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# Comparative study of methods for the quantification of biofilm on complete dentures

# *Estudo comparativo de métodos para quantificação de biofilme em próteses totais*

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**ABSTRACT:** This study compared the application and reliability of 4 methods for biofilm quantification (computerized, paper-weighing, point-counting, and planimetric) in complete dentures, verifying the correlation between them. The internal surfaces of 62 complete dentures were stained (5% erythrosine) and photographed. The slides were projected on paper, and the areas (total and biofilm-covered) were outlined with a pencil. These areas were measured with an equidistant point grid (point-counting method), a digital planimeter (planimetric method), and for the paper weighing method they were cut and weighed with a precision scale. For the computerized method, ImageTool software was used. In order to perform a validation test of the methods, all of them were applied to slide projections of geometric figures with known dimensions. The correlation tests showed high correlation values (r = 0.82 to 0.99) among the methods. The validation test (ANOVA) showed no statistically significant differences among the values obtained from the measurement of figures using all four quantitative methods and the real dimensions of these geometric figures. Quantitative methods were efficient and reliable for measuring quantity of biofilm in complete dentures, and may be useful in experimental studies on the efficacy of hygiene products. The computerized method was fast and easy to perform.

**DESCRIPTORS:** Denture, complete; Biofilms.

**RESUMO:** Este estudo comparou a aplicação e a confiabilidade de quatro métodos de quantificação de biofilme (computadorizado, pesagem de papel, contagem de pontos e planimétrico) em próteses totais, verificando a correlação entre eles. As superfícies internas de 62 próteses totais foram coradas (eritrosina a 5%) e fotografadas. Os diapositivos foram projetados em papel, e as áreas (total e com biofilme corado) foram contornadas com grafite. Estas áreas foram medidas com uma grade de pontos eqüidistantes (método de contagem de pontos), um planímetro digital (método planimétrico) e, para o método de pesagem de papel, foram recortadas e pesadas em balança de precisão. No método computadorizado, foram medidas com um software (ImageTool). Com o objetivo de realizar um teste de validação dos quatro métodos, estes foram aplicados também em diapositivos de figuras geométricas de dimensões conhecidas. Os testes de correlação mostraram altos valores de correlação (r = 0,82 a 0,99) entre os métodos. O teste de validação (ANOVA) não mostrou diferença estatisticamente significante entre as medidas reais dos desenhos geométricos e aquelas obtidas pelos quatro métodos quantitativos. Os métodos quantitativos mostraram-se eficazes e confiáveis na mensuração dos níveis de biofilme em próteses totais, podendo ser úteis em estudos experimentais da eficiência de produtos de higiene. O método computadorizado mostrou-se rápido e de fácil aplicação.

**DESCRITORES:** Prótese total; Biofilmes.

## INTRODUCTION

Several studies have focused on the efficacy of substances and methods for cleansing complete dentures. However, few studies have concentrated on the materials and methods used for quantification of biofilm (a parameter for the efficacy of hygiene procedures). Biofilm quantification methods vary greatly, making comparison of results difficult. In some experiments, biofilm is clinically quantified in vivo[2,7,9,10,17,19,21,27] using biofilm disclosing agents, protein evaluation and microbiological quantification. Due to the complexity of these clinical studies, several in vitro laboratorial procedures have been developed[20].

A small number of studies have evaluated and discussed the quantification methods used in clinical experiments[4,5,8,13,18,24]. In these studies, the

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need for standardization has been mentioned. One of the commonly used methods is biofilm disclosure associated with quantitative or scoring methods[20]. The aim of the present study is to evaluate the applicability and reliability of four methods for clinical evaluation of quantity of biofilm in complete dentures (computerized, paper-weighing, point-counting, and planimetric) associated with biofilm-disclosing agents.

## MATERIAL AND METHOD

Sixty-two complete denture wearers were selected for this study (22 men and 40 women, aged between 45 and 80 years). The internal surface of the denture (which consisted of pink-colored, thermally cured acrylic resin) was stained with an aqueous solution of 5% erythrosine (Art. 1355 Erythrosin, E. Merck, Darmstadt, Germany). The denture was positioned on a clamp (Universal Adriática S/A, São Paulo, SP, Brazil – intermediate shank at 0°), through its external surface. The stained surface was photographed with a camera (Canon EOS Elan II E QD, Canon Inc., Tokyo, Japan) fixed on a stand (CS-4 Copy Stand Testrite, Newark, NJ, USA) using a slide film (Asa 100, Kodak Brasileira Com. e Ind. Ltda. São Paulo, Brazil), with the focus centered on the median palatine raphe, halfway between the maxillary frenum and the posterior margin of the denture.

### Application of the biofilm quantification methods

For the computerized method (1), slides were scanned (CanoScan 2700F, Canon Inc., Tokyo, Japan; 680 dpi) and, using these images, the total internal surface of the denture and biofilm-covered areas were measured, using the software ImageTool 2.02 (Windows, Texas, USA) (Figure 1A). The paper-weighing (2), point-counting (3) and planimetric methods (4) were applied to 3 figures obtained by projecting the slides in a dark room, using a Kodak Ektagraphic III projector (Kodak Brasileira Com. e Ind. Ltda. São Paulo, Brazil) placed on a wooden post (1.70 m high) fixed to the ground. Slides were projected on paper (297 x 420 mm and 75 g/m$^2$, Chamex Print & Copy paper, Chame Chamex Brasil, São Paulo, Brazil), resulting in images amplified ten times (36 x 24 cm). The areas of interest (total and biofilm-covered) were outlined using a lead pencil. For the point-counting method, a grid with equidistant points (0.5 cm) was designed using the Page Maker software (Adobe, California, USA); this grid was copied onto an overhead transparency sheet (polyester film, 210 x 297 mm, 40 µm, Chamex, International Paper do Brasil Ltda., Mogi Guaçu, SP, Brazil). The figures were fixed to the working surface with masking tape, and the grid was superimposed on each figure, in order to count the number of points over the total surface and biofilm-covered areas (Figure 1B). For the planimetric method, the figures representing the areas

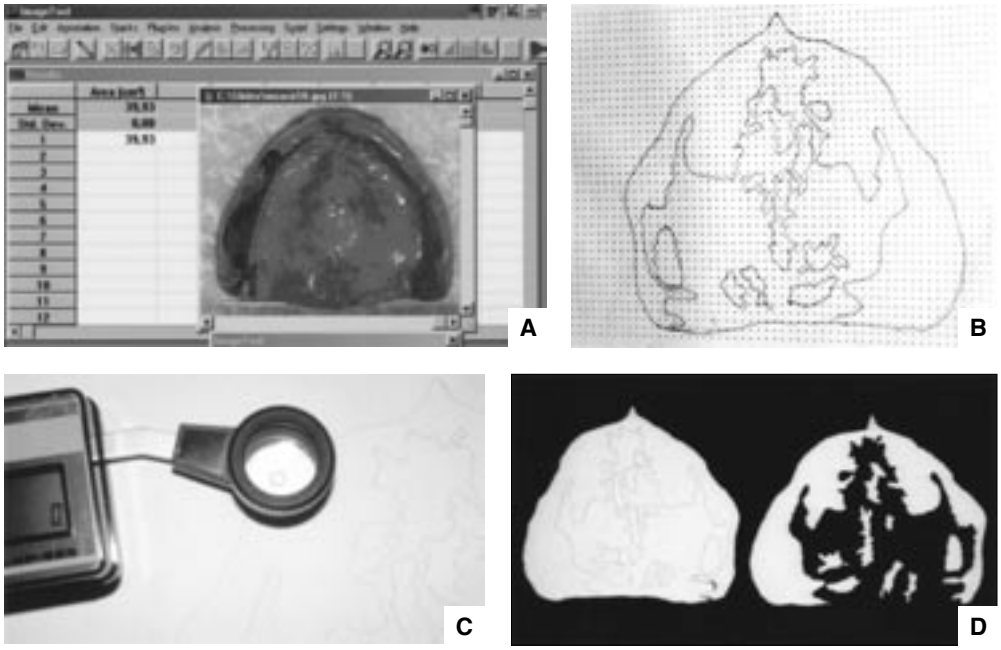



**FIGURE 1 -** ImageTool quantification method (**A**). Point-counting quantification method (**B**). Planimetric quantification method (**C**). Paper-weighing quantification method (**D**).

(total and biofilm-covered) were also fixed on a working surface and measured ($cm^2$) using a digital planimeter (Placom KP 92N, Tokyo, Japan) (Figure 1C). For the paper-weighing method, the areas of interest were cut using scissors (Tramontina, RS, Brazil), and the resulting shapes (Figure 1D) were weighed using a precision scale (Metler Toledo GMbH, Greifensee, Switzerland). For all the four methods, the percentage of biofilm was calculated as the ratio between biofilm-covered area (multiplied by 100) and total internal surface of the denture.

## Validation test

In order to perform a complementary validation test, a pair of compasses and a ruler were used to draw four geometric figures (Figure 2). All quantification methods studied were applied to all figures. Figures were prepared as follows: one white cardboard rectangle (288 x 192 mm), with drawings of 20 circles of various diameters (Figure 2A); one black cardboard rectangle (299 x 192 mm), to which 22 gray circles of various diameters were glued (Figure 2B); two black cardboard circles to which 10 gray cardboard circles of smaller diameter were glued (Figure 2C and 2D). For each figure, the total area of each rectangle (base × height) and circles ($\pi \times r^2$) were calculated. The percentage occupied by each inner circle was calculated as the ratio of its area (multiplied by 100) and the total surface of the geometric figure. All figures were photographed following the same procedure used for photographing the dentures. For the computerized method, the areas (total area of the geometric figure and each inner circle) were measured over the scanned slide. After projection of the slides on paper and outlining of the areas of interest, these were cut out and measured by the paper-weighing, point-counting (using a grid), and planimetric methods. In each method, the percentage of the area occupied by each inner circle was calculated as the ratio between its area (multiplied by 100) and the total area of the geometric figure.

In order to compare the percentage values obtained from each method and to evaluate the proportionality relation among them, the regression and correlation test (Pearson's test) were applied. The partial correlation test was applied in order to compare "r" values. This was done with the purpose to evaluate the best correlation of the association of the different methods. The validation test was performed with the analysis of variance (ANOVA). This test was applied with the purpose to evaluate statistical equality or difference among methods and between them and the real dimensions of the geometric figures.

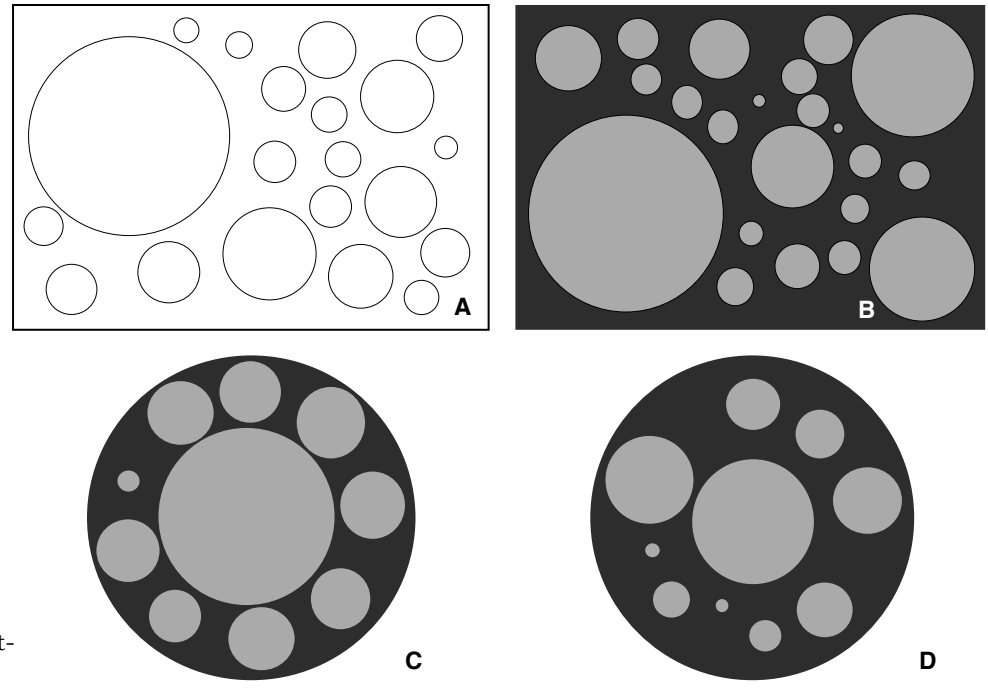



**FIGURE 2 (A, B, C, D) -** Geometric figures (validation test).

## RESULTS

The biofilm percentages are shown in Graph 1 and Graph 2. Table 1 shows the distribution of the number of complete dentures according to the percentage of biofilm.

The results of the correlation test are shown in Table 2. Table 3 shows the results of partial correlation.

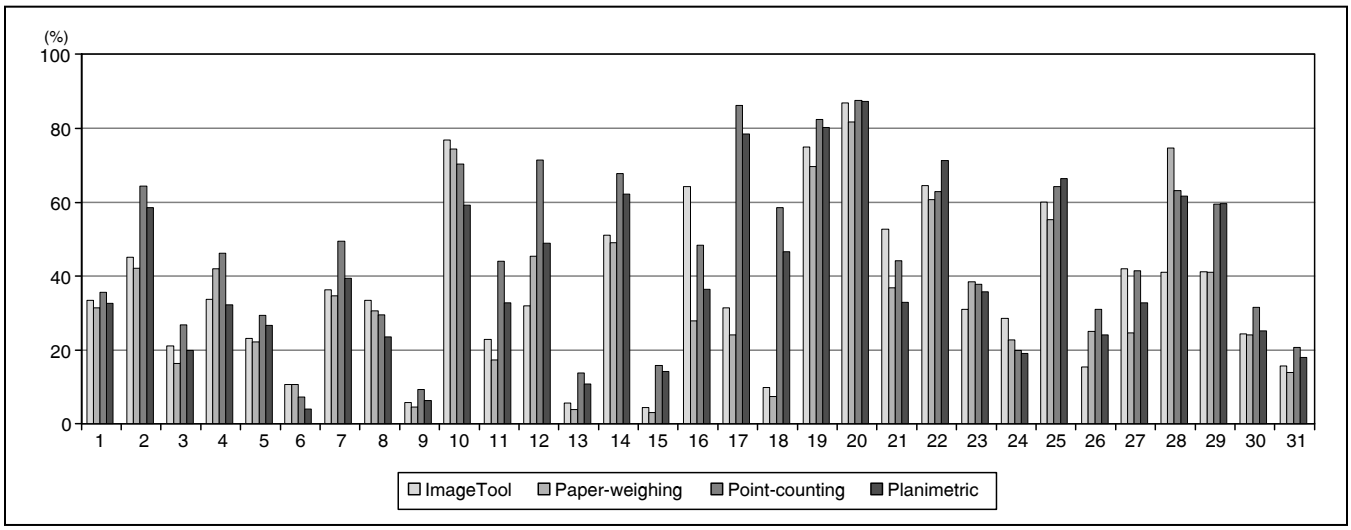
Table 4 shows the results of the validation test, while Table 5 presents the statistical test applied (analysis of variance - ANOVA).

## DISCUSSION

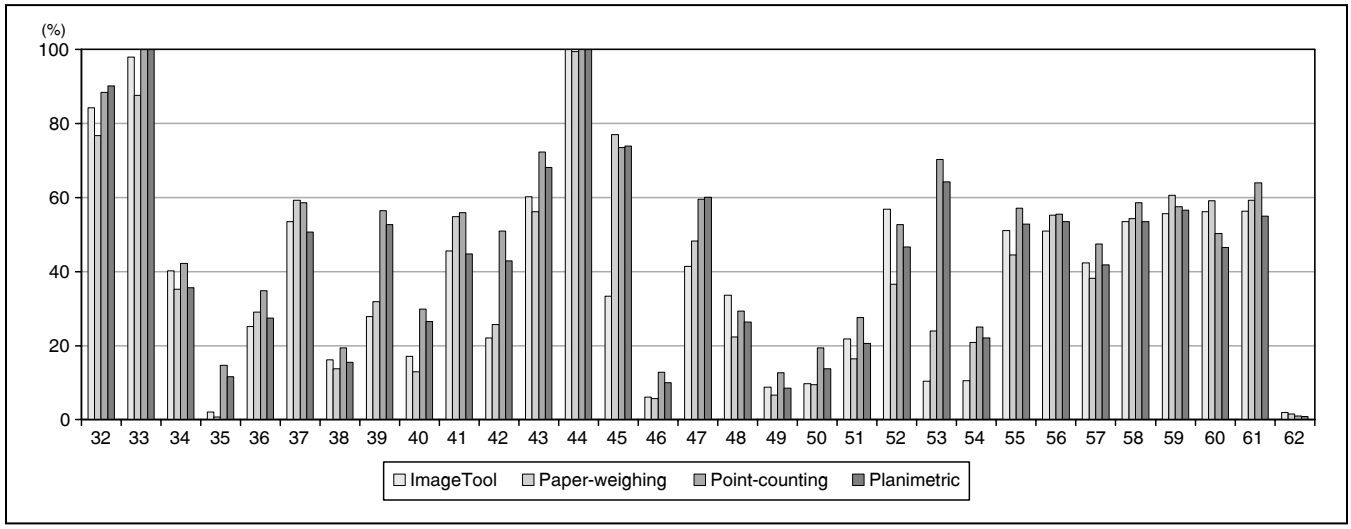
The difficulties for achieving reliable comparisons among methods for cleansing complete dentures are related to the fact that different methods are chosen for clinical quantification of biofilm levels[26]. The level of precision, accuracy, reliability, and validity of the quantification method chosen should be considered[18]. An accurate methodology could be attained by the use of disclosing agents associated with morphometric methods[4]. Another factor to be considered is the viability of different examiners apply a methodology, allowing the reproducibility of the diagnosis inter-examiners. This allows the comparison of results obtained by different research groups[1,17,18]. Distinction should also be made between methods that quantify and those that qualify the biofilm layer[20]. Although the protein quantification method[2,9] seems to be accurate, contamination by saliva may occur. Scanning microscopy is more adequate for obtaining qualitative information about the biofilm[2]. Microbiological quantification methods[19,21] are very accurate, but time-consuming. They also make the comparison of results difficult when different types of culture medium and methods are used for the collection of microorganism samples[20]. The disclosing agent method is the most widely applied to complete dentures. Its association to quantitative methods[3,6,9,15,19,23,27,29] is less common. However, few studies discuss the application and reliability of these methods[4,8,18,23,24].

In the present study, the photographic method was chosen because the quantification is known to be difficult through visual inspection[4]. The challenges presented by the standardization of denture positioning, lighting and contrast of the photographs were previously addressed[18]. In order to standardize such conditions, the use of digital imaging has been suggested[27]. In the present study, controlled conditions of lighting and film processing were applied. Previous analyses of this photographic method, used for comparing total surface areas (internal and external) of complete dentures, showed high correlation coefficients[6,23].

In the literature, several denominations to the methods that quantify the biofilm in complete dentures that are not related to subjective methods were found (scoring method). Those methods were denominated as quantitative methods in this study. In others studies, those methods were denominated as based on physical and analytical parameters[12], photographic[16], morphometric[3,4,] or with quantitative image superimposition[18]. The




**GRAPH 1 -** Percentage of biofilm in 31 maxillary complete dentures obtained using quantitative methods (1-31).




**GRAPH 2 -** Percentage of biofilm in 31 maxillary complete dentures obtained using quantitative methods (32-62).

**TABLE 1 -** Quantitative methods: distribution of the number of complete dentures according to the percentage of biofilm.

| Class intervals (percentage of biofilm) | ImageTool (1) | | Paper-weighing (2) | | Point-counting (3) | | Planimetric (4) | |
|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | n | % |
| 1) 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2) 0.01 to 25.49% | 23 | 37.1 | 25 | 40.3 | 13 | 21.0 | 18 | 29.0 |
| 3) 25.50 to 50.49% | 19 | 30.6 | 19 | 30.6 | 20 | 32.3 | 20 | 32.3 |
| 4) 50.50 to 75.49% | 15 | 24.2 | 13 | 21.0 | 23 | 37.1 | 18 | 29.0 |
| 5) 75.50 to 100% | 5 | 8.1 | 5 | 8.1 | 6 | 9.7 | 6 | 9.7 |

point-counting method[3,4] showed high correlation values, but this result was based on measurements performed twice by the same examiner. The planimetric method was reported as used for quantification analysis on natural teeth[14,25]. Both methods presented sensitivity for the detection of biofilm levels in complete dentures[8]. The paper-weighing method was previously applied to natural teeth[12] and complete dentures[22,29] and its reproducibility was tested[23]. The use of the computerized method is relatively new for complete dentures. Minagi *et al.*[19] (1987) used computerized analysis; however, dentures were fragmented before quantification. Sheen, Harrison[27] (2000) used computerized digital imaging, but they also highlighted the need for apply reproducibility tests on their method. The computerized method in this study was used for the evaluation of a hygiene product[6] and brushes[28] for cleansing complete dentures, but its validity was not tested.

Graphs 1 and 2 show that methods of biofilm quantification, although more challenging than scoring methods, offer objective and accurate results. Since these methods do not rely on ability, calibration, or number of examiners, they should be the methods of choice in clinical experiments for the evaluation of complete denture cleansers. The difficulty in differentiating biofilm from food residues and stains in the photographs is considered a limitation of these methods[27] and might explain the disagreement among some values in Graphs 1 and 2.

Although the examiner was trained on the four methods the time spent to measure the areas of interest was considerably high for all methods. However, the computerized method was clearly faster (average time: 20 minutes), since the measurements were performed directly from the scanned image and the program measured the selected area. On the quantitative methods of paper-weigh-

**TABLE 2 -** Regression and correlation tests (ImageTool-1, paper-weighing-2, point-counting-3 e planimetric-4).

| | Compared methods | | | | | |
|---|---|---|---|---|---|---|
| | 1 *versus* 2 | 2 *versus* 3 | 2 *versus* 4 | 1 *versus* 3 | 1 *versus* 4 | 3 *versus* 4 |
| | Statistical analysis of r value | | | | | |
| Value of r | 0.9481 | 0.8550 | 0.8747 | 0.8251 | 0.8285 | 0.9903 |
| Degrees of freedom | 60 | 60 | 60 | 60 | 60 | 60 |
| Value of *t* | 23.0962 | 12.7699 | 13.9796 | 11.3121 | 11.4681 | 55.2074 |
| Ho probability | 0.000% | 0.000% | 0.000% | 0.000% | 0.000% | 0.000% |
| | There was correlation significance at level of 1% ($\alpha = 0.01$) | | | | | |
| | Adherence test to the measured curve | | | | | |
| $\chi^2$ | 77.9065 | 81.7321 | 77.2748 | 69.7289 | 67.8253 | 26.7515 |
| Adherence probability (%) | 0.000% | 0.000% | 0.000% | 0.000% | 0.000% | 57.680% |

**TABLE 3 -** Results of the partial correlation test - R1 *versus* R2 (1 - paper-weighing/ImageTool; 2 - paper-weighing/point-counting; 3 - paper-weighing/planimetric; 4 - ImageTool/point-counting; 5 - ImageTool/planimetric; 6 - point-counting/planimetric).

| Correlated methods | | R 1st sample | R 2nd sample | Degrees of freedom (1st and 2nd samples) | Variance common | Z value | Ho probability | Significance |
|---|---|---|---|---|---|---|---|---|
| 1st | 2nd | | | | | | | |
| 1 *versus* 2 | | 0.9481 | 0.8550 | 59 | 0.1841 | 4.1339 | 0.00% | 1% ($\alpha = 0.01$) |
| 1 *versus* 3 | | 0.9481 | 0.8747 | 59 | 0.1841 | 3.5325 | 0.04% | 1% ($\alpha = 0.01$) |
| 1 *versus* 4 | | 0.9481 | 0.8251 | 59 | 0.1841 | 4.9164 | 0.00% | 1% ($\alpha = 0.01$) |
| 1 *versus* 5 | | 0.9481 | 0.8285 | 59 | 0.1841 | 4.8338 | 0.00% | 1% ($\alpha = 0.01$) |
| 1 *versus* 6 | | 0.9481 | 0.9903 | 59 | 0.1841 | 6.5237 | 0.00% | 1% ($\alpha = 0.01$) |
| 2 *versus* 3 | | 0.8550 | 0.8747 | 59 | 0.1841 | 0.6014 | 54.76% | Not significant |
| 2 *versus* 4 | | 0.8550 | 0.8251 | 59 | 0.1841 | 0.7824 | 43.40% | Not significant |
| 2 *versus* 5 | | 0.8550 | 0.8285 | 59 | 0.1841 | 0.6999 | 48.40% | 1% ($\alpha = 0.01$) |
| 2 *versus* 6 | | 0.8550 | 0.9903 | 59 | 0.1841 | 10.6576 | 0.00% | 1% ($\alpha = 0.01$) |
| 3 *versus* 4 | | 0.8747 | 0.8251 | 59 | 0.1841 | 1.3838 | 16.64% | Not significant |
| 3 *versus* 5 | | 0.8747 | 0.8285 | 59 | 0.1841 | 1.3013 | 19.32% | Not significant |
| 3 *versus* 6 | | 0.8747 | 0.9903 | 59 | 0.1841 | 10.0562 | 0.00% | 1% ($\alpha = 0.01$) |
| 4 *versus* 5 | | 0.8251 | 0.8285 | 59 | 0.1841 | 0.0825 | 93.42% | Not significant |
| 4 *versus* 6 | | 0.8251 | 0.9903 | 59 | 0.1841 | 11.4400 | 0.00% | 1% ($\alpha = 0.01$) |
| 5 *versus* 6 | | 0.8285 | 0.9903 | 59 | 0.1841 | 11.3575 | 0.00% | 1% ($\alpha = 0.01$) |

ing, point-counting and planimetric, besides the time spent to trace the areas, time was also spent to count the points, use the planimeter and cut the paper (average time: 40 minutes). Specifically for the weighing method, the time spent during weighing has to be considered. The computerized method can also be performed without a scanner, if a digital camera is connected to the computer[27,28]. Hutchins, Parker[11] (1973) and Silva *et al.*[29] (2002) have described the ideal characteristics of a good disclosing agent for complete dentures. They call attention to the importance of the characteristics

**TABLE 4 -** Validation test: percentage of the inner circle areas (real dimensions and measurements obtained using quantitative methods).

| | Circles | RD | IT | PW | PC | PN |
|---|---|---|---|---|---|---|
| Figure A | 1 | 21.13 | 20.79 | 20.99 | 20.38 | 21.21 |
| | 2 | 0.82 | 0.80 | 0.76 | 0.82 | 0.83 |
| | 3 | 1.45 | 1.38 | 1.37 | 1.44 | 1.43 |
| | 4 | 2.05 | 2.01 | 1.98 | 2.02 | 2.00 |
| | 5 | 4.61 | 4.58 | 4.27 | 4.44 | 4.47 |
| | 6 | 2.05 | 2.02 | 1.98 | 1.98 | 1.15 |
| | 7 | 0.57 | 0.64 | 0.60 | 0.62 | 0.67 |
| | 8 | 1.19 | 1.21 | 1.17 | 1.30 | 1.18 |
| | 9 | 2.62 | 2.68 | 2.63 | 2.70 | 2.62 |
| | 10 | 0.96 | 0.94 | 0.93 | 1.09 | 0.87 |
| | 11 | 0.89 | 0.91 | 0.86 | 0.99 | 0.90 |
| | 12 | 0.57 | 0.61 | 0.58 | 0.62 | 0.58 |
| | 13 | 0.26 | 0.30 | 0.27 | 0.27 | 0.25 |
| | 14 | 2.87 | 2.88 | 2.96 | 2.94 | 2.90 |
| | 15 | 0.63 | 0.64 | 0.63 | 0.68 | 0.64 |
| | 16 | 1.03 | 1.07 | 1.04 | 1.03 | 1.05 |
| | 17 | 1.03 | 1.10 | 1.12 | 1.13 | 1.56 |
| | 18 | 1.64 | 1.65 | 1.76 | 1.68 | 1.70 |
| | 19 | 0.36 | 0.39 | 0.38 | 0.41 | 0.41 |
| | 20 | 0.57 | 0.33 | 0.36 | 0.31 | 0.32 |
| Figure B | 1 | 21.13 | 21.05 | 20.88 | 20.35 | 20.65 |
| | 2 | 0.75 | 0.81 | 0.78 | 0.84 | 0.78 |
| | 3 | 0.32 | 0.33 | 0.31 | 0.34 | 0.35 |
| | 4 | 1.03 | 1.10 | 1.08 | 1.18 | 1.11 |
| | 5 | 0.51 | 0.57 | 0.58 | 0.54 | 0.54 |
| | 6 | 5.63 | 5.93 | 6.05 | 5.80 | 6.00 |
| | 7 | 0.41 | 0.48 | 0.44 | 0.49 | 0.47 |
| | 8 | 0.46 | 0.52 | 0.49 | 0.56 | 0.50 |
| | 9 | 0.57 | 0.60 | 0.61 | 0.54 | 0.57 |
| | 10 | 3.69 | 3.75 | 3.59 | 3.50 | 3.72 |
| | 11 | 0.05 | 0.06 | 0.06 | 0.07 | 0.03 |
| | 12 | 8.20 | 8.39 | 8.45 | 8.26 | 8.25 |
| | 13 | 0.51 | 0.57 | 0.52 | 0.54 | 0.56 |
| | 14 | 0.69 | 0.69 | 0.70 | 0.71 | 0.67 |
| | 15 | 1.32 | 1.35 | 1.31 | 1.39 | 1.33 |
| | 16 | 0.08 | 0.09 | 0.08 | 0.10 | 0.07 |
| | 17 | 0.32 | 0.35 | 0.32 | 0.38 | 0.31 |
| | 18 | 0.57 | 0.58 | 0.53 | 0.56 | 0.53 |
| | 19 | 1.94 | 1.98 | 1.89 | 2.01 | 1.90 |
| | 20 | 0.46 | 0.49 | 0.47 | 0.52 | 0.50 |
| | 21 | 0.89 | 0.90 | 0.84 | 0.94 | 0.66 |
| | 22 | 2.33 | 2.37 | 2.21 | 2.29 | 2.27 |
| Figure C | 1 | 28.03 | 28.91 | 28.18 | 28.99 | 28.80 |
| | 2 | 0.35 | 0.40 | 0.39 | 0.44 | 0.33 |
| | 3 | 3.11 | 3.33 | 3.32 | 3.71 | 3.35 |
| | 4 | 2.34 | 2.49 | 2.41 | 2.77 | 2.54 |
| | 5 | 3.54 | 3.78 | 3.59 | 3.93 | 3.83 |
| | 6 | 3.11 | 3.17 | 3.28 | 3.57 | 3.26 |
| | 7 | 3.77 | 3.90 | 4.00 | 4.15 | 3.95 |
| | 8 | 4.00 | 4.53 | 4.47 | 4.81 | 4.55 |
| | 9 | 3.11 | 3.53 | 3.38 | 3.79 | 3.50 |
| | 10 | 3.77 | 3.96 | 3.95 | 4.22 | 4.31 |
| Figure D | 1 | 14.10 | 14.37 | 14.00 | 14.21 | 14.24 |
| | 2 | 0.17 | 0.16 | 0.17 | 0.23 | 0.15 |
| | 3 | 1.13 | 1.12 | 1.08 | 1.28 | 1.02 |
| | 4 | 0.11 | 0.15 | 0.15 | 0.15 | 0.12 |
| | 5 | 0.81 | 0.85 | 0.84 | 0.98 | 0.81 |
| | 6 | 2.67 | 2.85 | 2.80 | 3.01 | 2.73 |
| | 7 | 3.84 | 4.09 | 4.19 | 4.29 | 4.13 |
| | 8 | 1.93 | 2.17 | 2.13 | 2.42 | 2.20 |
| | 9 | 2.41 | 2.66 | 2.62 | 2.87 | 2.61 |
| | 10 | 2.31 | 7.26 | 7.34 | 7.63 | 7.41 |

RD: real dimension, IT: ImageTool, PW: paper-weighing, PC: point-counting, PN: planimetric.

**TABLE 5 -** Analysis of variance results (validation test).

| Analysis of variance | | | | | |
|---|---|---|---|---|---|
| Source of variation | SS | DF | MS | (F) | Ho prob. |
| Between methods | 0.1053 | 4 | 0.0263 | 0.32 | 13.8261 |
| Residue | 19.7137 | 244 | 0.0810 | | |
| Total variation | 97.2552 | 309 | | | |
| Means - single factor of variation: methods | | | | | |
| Real dimension | 0.10669 | | | | |
| Paper-weighing | 0.09415 | | | | |
| ImageTool | 0.13771 | | | | |
| Point-counting | 0.14318 | | | | |
| Planimetric | 0.11887 | | | | |
| Statistically equal means | | | | | |

SS: Sum of squares, DF: degrees of freedom, MS: mean squares, prob:. probability.

of good coloration and easy removal from the denture. It is important to note that when biofilm is to be measured on denture bases made of clear acrylic resin, a colored background to contrast with the disclosing agent should be used[11,29]. In the present study, clear-base dentures were not selected, in order to eliminate this variable.

The point-counting procedure presented the highest values for biofilm level (n = 41; 66.1%), while the paper-weighing method showed the lowest values (n = 37; 59.6%) (Graphs 1 and 2). These results are in agreement with those observed by Fernandes *et al.*[8] (2002). However, the data in Table 1 show that, for all 4 methods, within each class interval, the distribution of the number of prosthesis did not vary, showing that most dentures were placed within the 2 to 4 class intervals (1% to 75% of the internal surface covered by biofilm) and few of them showed high levels of biofilm (class interval number 5 – 75% to 100% of the internal surface covered by biofilm). The data in Table 1 confirm the standard of the results in Graphs 1 and 2, so to the paper-weighing method the high number of complete dentures (25) was in class interval number 2 (up to 25% of biofilm) showing lower biofilm levels, and for the point-counting method most dentures were in class interval number 4 (from 51% to 75% biofilm), showing higher biofilm levels.

The validation tests were used to evaluate the four quantitative methods applying them to known dimensions (real values). No significant differences were found between measurements obtained by all four methods and between them and real dimensions of the geometric shapes. The same tendency presented on Graphs 1 and 2 was also observed with the method of point-counting showing the highest average, and the paper-weighing method the lowest one (Table 5). The paper-weighing method showed the closest results when compared with the real dimensions, followed by the planimetric method.

Correlation results showed high (0.82 to 0.99) and significant ($p < 0.01$) coefficients. Percentages (Graphs 1 and 2), distribution of number of dentures (Table 1) and high correlation values (Table 2) suggest that all four methods can be used for quantification of biofilm in complete dentures. The statistical significance (Table 3) of correlation factors for the association of paper-weighing/ImageTool, compared to the associations paper-weighing/point-counting and paper-weighing/planimetric methods indicates the best correlation for the first association. This result is satisfactory, since the paper-weighing method presented high correlation values in a previous study of duplicate measurements on 222 slides of complete denture photographs[23]. These results indicate that the computerized method (ImageTool) is reliable, since its correlation with the paper-weighing method was high. The correlation values between the association paper-weighing/point-counting and paper-weighing/planimetric were lower (0.85 and 0.87, respectively, Table 3). The planimeter is very difficult to be applied in small areas (0.2 to 0.5 $cm^2$) and its application to even smaller areas (0.1 $cm^2$) is virtually impossible. The point-counting method can be performed directly on the slide projection, but it is the most challenging method, presenting the greatest possibility of error.

Methods for biofilm quantification should be regarded as important procedures by those who work with complete dentures as the maintenance of prosthetic devices represents a challenge for patients and practioners. Nevertheless, biofilm quantification methods are frequently unknown or applied either in a negligent manner or without compliance to adequately established criteria.

## CONCLUSIONS

According to the results obtained, it may be concluded that:

1. Quantitative methods showed efficacy in the clinical measurement of biofilm levels on complete denture surfaces. Therefore, these methods may be useful in studies in order to evaluate the efficacy of denture cleansers.
2. The correlation coefficients obtained varied from 0.82 to 0.99. This represents a high correlation rate among the four quantitative methods evaluated in this study.
3. Considering the time spent in the application of each procedure, the computerized method may be the first choice for quantification of biofilm on the surface of complete dentures.

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