolic activity is high and there is a dramatic change in pH from the initial resting level. This is a result of fermentable carbohydrates broken down by cariogenic microorganisms forming acidic end products. When the two microelectrodes are inserted into the plaque area, even if they are in the same approximal site, both the time delay and the difficulty involved in reaching exactly same localization in the dental plaque are also strong explanations of the minor differences that were found between the registrations with the two pH systems. However, in the latter phase of the pH curve (15–30 min), the metabolic process is slower and the time span between measurements is therefore a subordinated factor. An interesting finding was the high correlation found between the two reference systems when comparing the total area of the pH response (AUC). For the skin reference electrode, $AUC_{5.7}$ was 13.1±12.1 and $AUC_{6.2}$ 24.9±15.4 (min × pH). The corresponding values for the glass capillary reference electrode were 11.5±7.4 for $AUC_{5.7}$ and 24.2±9.6 (min × pH) for $AUC_{6.2}$, resulting in correction coefficients of r=0.81 respective r=0.86 for the two pH levels.

From a technical point of view, the skin reference electrode was regarded as being easier to handle as it requires less cooperation from the test subject. The traditional system in which a salt bridge is created by having the test subject dip one finger into a KCl solution, into which the reference electrode is also inserted, is known to be complicated for test subjects such as young

Table 1 Mean (±SD) plaque pH data at the different time points registered using the skin-placed and glass capillary reference electrodes for intraoral measurement in combination with the microtouch method. The mean differences between the two systems are also presented

Time (min)	Skin electrode	Glass capillary electrode	Difference in mean pH units
0	6.64±0.48	6.73±0.28	−0.09
2	5.41±0.56	5.63±0.43	−0.22
5	5.20±0.53	5.23±0.46	−0.03
10	5.20±0.57	5.10±0.30	+0.10
15	5.24±0.55	5.29±0.39	−0.05
20	5.40±0.61	5.44±0.49	−0.04
30	5.59±0.64	5.58±0.47	+0.01

children and some elderly people. The plate has a larger contact area, is more stable and is not fragile compared with the glass reference electrode. It is placed closer to the sensing electrode than the glass capillary electrode, which may reduce "noise" [3]. The skin electrode is also comfortable for the operator to use, as full concentration can be kept on the hand-held electrode. The results of this study revealed no significant differences in pH readings between the two systems. As a result, this type of electrode would be suitable for all groups of subjects, especially those that have difficulty cooperating with the conventional glass capillary reference electrode. Since this study was performed, the skin reference electrode has been used successfully by us in bedridden subjects in a nursing home.

In the present study, it was important to measure and compare the two methods at several sites in one and the same subject. The handling of both systems by one and the same operator and the independent readings increase the level of objectivity [1, 8]. It can be concluded that the use of the skin reference electrode, in combination with the microtouch electrode, has high validity and may very well replace the traditional reference electrodes for intraoral use.

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