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Accuracy of Endodontic Microleakage Results: Autoradiographic vs. Volumetric Measurements

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The correlation between autoradiographic and volumetric leakage measurements was evaluated. Seventy-two anterior teeth with a single canal were selected and divided into three groups of 24. Group 1 served as control (no obturation), group 2 was obturated with gutta-percha only, and group 3 was obturated with gutta-percha and endodontic sealer. Samples were placed in a vertical position in 48-well cell culture plates and immersed in 1 ml of [^{14}C]urea for 14 days. One-mm-thick horizontal serial sections were cut with a diamond disk cooled with liquid-nitrogen gas. Linear penetration was recorded by five independent evaluators from autoradiographs. Volumetric results were based on counts per minute registered in a liquid scintillation spectrometer. Pearson's correlation coefficient test was used to determine the lineal correlation between both methods of evaluation. No acceptable correlation values were found in any of the three groups (group 1, $r = 0.34$; group 2, $r = 0.23$; group 3, $r = 0.20$). Our results indicate that there is no correlation between linear and volumetric measurements of leakage.

Root canal microleakage has been referred to for a number of years as the major cause of endodontic failures (1, 2). Since Dow and Ingle's study in 1955 (3), apical microleakage has been frequently used to evaluate endodontic materials and techniques, some of which have been labeled adequate or unsuitable based on the findings of such investigations. Regardless of the numerous studies published, only limited attention has been paid to the evaluation of the methods used to measure leakage.

Although several different methodologies have been suggested to assess microleakage (4–7), linear measurements by dye or radioisotope penetration are by far the most frequently used. Johnson and Zakariasen (8) indicated that linear measurements provide only a two-dimensional view of a three-dimensional body and do not allow for differentiation in density of leakage. In addition, Delivanis and Chapman (9) indicated that it is impossible to obtain objective results by autoradiographic linear measurements, because the results attained are based only on subjective qualitative judgments.

Radioactive solutions may provide quantitative and objective volumetric results by detection of counts per minute in a liquid scintillation spectrometer. Nevertheless, the correlation between linear and scintigraphic measurements of leakage has not been established, nor has the consistency of these methods been compared.

The purpose of this study was to determine the correlation between linear and volumetric measurements by radioisotope penetration.

MATERIALS AND METHODS

Specimen Distribution and Selection

Specimens included in the study were freshly extracted roots of anterior teeth, with single principal canals, complete apical formation, canal curvatures of <25 degrees, and minimum root length of 12 mm. Any specimen having root caries, fractures, internal or external resorptions, previous endodontic treatment, and/or calcifications were discarded. Seventy-two specimens were selected and randomly divided into 3 groups of 24 each. Specimens in group 1 served as controls (no obturation). Group 2 was obturated using the lateral condensation technique with only gutta-percha (Hygenic Corp., Akron, OH), simulating poorly obturated canals. Group 3 was obturated with gutta-percha in combination with an endodontic sealer (Tubli-Seal, Kerr Mfg. Co., Romulus, MI).

Immediately after extraction, the specimens were stored in 1% benzalkonium chloride solution (Antibenzil, Farmacéuticos Altamirano de México, Mexico City, Mexico) and were refrigerated. Crowns were removed perpendicular to the roots' long axis at the cementoenamel junction with a diamond disk (Komet, Lemgo, Germany). Calculus and soft tissue were eliminated with a scalpel blade (Becton-Dickinson, Franklin Lakes, NJ). Specimens were inspected for root fractures and cementum integrity under a stereomicroscope (Nikon SMZ-2T). Canal length was recorded by introducing a #10 Flex-R file (Union Broach, Emigsville, PA) until it was seen just at the apical foramen. Working length was established as 1 mm short of this distance. Final selections were made after preliminary radiographs, taken from both the mesial-distal and buccal-lingual aspects, were studied.

TABLE 1. Mean leakage values in mm and cpm

	n	Linear (mm)	Volumetric (cpm)
Group 1	24	Mean 8.43 SD 1.20	Mean 1172.62 SD 467.35
Group 2	24	Mean 3.12 SD 2.18	Mean 1158.54 SD 463.31
Group 3	24	Mean 0.68 SD 1.19	Mean 876.87 SD 352.60

SD, standard deviation.

universal scintillation liquid (Insta-Gel, Packard, Downers Grove, IL). Counts per minute were registered in a liquid scintillation spectrometer (Beckman, LS6000TA).

Statistical Analysis

Linear penetration was measured from autoradiographs by five independent evaluators. Means for every specimen were used to calculate the mean ± SD values/group. Two volumetric measurements were recorded/specimen, of which only the mean values were retained. As with linear measurements, means ± SDs were calculated for every group. Differences among the three groups for both linear measurements and counts per minute were compared using one-way analysis of variance (ANOVA). Groups were individually compared with Duncan's multiple range test. Pearson's test was used to determine the correlation coefficient values between the two methods of evaluation.

RESULTS

Table 1 summarizes the mean ± SD values calculated for every group. Controls (group 1) showed the highest amount of leakage, both in linear and volumetric measurements. Group 2 showed significantly less leakage than group 1, as demonstrated by the autoradiographic measurements; however, in volumetric results, only a slight difference was observed. According to both methods of evaluation, group 3 had the lowest leakage values.

Statistical analysis by one-way ANOVA showed significant differences among the three groups at a significance level of $p < 0.0001$ for linear penetration and $p < 0.05$ for volumetric measurements.

Individual comparisons for linear measurements by Duncan's multiple range test ($\alpha = 0.05$) showed significant differences for every combination. However, in volumetric measurements, there was no significant difference between groups 1 and 2.

Pearson's test showed no acceptable correlation values between the evaluation methods in any of the three groups (group 1, $r = 0.34$; group 2, $r = 0.23$; group 3, $r = 0.20$). Counts per minute were recorded on a second occasion, and the results obtained were not significantly different in either reading. However, in linear penetration, there was inconsistency between the measurements recorded by the different evaluators.

DISCUSSION

For a number of years, evaluation of materials and techniques by assessment of apical or coronal microleakage has been one of the most important topics in endodontic research. A variety of methodologies have been suggested for this purpose, but only a limited number of reports have focused on their evaluation (9, 11). Therefore, the procedures used are insufficiently standardized and, to date, no consensus can be drawn from previous investigations. Several authors have expressed their concern about this problem (12–14). This indicates the need for further studies to determine the accuracy and value of individual procedures. Microleakage studies might then be standardized.

Among the most important procedures that require evaluation are those that could affect the accuracy of results. Most investigators assess leakage by measuring the linear penetration of dye or radioactive solutions. As has been pointed out in previous reports (8, 9), this method of evaluation is based on qualitative subjective observations. In a study by Delivanis and Chapman (9), discrepancies in linear measurements were reported when the same observers were asked to do a re-evaluation 1 wk later. Our results agree with this finding. Inconsistency in the measurements made by different evaluators was observed. If measurements are based on the judgment of one evaluator only, the accuracy of results could be affected. Increasing the number of observers may reduce the problem, but the measurements are still subjective.

Different techniques to assess volumetric leakage have been suggested. Jacobson and von Fraunhofer (15) described an electrochemical method to assess leakage quantitatively. Even though their method allows leakage to be monitored continuously throughout the test period and objective results can be obtained, it has a significant limitation. Partial leakage cannot be measured because the electrolytes must penetrate to the full length of the filling material for leakage to be registered (16).

Zakariasen et al. (5) proposed a technique for measuring volumetric leakage by spectrophotometric recovery of dye solutions. This method provides a quantitative and objective volumetric measurement of leakage. When radioactive solutions are used, similar evaluations can be obtained by detection of counts per minute in a liquid scintillation spectrometer. In contrast to the spectrophotometric dye-recovery method, this technique does not require the dissolution of the root structures or the construction of a linear regression curve. Furthermore, the results of the present study indicate that these measurements are a consistent method of evaluation. However, most studies assessing radioisotope penetration base their results on autoradiographic linear measurements only. Two studies by Besse et al. (17) and Jacobsen et al. (18) used liquid scintillation spectrometry. However, no comparisons can be made with the present study, because neither study reported autoradiographic linear measurements.

Although most studies that have compared linear and volumetric measurements have been made using spectrophotometric dye recovery, comparisons can still be made. Two previous reports by Beatty and Zakariasen (19) and Johnson and Zakariasen (8) stated that there was a high correlation between linear and volumetric measurements. However, our results—as well as those reported by Madison and Zakariasen (20) and Zakariasen et al. (5)—do not confirm their findings. In the present study, no correlation was found between the two methods of evaluation. Inconsistency and subjectivity of linear measurements were thought to be the cause of this lack of correlation.

In the present study, only the cervical surfaces of the roots were sealed with nail polish to avoid leakage through the exposed dentinal tubules. In a pilot study, we observed that nail polish retained radioisotopes significantly more than uncoated root surfaces. The increment in exterior surface radioactivity reduces the reliability of volumetric measurements. It is interesting that no

leakage occurred through the root structures when specimens had complete cementum integrity. All specimens selected had a complete cementum layer on all surfaces, as was demonstrated by stereomicroscopic examinations.

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