**ABSTRACT**

**Aim** This study was performed to evaluate the effect of a glucose rinse and of plaque accumulation on pH of tooth surface in healthy adolescents with a device used in gastroenterology and never tested in the oral cavity.

**Methods** Values of pH were monitored in 12 adolescents using a portable device (pH-day 2® Menfis, bioMedica S.r.l., Bologna, Italy) with a disposable antimonium electrode kept in contact with the interproximal surface of the upper molars for 40 minutes respectively before and after a one-minute rinse with 10 ml of a 10% glucose solution. The same procedure was repeated in the same subjects after 72 hours of plaque accumulation.

**Results** The device tested resulted difficult to use on the tooth surface because of the size of the active part of the probe. The glucose rinse caused a statistically significant decrease of the mean pH, restrained in basal conditions (d = -0.16, p <0.05), clinically relevant after plaque accumulation (d = -1.24, p <0.05). Time in minutes of pH < 6 grew considerably only in case of combination of plaque accumulation and glucose rinse (d = 20.90, p <0.05). A Stephan’s curve of drop and recover of the pH values was not recorded.

**Conclusions** The continuous recording of pH of natural plaque present on the tooth could represent an alternative to other techniques found in literature. More studies are necessary to verify the suitability of this new device for the monitoring of pH in the oral cavity.

**Keywords** Glucose rinse; pH; Plaque accumulation.

**Introduction**

In recent years researchers have been trying to identify simple methods which can easily be used in clinical as well as research settings for plaque pH registration. This pilot study aimed to test a device commonly used in gastroenterology for 24-hour monitoring of pH of oesophagus and stomach (pH-day 2® Menfis; bioMedica S.r.l., Bologna, Italy) which has never been used in the oral cavity.

Dental caries is still a common disease among children and adolescents. Before approaching the adolescent with caries, the healthy patient represents an important model in order to understand how negative ecological conditions on dental biofilm interact with the defensive mechanisms of the oral cavity. It has been suggested that in healthy subjects pH does not follow the typical Stephan’s curve of pH fall after a glucose challenge [Stephan, 1944] and that a drop of pH after assumption of fermentable carbohydrates is not predictable [Fejerskov et al., 1992] in this kind of patients. Development of dental caries is strictly related to diet, saliva secretion and presence of a bacterial biofilm on the dental surfaces [Loesche, 1986; Marsh, 1994; Kleinberg, 2002; Selwitz et al., 2007]. The frequent assumption of fermentable carbohydrates and the consequent variation of pH caused by the bacterial flora can induce alterations in the biofilm in which the microorganisms are organised [Rosen and Weisenstein, 1965; van Houte et al., 1996]. A low pH creates conditions favourable to acidogenic bacterial species capable to maintain the pH low and cause demineralisation of the dental enamel [Marsh, 2006; Welin-Neilands and Svensäter, 2007]. Environmental acidification seems to be the main determinant of the phenotypic and genotypic changes that occur in the microflora during caries [Takahashi and Nyvad, 2011].

In order to achieve a better understanding of the ecological balance on dental enamel and of the role of pH in the maintenance of this balance it is necessary to develop study models which interfere as little as possible with the oral homeostasis. Since pH tends to drop with dehydration of the dental surface, the measurement should be performed in the presence of saliva [Abelson and Mandel, 1981]. The measurement of pH should not be performed on a dry surface, keeping the patient’s
mouth open and the lips away from the teeth. Also it should not disaggregate the plaque on the dental surface. Moreover, a huge amount of biofilm like the one used in the Zurich method [Graf and Mühlmann, 1965; 1966; Graf, 1970; Imfeld, 1983] may produce patterns of pH drop and recover not reproducible in the healthy patient and the kind of biofilm analysed in that model is formed on an artificial surface [Preston and Edgar, 2005].

The aim of the present study was to evaluate the impact of a glucose rinse and plaque accumulation on the pH of the dental surfaces of healthy adolescents with a new device capable of a continuous recording of pH without interfering with the salivary clearance of the patient.

Methods

A sample of 12 adolescents 15 to 18 years-old was selected among the patients of the Dental School of the University Insubria of Varese, Italy. All the subjects were free from systemic and periodontal diseases and had no history of caries. A consent form explaining the aim and the characteristics of the clinical trial was read and signed by the parents of the participants. Each subject was assigned an identifying code according to the Italian law on privacy. The research was ethically conducted in accordance with the Declaration of Helsinki and consent of an appropriate ethical committee was obtained prior to the start of the study.

The subjects refrained from eating, drinking and tooth cleaning for two hours before pH evaluation. All measurements were performed in the morning.

Assessment of the acidogenicity of the dental surface was performed with a portable device used in gastroenterology for 24-hour monitoring of pH of oesophagus and stomach (pH-day 2® Menfis, bioMedica S.r.l., Bologna, Italy). This device records pH continuously with a sampling period of 1-6 seconds, an accuracy of 0.1 p, and a pH range of 0.1-14. The active part of the probe is a unique disposable device consisting of an antimonium electrode with a reference electrode covered by resin (2.0 mm diameter) connected to the pH-meter with a thin and flexible cable which can be easily brought through the mouth-angle of the subject. The electrode was kept in contact with the interdental space of the upper molars (teeth 16 and 17) using an orthodontic elastic separator positioned between these teeth, which permitted to hold the active part of the probe in the interproximal space without interfering with the oral homeostasis.

The pH was monitored for 40 minutes in basic conditions (“at rest”). Subsequently the subjects rinsed for 1 minute with 10 ml of a 10% glucose solution. The pH was monitored again for 40 minutes. The test was repeated with the same pattern on the same subjects after 72 hours of plaque accumulation during which the subjects refrained from tooth brushing and other tooth cleaning activities. The device produced a graphic of the pattern of pH values for each test. A single researcher analysed all the graphics. For each test, the device also computed the mean pH value and the time in minutes during which the pH was below the value of 6.

Paired t-tests were used to study whether the means of pH and of time below the value of 6 differed for the 12 subjects in the experimental conditions. The non-parametric version of the paired samples t-test, the Wilcoxon signed rank test, was also used with similar results, which are therefore reported.

Results

The experimental procedure was completed without complications and was well tolerated by the patients.

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The effects of a glucose rinse and a 72-hour plaque accumulation on the pH of the dental surface in healthy
adolescents were evaluated with a device used for monitoring pH of the stomach never tested in the oral cavity. Plaque accumulation or glucose rinse alone did not produce an important pH-lowering effect on the dental surface. It was necessary to combine glucose rinse and plaque accumulation in order to observe a significant and clinically relevant drop of pH. One weak point of this study is the limitation in the sample size. However, the approach to analysis was very conservative with results confirmed by non-parametric tests. In fact the Wilcoxon signed-rank test did not assume that the difference between the sets of pH values was normally distributed.

A typical fall of pH following a curve of decrease and a slow recover towards basal values is well known in the literature since 1940, when Stephan [1944] presented his studies in caries patients; the author used a “touch” technique keeping an antimony electrode connected with a pH-meter on the dental surface. Different methods of pH recording of the dental surface are proposed in the literature. The most popular is the Zurich method of telemetric measurement with the electrode fixed in a removable prosthetic appliance (the “indwelling” electrode). This method allowed a continuous evaluation of pH [Graf and Mühlemann, 1966; Graf, 1970] and the study of the effect of food, beverages and different dental hygienic devices on the pH of plaque [Imfeld, 1983]. Imfeld [1992] tested several types of food with this procedure and classified as “tooth friendly” food that did not lower the pH below the critical value of 5.7 when in contact with accumulated plaque. The plaque in the Zurich method is accumulated on an artificial surface and is not reproducible in healthy patients [Preston and Edgar, 2005].

In our study we obtained a continuous registration of pH values using the “touch” technique and fixing the active part of the pH-meter in the interproximal dental space. The device resulted challenging to position and to keep in contact with the interproximal dental space because of the dimension of the recording part of the probe. However, with the help of an orthodontic elastic separator and an accurate control of the patients’ movements it was possible to keep the probe in position for 40 minutes. As stated by Schachttele and Jensen [1982] it is important to record pH at sites where caries is frequent in order to obtain data relevant to disease, so the interdental surface represents the gold standard to analyse acid production. The device tested in our study appears to be more suitable for measuring pH of saliva rather than the dental surface. The reference electrode and the active antimony electrode are positioned in the same tip connected to the pH-meter with a thin and flexible cable, recording on the dental surface resulted difficult considering the dimensions of the active tip. However the possibility of 24 hours of monitoring and the portability of the instrument represent an interesting opportunity for several experimental applications. A pH decrease was observed when the dental surface

<table>
<thead>
<tr>
<th>AT REST</th>
<th>AFTER GLUCOSE RINSE</th>
<th>AFTER 72 HOURS OF PLAQUE ACCUMULATION</th>
<th>AFTER 72 HOURS OF PLAQUE ACCUMULATION AND GLUCOSE RINSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Mean pH</td>
<td>Minutes of permanence at pH&lt;6</td>
<td>Mean pH</td>
</tr>
<tr>
<td>Patient 2</td>
<td>6.4</td>
<td>5.8</td>
<td>6.3</td>
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<td>Patient 3</td>
<td>7.1</td>
<td>0.1</td>
<td>6.6</td>
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<td>Patient 4</td>
<td>6.7</td>
<td>2.1</td>
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<tr>
<td>Patient 5</td>
<td>6.4</td>
<td>14.1</td>
<td>6.2</td>
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<tr>
<td>Patient 6</td>
<td>6.8</td>
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<td>Patient 8</td>
<td>6.9</td>
<td>0.1</td>
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<td>Patient 9</td>
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<tr>
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<tr>
<td>Patient 11</td>
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<td>6.5</td>
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<td>Patient 12</td>
<td>6.2</td>
<td>7.2</td>
<td>6.1</td>
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**TABLE 1** Comparison of mean pH values and time in minutes of permanence at pH<6, at rest and after a glucose rinse.

**TABLE 2** Comparison of mean pH values and time in minutes of permanence at pH<6 after 72 hours of plaque accumulation, at rest and after a glucose rinse.
was dehydrated moving away the lips from teeth and obstructing the normal circulation of saliva in the mouth. A rapid recover to the basal values occurred when the patient closed the mouth, swallowed and restored the normal salivary clearance. This observation is in agreement with the results of Abelson and Mandel’s study [1981], in which the pH tended to increase when the access of saliva to the dental surface was increased. Also other authors have stated the crucial role of saliva in controlling the equilibrium of de- and re-mineralisation and the importance of saliva in the design of experiments regarding plaque pH [Edgar and Higham, 1995; Hay, 1995]. Lingström and coworkers [2000] recorded lower levels of pH after a sugar rinse under conditions of limited salivary access.

After 72 hours of plaque accumulation healthy patients still showed a very thin layer of plaque, more evident in the interdental spaces where self cleaning guaranteed by tongue, mucosa movements and saliva circulation on teeth are less efficient. As reported by Dawes and Dibdin [1986] plaque thickness can affect the pH fall after the sucrose challenge, in particular a too thin or very thick plaque layer can produce a small pH fall. Without plaque accumulation the glucose rinse produced very weak, albeit significant, variations of the pH values in comparison with the values obtained at rest (Table 1). Similar results were obtained by Kleinberg [1961] who also used an antimony electrode and he postulated that high sugar concentrations could cause substrate inhibition. Frostell [1969] removed natural dental plaque and tested it in vitro and found that 50% glucose solution caused lower and more prolonged minimum pH values than when using a 5% glucose solution.

Conclusion

Our experimental procedure could represent an interesting approach to pH measurement with less interference with the homeostasis of the oral cavity. The disposable antimony probe used seems to be more suitable for recording of pH of saliva rather than of the dental surface. The presence of the reference and active electrode in the same tip represents a simplification in the procedure but requires that both electrodes needs to contact the same surface, so a smaller tip could be more suitable for the use on the dental surface. More studies are necessary to confirm this observation. Limited variations of pH were observed after plaque accumulation without the glucose rinse (Table 2). This range of pH values is above the level of enamel demineralization and therefore has no clinical relevance. In order to achieve a considerable drop of pH on the dental surface it was necessary to combine plaque accumulation and glucose rinse. In this case the time of permanence at values of pH that can cause enamel demineralisation grew considerably (Table 2). This observation supports the dynamic relationship between the parameters responsible of clinically significant variations of pH on the dental surface as reported by other authors [Marsh, 2006; Lingström et al., 2000].

The continuous recording of pH of natural plaque present on the dental surface could represent an alternative to other methods found in literature, however further studies are necessary to validate the device tested.

References