VALIDATION OF AN ALTERNATIVE ABSORBENT PAPER FOR COLLECTING GINGIVAL CREVICULAR FLUID

Validação de um papel absorvente para coleta de Fluido Crevicular Gengival

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INTRODUCTION

The presence and volume of gingival crevicular fluid (GCF) can be indicative of changes to periodontal tissues that are the consequence of inflammatory reactions of the host, triggered by aggression from the dental biofilm (Linden et al., 2002). GCF is an inflammatory exudate and as such contains components known as biological markers (Loos & Tjoa, 2005) inflammatory mediators and antibodies that originate from connective tissues (Ciantar & Caruana, 1998; Loos & Tjoa, 2005).

GCF is constantly secreted, initiating before periodontal structural changes can be detected by physical means (Del Fabbro et al., 2001). According to Del Fabbro et al., (2001) and Griffths (2003), the volume of GCF collected is directly proportional to the stage of periodontal inflammation, which underscores its importance as an assessment instrument.

GCF can be collected by a variety of methods, such as suction, lavage or absorption (Gustafsson, 1996; Ciantar & Caruana, 1998; Griffths, 2003). Absorption into strips of absorbent paper (Periopaper®) is traditionally used and is considered noninvasive and easy to carry out (Deinzer et al, 2000). However, no studies so far have validated
alternative methods for collecting GCF, specifically with respect to their absorption capacity and to the accuracy of measurements in comparison with Periopaper® (Deinzer et al., 2000; Ozkavaf et al., 2001). Yet it is believed that the validation of alternative methods could contribute to establishing adequate options easily employed in the clinical practice, with reduced operational costs.

Therefore, the objective of this study was to validate an alternative method for collecting GCF.

MATERIAL AND METHODS

Type of study: in vitro study.

Construction of the standard curve

A) Reference group (R): Periopaper® and Periotron® 8000

A standard curve was constructed based on known volumes of saliva, starting with 0.1 microliters (µl) and increasing in 0.1 µl increments up to 1.0 µl. These volumes were pipetted by a trained examiner using a Hamilton® syringe (Hamilton, Reno, Nevada, USA). Another examiner, blind to the volumes, then measured each volume three times using Periopaper® (OraFlow, PlainView, New York, NY, USA). Each volume was pipetted three times and read on Periotron® 8000 (OraFlow, PlainView, New York, NY, USA) after predefined time intervals [immediately after collection (T1), after 15 seconds (T2) and after 30 seconds (T3)], resulting in a total of 90 Periopapers®.

B) Test group (T): strips of absorbent paper and Periotron® 8000

Initially, standardized strips were produced from 80 gram qualitative absorbent paper (Figure 1), using a digital pachymeter (Mitutoyo®, Suzano, SP, Brazil), with the same measurements as Periopapers® (2.55 mm wide, 0.16 mm thick, and 14.19 mm long; 8.07 mm of the length was identified as a handling area with a blue tape). Volumes previously established by a trained examiner, starting with 0.1 microliters (µl) and increasing in 0.1 µl increments up to 1.0 µl, were then applied to the strips, and an examiner blind to these volumes measured them on Periotron® 8000. As described above, each volume was pipetted three times and measured on Periotron® 8000 at the predefined intervals (T1, T2 and T3), making up a total of 90 strips of absorbent paper.

STATISTICAL ANALYSIS

Student’s t test for independent samples was used to compare the means obtained for measurements taken in groups R and T. One-way analysis of variance (ANOVA) was used to compare the means for the three different intervals between collection and measurement (T1, T2 and T3) for each method. Data are shown as total means and 95% confidence intervals for each group and for each of the experimental intervals. Mean differences between the two methods and respective 95% confidence intervals were calculated in order to estimate the general variability. Scatter plots were produced in order to demonstrate the correlation between the means of groups R and T. Simple linear regression models were constructed to estimate the extent to which group T was capable of predicting group R. Linear regression coefficients (β) and 95% confidence intervals were calculated for each interval. Furthermore, in order to test the hypothesis that group T has a similar absorption capacity to group R, intraclass correlation coefficients (ICC) were calculated for all three intervals. Data were analyzed using the statistical package Stata SE 10.0 (Stata Corp., College Station, TX, USA). Significance was set at 5%.

RESULTS

Table 1 lists the means of Periotron® values for readings.
in groups R and T at experimental intervals T1, T2 and T3, as well as the differences between these means (95% confidence interval). No statistical differences were observed in the groups between the means obtained at T1, T2 and T3. Comparisons between the two groups at each experimental interval also revealed no statistical differences.

**DISCUSSION**

The objective of this in vitro study was to validate an alternative absorbent paper for collecting GCF. According to our results, the test paper showed a similar behavior compared to the reference regarding the absorbent capacity. It was also observed that different time intervals elapsed between GCF collection and the readings on Periotron® did not provoke significant changes to the volumes measured.

GCF can be collected via suction, lavage or absorption. (Ciantar & Caruana, 1998; Deinzer et al., 2000; Griffiths, 2003) Suction methods usually employ microcapillaries/micropipettes and can measure different volumes. However, these methods are technique-sensitive and demand a long time (around 30 minutes per site) to ensure an accurate collection of small volumes (Griffiths, 2003). In addition, according to the literature, suction methods may cause traumas, affecting the measurement of volumes collected and its components. With relation to lavage, although Gustaffson (1996) states that this should be the method of choice when analyzing GCF components, it has the limitation of not providing information related to the volume of fluid collected.

The absorption method was selected for assessment in the present study because it is easy to carry out, minimally invasive, and is traditionally the method of choice for GCF in the literature (Deinzer et al., 2000; Griffiths, 2003). Several different types of absorbent paper are available and have been assessed previously: Durapore, Millipore (Giannopoulou et al., 2003), Whatman chromatographic (Johnson et al., 1999) and absorbent paper points (Serra et al., 2003). However, none of these papers has had its validity tested against Periopaper®, the reference absorbent paper widely recognized as the method of choice for GCF collection via absorption (Deinzer et al., 2000; Ozkavaf et al., 2001).

In this study, we employed strips of 80 gram qualitative absorbent paper. These were cut to the exact dimensions of the reference paper (Periopaper®) using a digital pachymeter. Strip dimensions were observed with great care, since differences in size could influence the absorption capacity and consequently change the values. Furthermore, Periopapers® have an area demarcated in orange (the handling strip) which cannot be placed between Periotron® reading plates without risking incorrect readings (Preshaw et al., 1996; Ciantar & Caruana, 1998; Chapple et al., 2005; Oradigm, 2008). We therefore demarcated, in blue, the same area on our test strips, thus creating the same conditions as those observed for the reference paper.

According to Figueredo & Gustaffson (1998), one of the major advantages of using absorption to collect GCF is the possibility of measuring its volume, which leads us to a discussion of the best way of reporting the components of this exudate. It has been suggested that GCF components can be reported in total quantities (mg), concentrations (ng/ml) and collection time (Ciantar & Caruana, 1998; Griffiths, 2003; Hanioka et al., 2005). Although some authors claim that the fluid volume is dependent on the inflammatory expression present at the site (Del Fabbro et al., 2001; Griffiths, 2003), and consequently it is important to describe its components, other authors question the need for such calculations. There are also investigators such as Chung et al (1997), who prefer to report the components in

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**Table 1**

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<th>T1</th>
<th>Difference</th>
<th>T2</th>
<th>Difference</th>
<th>T3</th>
<th>Difference</th>
<th>Intragroup P</th>
</tr>
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<tbody>
<tr>
<td>R</td>
<td>73.8 (50.1-97.4)</td>
<td>9.2 (4.5-13.7)</td>
<td>69.9 (45.9-93.8)</td>
<td>8.5 (0.35-16.7)</td>
<td>66.6 (44.9-88.3)</td>
<td>8.5 (4.7-12.3)</td>
<td>0.91</td>
</tr>
<tr>
<td>T</td>
<td>64.6 (42.3-86.9)</td>
<td>61.4 (43.4-79.3)</td>
<td>58.1 (35.4-80.8)</td>
<td>0.58</td>
<td>0.58</td>
<td>0.60</td>
<td>0.91</td>
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<tr>
<td>Intergrup P</td>
<td>0.58</td>
<td>0.92</td>
<td>0.96</td>
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<tr>
<td>ICC</td>
<td>0.95</td>
<td></td>
<td>0.92</td>
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concentrations and absolute quantities. Nevertheless, Zhang et al. (2002) have clearly demonstrated that the initial differences observed in concentrations of PMN and IL-8 in the GCF of sick periodontal sites disappeared after adjustment for volume, suggesting that it is therefore important to calculate the volume. Finally, Griffiths (2003) states that correct interpretation of results is dependent on the volume of GCF collected.

In response to the importance of quantifying GCF volume, a number of methods have been described for collecting GCF via absorption, such as colorimetry, weighing and the use of electronic apparatus (Periotron®). Colorimetry is a valid method which uses ninhydrin or fluorescein to mark the areas of absorption. However, Griffiths (2003) alerts to the fact that rapid evaporation may interfere with results. Furthermore, the stains employed mean that GCF components cannot be analyzed. The weighing method, on the other hand, requires a highly sensitive apparatus that is not specific to this type of analysis and is subject to evaporation – an inconvenience that can be observed by the decreasing figures obtained on the balance when measurements are taken. (Griffiths, 2003) For this study, we chose to use Periotron®, an electronic system that calculates volume based on magnetic impedance, i.e., on how wet the absorbent paper is. (Preshaw et al., 1996; Ciantar & Caruana, 1998; Chapple, 1999; Chapple et al., 2005; Oraflow, 2008). This machine is widely used in research, was developed specifically for this use and is easy to operate (Chapple, 1999; Griffiths, 2003).

In the analysis of results, the manufacturer’s recommendations for constructing standard curves were followed. Precautions were also taken with regard to environmental conditions (temperature), and measurements were repeated. (Preshaw et al., 1996; Tözüm et al., 2004; Oraflow, 2008) The standard curve was constructed using a Hamilton® syringe to measure small volumes. Each volume was pipetted and measured three times, using a fresh strip at each time, in both experimental groups. Preshaw et al. (1996) state that each volume should ideally be measured three times when calibrating the Periotron®, a recommendation that is in agreement with the reports of Chapple et al. (1995) and Chapple (1999). Nevertheless, other authors, such as Ciantar & Caruana (1998) and Deinzer et al. (2000) deem it necessary to repeat each measurement five times. A study by Tözüm et al. (2004) however, showed that there were no differences between measurements taken three, five or 20 times.

Evaporation is considered to be a technical difficulty particularly affecting small volumes (Tözüm et al., 2004). For this reason, we decided to measure GCF volumes after three different time intervals between GCF collection and readings on Periotron® 8000, namely, immediately after collection and 15 and 30 seconds later. Our results showed that waiting 30 seconds did not interfere with the values measured, a finding that contrasts with that reported by Tözüm et al. (2004), who observed significant losses after intervals of 30 and 60 seconds and suggested that 5 seconds was a safe interval for transferring the absorbent paper strip to the Periotron®.

According to the results observed, the alternative collection method using paper strips showed a similar absorption capacity for known volumes when compared with Periopaper® (R = 73.8 and T = 64.6). Furthermore, intergroup regression coefficients obtained for each of the three experimental intervals were very close to 1, which indicates that for each measured unit changed in the RT group, the measurement made using the R group method will change by approximately 1 unit. We can therefore state that the figures obtained revealed excellent consistency and correlation between both methods.

CONCLUSION

Our findings suggest that GCF collection using strips of 80 gram qualitative absorbent paper combined with volume quantification on Periotron® is a promising and appropriate method for the analysis of GCF. Furthermore, intervals of up to 30 seconds between collection and reading do not seem to interfere with measurements.

ACKNOWLEDGEMENTS

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ABSTRACT

Gingival crevicular fluid (GCF) is a marker of periodontal inflammatory status and thus its volume and composition are important to assess the pathogenesis of this inflammatory condition. This study investigated an alternative absorbent paper (test group, T) for collecting GCF comparing to the reference paper (R, Periopaper®) Standard curves for R and T were plotted after the tests at three time intervals between GCF collection and readings on the Periotron® [immediately after collection (T1) and after 15 (T2) and 30 (T3) seconds]. Mean results as read in Periotron® units (PU) were compared (t test for independent samples), and no differences were found between R and T at T1 (R = 73.8, T = 64.6), T2 (R =
69.9, T = 61.4) or T3 (R = 66.6, T = 58.1). Intragroup assessment (ANOVA) also revealed no difference for R and T at T1, T2 or T3. The intraclass correlation coefficient between groups was excellent (T1 = 0.95, T2 = 0.92 and T3 = 0.96). These results suggest that the alternative GCF collection method using paper strips is accurate and that intervals of up to 30 seconds between GCF collection and reading on the Periotron® do not influence the measurements.

**UNITERMS:** Gingival crevicular fluid, validation studies, periodontics.

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