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QUANTITATIVE MARGIN ANALYSIS IN THE SCANNING ELECTRON MICROSCOPE.

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Abstract

Interface between restorative materials and tooth hard substances must be morphologically as perfect as possible to avoid plaque accumulation and subsequent secondary caries or pulpal diseases. Therefore the marginal behavior of restorations is an important parameter to predict their longevity.

Morphologically, the quality of margins is characterized by different well defined criteria. Using a replica technique it is possible to assess the complete marginal circumference of restorations in the SEM. Margins of restorations show a large variety of their morphology. This publication describes a method to quantify the quality of dental restorations.

The restoration margins are traced on the SEM screen with a digitizer and an interface to measure the margin's length. Simultaneously the margin quality is assessed and assigned to the corresponding lengths. The % distribution of the quality criteria for each restoration is then calculated. Using a comparative light microscope, the replicas are aligned and mounted identically in the SEM for longitudinal studies.

The results presented are limited to tests for the accuracy of the method. Using 5 criteria to characterize the margin quality, it was found that the difference between two measurements by the same operator, 4 weeks apart was 3% ± 2.6%. The largest difference for one group was 9%. In another accuracy test where 4 criteria for margin characterization were used, the difference between two measurements was 1.9 % ± 0.9 %. The largest difference between two groups found was 3.4 %.

This method can be used for longitudinal studies in vivo, but also for in vitro screening tests with new materials.

Key Words: Dental restorations, margin quality, replica technique, in vivo and in vitro testing, computer assisted analysis.

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Introduction

The prevalence of dental caries worldwide is high (Burt 1981, Thylstrup and Fejerskov 1986, WHO 1982). Caries is characterized by the destruction of the tooth hard tissues (enamel and dentin) by organic acids formed from microorganisms in the dental plaque, when substrates (mono- or disaccharides) are present (Thylstrup and Fejerskov 1986). The destructive process is very complicated and is dependent on many co-factors such as age and quality of the plaque, quality of the substrate, frequency of substrate availability, quantity and quality of the saliva, fluorides, etc. (Thylstrup and Fejerskov 1986). The small carious lesion consists of a subsurface decalcification, which is reversible up to a certain extent (Holmen et al. 1985a, 1985b). If the caries process progresses a cavity occurs. At this point the diseased tissue is usually completely removed using mechanical or chemical (Schutzbank et al. 1978, Kurosaki et al. 1974) means. If the dietary habits of the patients change drastically, and if the cavities are accessible to the daily cleaning processes it is possible for such lesions to come to rest, due to calcifications. In these rare cases no filling therapy is required.

It is common practice to replace the missing tissue with dental restorative materials. Dental restorations may be placed with gold, amalgam, composite resins or glass ionomer cements (Charbenau et al. 1981, Phillips 1973). Experiments with adhesively luted ceramic inlays are promising (Herder and Roulet 1988). All dental restorative materials must be able to restore the form and function of the decayed tooth. In addition, they must protect the dental pulp from physical, mechanical, chemical or bacteriological trauma (Charbenau et al. 1981). Consequently it is important that restorations are able to maintain an impermeable seal and a perfect morphology at the restoration-tooth interface.

When a restoration does not provide the required seal, there is a pathway from the oral environment to the dentinal tubules connected with the pulp. Thus noxious substances may penetrate from the oral cavity to the pulp and induce pulpitis, causing pain and thus requiring further treatment, e.g., root canal treatment

(Brännström 1984, Schroeder 1981). Such noxious substances are usually toxins produced by the microorganisms of the dental plaque. If the marginal openings allow the microorganisms themselves to penetrate, they may migrate to the base of the cavity and further damage the pulp with their toxins (Brännström and Nyborg 1973, Brännström and Nordenvall 1978). Microorganisms are found under most dental restorations placed by conventional methods (Brännström and Nyborg 1971).

If the morphology at the tooth-restoration interface presents niches to harbor a sufficient amount of microorganisms, recurrent caries may occur if patients are not able/willing to remove the plaque and enough substrate is available. Thus, recurrent caries usually occurs when restorations have overhanging margins or marginal openings. In these cases the dentist will usually replace the restoration, which usually leads to larger reconstructions, and subsequently removes more of the natural tooth hard substances (Lutz 1984).

Therefore assessing the margin quality of dental restorations is important when evaluating new restorative materials or new application techniques.

Methods for Assessing the Margin Quality (Review of the literature)

The quality of restoration margins can be assessed in vivo or in vitro. In the following paragraph the common methods will be briefly discussed.

Most researchers preferred the in vitro methods because direct evaluation is possible. Most in vitro work was done with dye penetration (Crim and Mattingly 1981, Crim and Chapman 1986, Fuks et al 1985, Lutz 1980, Lutz et al. 1986, Roulet 1976). Usually 0.5% - 2 % aqueous fuchsin solutions were used (Crim and Mattingly 1981, Crim and Chapman 1986, Fuks et al. 1985, Lutz 1980, Lutz et al. 1986). Anilin blue in 60% alcohol has shown superior penetration, especially if applied with a vacuum technique (Roulet 1976). However, careful interpretation is needed because anilin blue becomes transparent when the pH is high. If calcium hydroxide liners are used, this dye may not be used. Enhanced contrast was achieved, if a fluorescent dye (De Trey 1976, Derkson et al. 1986) or a silver staining technique was used (Wu et al. 1983, Dumsha and Biron 1984, Gordon et al 1986). The use of radioactive tracers guaranteed excellent penetration and the leakage was detected easily (Hembree and Andrews 1980, Hembree 1986, Wu et al. 1983). However, special equipment and permission is required to handle radioactive substances. With radioactive isotopes, the leakage is shown by exposing the sections to microradiographic slides. On these microradiographs the detection of the restoration contour and the tooth restoration interface is difficult, if there was no leakage. With all dye penetration methods interactions between dye and tooth hard tissue or the restorative material may occur. These methods are all destructive since they require sectioning the restorations and are thus not suitable for longitudinal studies. Hansen (1982) has described an in vitro method which allows longitudinal follow-up of the margin quality. He measured the maximum width of the marginal gap with a light microscope. This is an indicator that the restoration is leaking. A nondestructive approach is to penetrate the restorations with a fluorescent dye (DeTrey 1976) or a 0.5 % basic fuchsin solution in propylene glycol (Tsuchiya et al. 1986) and to rate the degree of penetration from the surface of the restoration. The % of areas that leak on the entire perimeter of the restoration can be indicated. Leinfelder (1986) used a similar approach. He took advantage of the pH change, caused by the soluble calcium hydroxide liners, which were detected by placing litmus paper on the surface of the restoration. This procedure is very sensitive and can also be used in vivo.

The first systematic approach to the evaluation of restorations was described by McCune et al. (1967). The criteria used in that research are known as the US Public Health Service Criteria or the Ryge criteria (Cvar and Ryge 1971, Ryge 1981). The restorations are systematically evaluated using a mirror and an explorer and the quality of the margin evaluated using the criteria: "anatomical form", "cavo surface marginal discoloration" and "marginal adaptation". However, this method is problematic. Leinfelder et al. (1982) admitted that the smallest ledge that can be detected is 100 µm and Dedmon (1982) has shown that among one dental school there was significant inconsistency within and among the faculty members as to the maximum marginal opening acceptable. Since these criteria are quite coarse, their use in the evaluation of modern materials with excellent characteristics is of limited value unless the experimenters are willing to accept 2 and 3 years evaluation times.

This is why researchers were working on more sensitive evaluation methods, especially for wear, which can detect the formation of ledges at the cavo surface margins (Leinfelder et al. 1983) or can evaluate the clinical behaviour of dental amalgams (Goldberg et al. 1980, Osborne et al. 1980 a and b, Mahler 1979, Mahler and Marantz 1979 a and b). In these studies stone models and/or standardized photographs were used to rate the set of restorations to be evaluated. This can be done by ranking the models/photographs from excellent to poor or by comparing the models/photographs with well defined standards. However, as the clinical assessment technique, all of the methods have one basic drawback. The better the material, the more difficult it is to see changes in the quality of the restoration margins.

The replication technique with polymethyl-methacrylates (Roulet 1978) brought large improvements. In the technique described by Roulet (1978), the first stage replica was obtained with a polymethylmethacrylate material. Unfortunately, undercuts lead to fractures of the replica material. However, the advantage of this technique was the possibility to process the first stage (negative) for the SEM investigation. Silicone impression materials were better (Bergvall and Bränström 1971, Flinn 1978, Lee and Swartz 1970, Lutz 1980, Roulet and Michellod 1984, Roulet 1987). These impressions are usually cast with epoxy resins to obtain replicas for the subsequent SEM analysis. The reduction of artifacts (Michellod 1984), large depth of focus and extremely large range of magnification enabled the researcher to easily assess the quality of restoration margins. Since the replica technique is non-destructive, it can be applied to in vitro and in vivo tests and is also well suited for longitudinal studies. In an earlier work, photographs were taken under standardized conditions in the SEM, mounted, and the margins quantitatively and qualitatively analyzed (Roulet 1978). The percentage portion of marginal openings was used to rate the quality of the restorations. In addition, the various different aspects of the margins were described. However, this was not sufficient to characterize the quality of the restorations, especially if high quality margins were obtained.

Lutz (1980) has reduced the many aspects of the margin morphology to the following criteria: "perfect margin", "marginal opening", "overhang", "underhang", "restoration margin fracture" and "enamel margin fracture". The restoration margins were looked at in the SEM on replicas at a constant magnification. The total length of the restoration margin was observed and the distribution of the above criteria was estimated in percents for each image. For each restoration the distribution was calculated as the mean of the values determined for each screen. This technique allows early discrimination between different restoration materials and application techniques. It was widely used at the University of Zürich Dental School by Lutz (1980) especially in the development of better composite resins and application techniques thereof. However, the technique is quite cumbersome and there is a definite potential for error because the operator has to estimate the proportional distribution of the criteria on the image. In addition, the operator protocols the estimates by hand.

Purpose of the Paper

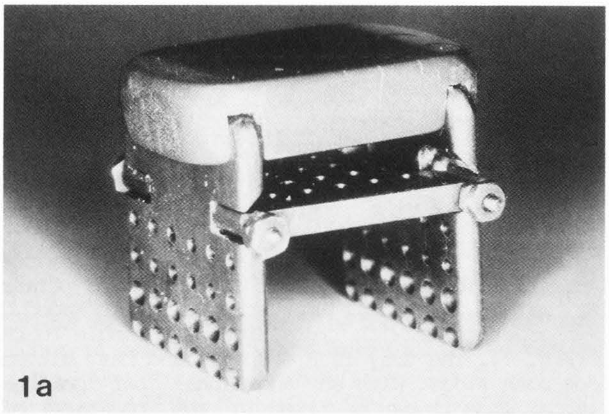
The purpose of this paper is to describe a computer assisted technique to easily determine the percent distribution of well defined criteria based on the morphology of the margins of dental restorations.

The Technique

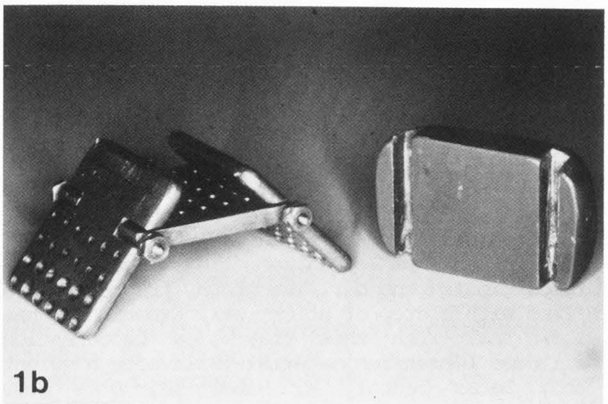
Restorations are placed in vitro in extracted teeth or in vivo. After polishing the restorations the teeth are carefully cleaned with a nonabrasive toothpaste and a soft bristle brush.

Impression technique. Whenever possible the impression is taken with a polyvinysiloxane impression material (President light body, Coltene AG CH - 9450 Altstätten). For in vivo experiments placing a rubberdam has increased the quality of the replicas because it is easier to clean the site and to prevent contamination from oral fluids. The possible inhibition of the set of the impression material by the sulfides in the rubber leaves only a smeary surface where the rubberdam was in contact with the impression material. The reproduction of the restoration's surface is not disturbed if the rubberdam is 1 mm away from the margins. To obtain replicas of the entire Class II margin, a special impression tray is needed in order to take an impression of a single tooth (Fig. 1). The tray is H - shaped and has hinges on both sides. With an additional bar on the upper side it can be fixed in the position of the H shape (Fig. 1 a). The lower part is then filled with impression material and additional impression material is syringed into the proximal area. Then the filled tray is positioned onto the tooth. After the impression material has set the bar-lock is removed and the shape of the tray changed to widen the lower part by rotating the wings of the tray at the hinge axes (Fig. 1 b). In this situation the impression material is torn at the narrowest section i.e. the proximal area. After removal, the original H - shape is restored by repositioning the bar. The torn fragments are fixed with a drop of sticky wax. Experience has shown that with this tray , a condensation silicone impression material (Silasoft N light body, Detax Dental, K. Huber KG, D - 7500 Karlsruhe 1), is better, due to its lower tear resistance (Craig 1980). Both materials show sufficient detail reproduction. The in vivo restorations are usually reevaluated after 6 months, the in vitro restorations after 2500 cycles of thermocycling from 5 oC to 55 oC.

Replica production and mounting. The impressions are first boxed using a condensation silicone impression material and PVC tubes. The replicas are cast with an epoxy resin (Stycast 1266, Emerson and Cuming Europe N.V., B - 2431 Westerlo Oevel). The mixed resin is first evacuated for 15 minutes to eliminate the air bubbles and then heated to 37 oC to increase its fluidity. The cast replicas are then mounted identically on SEM holders with a PMMA resin (Palavit, Kulzer GmbH, D - 6328 Friedrichsdorf). To be identically mounted (which is necessary to avoid errors from different projections) we developed a new procedure using a double comparative stereomicroscope (Vergleichsbrücke + Makroskop, Leitz GmbH, D - 6330 Wetzlar). This microscope is routinely used in criminalistic laboratories to compare fingerprints, etc.,(Fig. 2). With this microscope it is possible to observe two specimens simultaneously. It is possible to align the second sample identically with the fixed first sample by either different colored illumination (e.g. red and blue light) or by assembling split pictures. We use a special combination of precision driven stages (Fig. 3). and add a flat reference plane to the round SEM holders. Then we are able to transfer the samples into the SEM in an identical three dimensional position. The samples are then



1a



1b

Figs. 1a and 1b. Individual impression tray to obtain replicas from the approximal area too. Fig. 1a shows the impression tray in the closed position used to take the impression, Fig. 1b shows the open position used to remove the impression. During opening, the impression material is torn at the thinnest part. Afterwards the impression is repositioned in the original position and the replica poured.

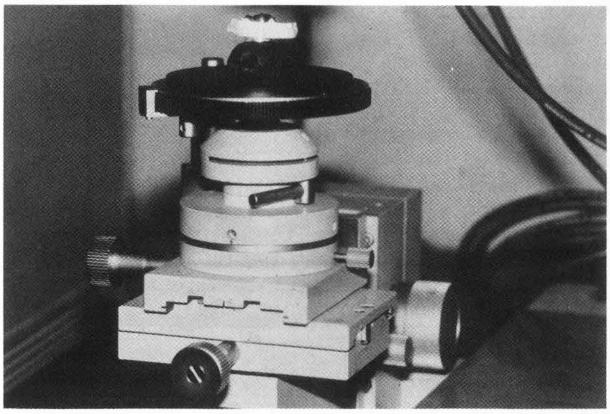


Fig. 3. Combination of precision driven tables to allow individual positioning of the second sample in order to align it precisely with the first sample.

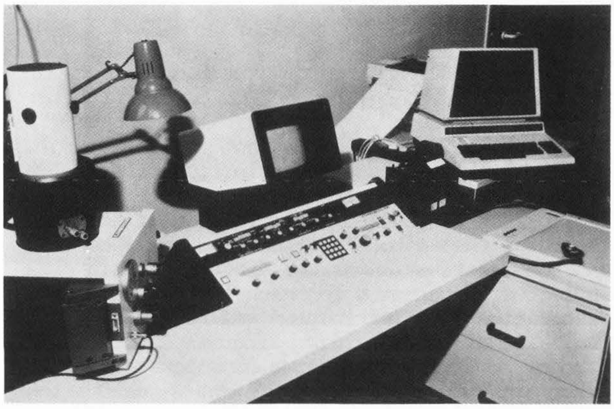


Fig. 4. Experimental set up showing the Stereoscan 100 on the left and the digitizer with the CBM computer on the right side.

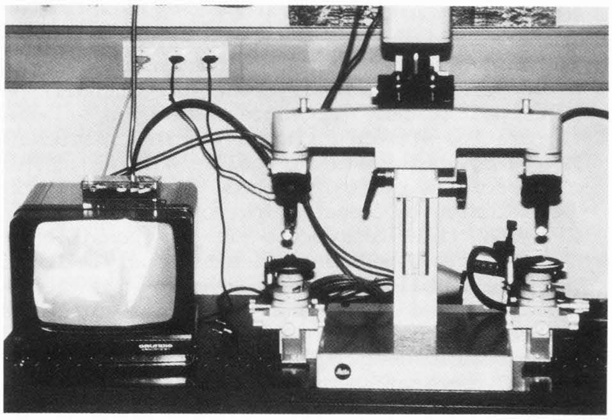


Fig. 2. Double comparative stereomicroscope used to mount two samples identically.

coated with a 20 nm thick layer of gold using a sputter coater (SCD 030, Balzers Union, FL - 9496 Balzers).

SEM Analysis. Thereafter the specimens are analyzed in the SEM (Stereoscan S 100, Cambridge Instruments Ltd. D - 4600 Dortmund), which is connected to a CBM 8032 microcomputer (Commodore GmbH, D - 6000 Frankfurt) and a digitizer (Hipad Model DT 114 G, Bausch and Lomb, Huston Instruments Div., Austin, Tx.) through an especially designed interface. The experimental set up is shown in Fig. 4 and schematically in Fig. 5. Its function can be explained as follows: a standard TV - video signal (0 - 1 V) is obtained from the video system board No. 852296 from the S 100 on socket 25. On the same board it is also possible to interfere with the video signal given to the TV screen of the SEM. The interface recognizes the coordinates given by the digitizer and monitors the electron beam position by registering the vertical synchronization pulse, the horizontal synchronization pulse and the line start.

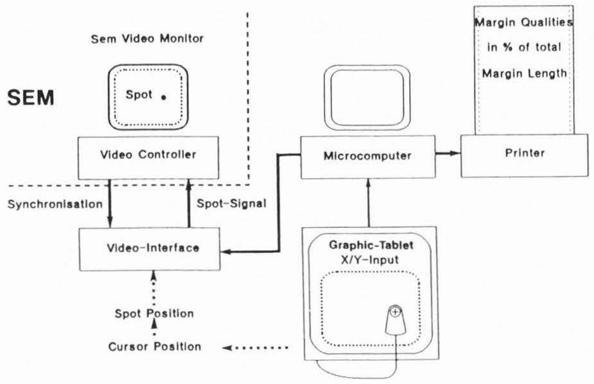


Fig. 5. Connections of the main components of the experimental set up for the quantitative margin analysis. REM = Stereoscan S 100, Microcomputer = CBM 8032, Graphic-Tablet = Hipad.

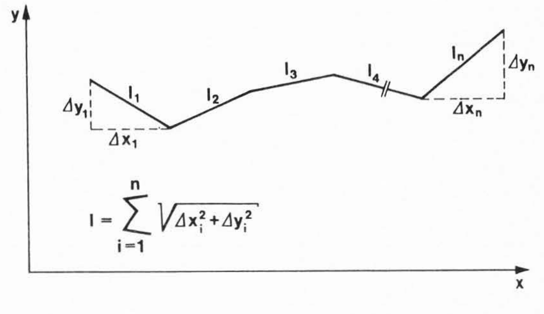


Fig. 6. Principle of the length measurements between different points.

Knowing the line number and the time within a single line (quartz time base controlled with 4 MHz), the interface is able to correlate the coordinates of the digitizer with the spot position on the screen. If both data are congruent, the interface generates a white pulse of 150 ns duration, which is then superimposed onto the TV image and thus visible on the TV screen at the corresponding position. With this mechanism it is possible to control the position of the spot on the screen by the position of the cursor of the digitizer. The computer program is designed such that the coordinates of every point defined with the digitizer are stored and used for further calculations. There is practically no upper limit of points which can be used for the calculations. The lattice space in the x and y directions can be defined in the program, which allows compensation for the errors given by the projection distortion in the SEM. When the input of a sequence of points is terminated, the operator induces the calculation of the length given by connecting all points with straight lines. The calculations are done using the Pythagorean theorem ("The sum of the squares of the legs of a right triangle is equal to the square of the hypotenuse"). Thus, the distance between two points is the hypotenuse and the delta x and delta y values are assigned to the legs of the triangle. The distance between the first and the last point is the sum of such distances between the single points (Fig. 6). With this procedure it is possible to approximate the length of curved margins also. The computer has then prepared different data files in which the measured length is stored. The first file keeps the measured length. To enhance the user comfort, the measured length is always stored automatically. The others are assigned to different characteristics defined according to the needs of the experiment. They are defined as sectors on the digitizer and when the cursor is positioned in the corresponding area, the defined criteria e.g. "excellent margin" (Fig. 7), "marginal opening" (Fig. 8), "positive ledge" (Fig. 9), "negative ledge" (Fig. 10) "restoration margin fracture" (Fig. 11) or "enamel margin fracture" (Fig. 12) is written on the computer screen. Thus the operator has an optical control of the data file he/she is storing. All data stored into the different files are also printed out by the matrix dot printer connected to the system. A pulse from the digitizer terminates the booking process and reactivates the measuring mode. After the entire perimeter of the restoration has been measured and the morphological criteria were assigned as described, the computer gives a percentage length distribution of the criteria, e.g., 95 % "excellent margin", 5 % "positive ledge", which is characteristic for the quality of the restoration. Non parametric tests are recommended to statistically evaluate the results because the data are usually not homoscedastic (Neter and Wasserman 1974).

Restorations are assessed before and after exposure to thermocycling or after placement and after 6 months in vivo. The observed changes or stability can be used to predict the longevity of the restoration. Since all morphological changes can be detected with the system, the behaviour of the restorations in vivo or after in vitro stress can be characterized, e.g., a large increase of negative ledge after 6 months of use is an indicator of high wear or degradation of the material. Such a restoration does not have a good prognosis for longevity. If restorations show some fractures at the restoration's margins and in the enamel at baseline in vitro and the percentage of these damages is highly increased after thermocycling, this indicates a traumatizing contouring/finishing technique. It is also reasonable to predict poor longevity of such restorations. The actual version requires measurements with constant magnification. We use 200 - 400 x depending on the overall quality of the margins. Variable magnification within one measurement would speed up measuring time and enhance user comfort. We are currently working on modifying the program to automatically include the original magnification given on the SEM data line into the calculations.

Error Determination

Step 1: Standards were repeatedly measured and the obtained values compared with the SEM's internal standard. It was clearly demonstrated that after calibrating the system to compensate for the projection distortion in the magnification used (200 x), the measuring error was so small that it could be neglected if the targets to aim at were clear.

Step 2: Determining whether the operator was able to reproducibly measure the length of the perimeter of restorations.

Material and Methods. Experiment A: 25 Class III composite restorations were placed in vitro and 25 Class III composite restorations were placed in vivo using the enamel etching technique. Replicas were obtained as described above at baseline and after 1500 cycles from 5 oC to 55 oC or respectively after 6 months in vivo. The 50 pairs of replicas were analyzed using the quantitative margin analysis in the SEM by one operator.

Experiment B: 61 cylindrical composite restorations were placed in flat ground roots of human teeth using dentin adhesives. Replicas were obtained as described above at baseline and after thermocycling (1500x) and analyzed in the SEM as in experiment A. In both experiments the length of the perimeter was recorded for the first and the second replica in digitizer units. The lengths of every data pair were compared and the percent differences calculated. The mean difference and the standard deviation were then calculated for both experiments.

Results. In experiment A the mean length difference was 8 % ± 6.3 %. In experiment B a mean length difference of 4.8 % ± 4 % was found.

Discussion. Assuming that the perimeter of the restorations did not change and the replicas were precise, the length differences found are operator errors. The differences in experiments A and B can be explained as follows: The results of the quality evaluation in experiment A was 91 % excellent margins. This means that the slightly different structure that the composite resin had in comparison with enamel was the only criteria to recognize the margin. In experiment B the margin quality was much more inferior, since the ratings 1 and 2, which was equivalent to "excellent margin" was given only in 29 %, meaning that the margins were detected much easier.

In step 3 the reproducibility of the ratings given was tested, i.e., we checked to see if an evaluator was able to reproduce the ratings he/she was assigning.

Material and Methods to step 3. Experiment A. Five MOD composite restorations placed with a light cured composite resin and an incremental technique were placed in vitro. Replicas were obtained as described above at baseline and after subjecting the restorations to thermocycling and cyclic loads as described by Roulet (1987). Both sets of replicas (n=5) were measured twice approximately 3 weeks apart, without the operator knowing it was the same group. The criteria "excellent margin" (Fig. 7), "marginal opening" (Fig. 8), "positive ledge" (Fig. 9), "negative ledge" (Fig. 10), "restoration margin fracture" (Fig. 11), and "enamel margin fracture" (Fig. 12) were used in this experiment to characterize the behaviour of posterior composites. The two data sets obtained for each criteria were then compared using paired t-tests.

Experiment B: In order to improve the reproducibility, a rating scale using photographs was designed. The ratings were only numbered (1 - 4) instead of named, but the operator always had a set of standard photographs available to compare with the situation to be evaluated. This rating scale was tested first with approximately 60 photographic examples which were given to the members of the department who were asked to assign the ratings according to the reference photographs. After a few modifications, consistency in the rating was obtained. Eight Class V composite restorations applied with the use of dentin bonding agents, were placed in extracted teeth and replicas obtained as described above. The replicas were assessed twice by the same operator, not knowing they were the same replicas, with an interval of 6 weeks.

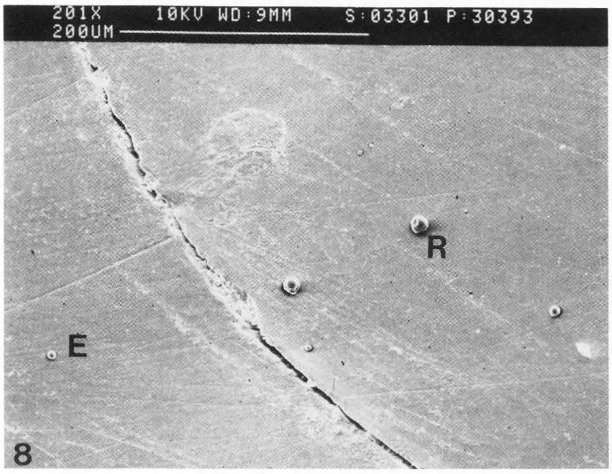
Results. In experiment A the statistical analysis revealed no significant difference for all the 6 pairs of data (n = 5) between the 2 measurements. The mean difference between two measurements was 3 % ± 2.6 %. The largest difference found for the comparison of two groups was 9 %. In experiment B no statistical differences with a t - test between the two sets of measurements for any of the criteria used were found. The mean difference was 1.9 % ± 0.9 % and the maximum difference between the two groups was 3.4 %.

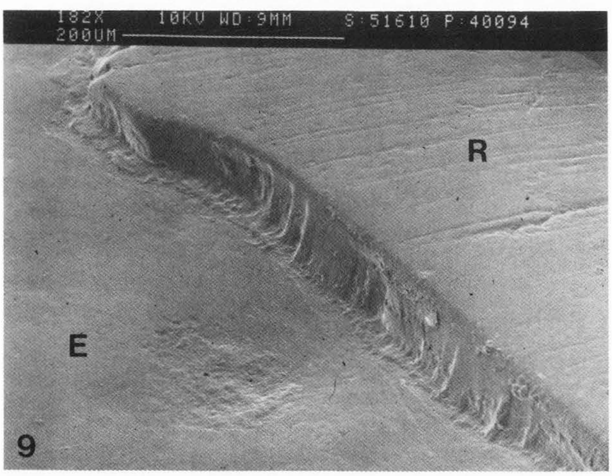
Discussion: In both experiments the operator errors in rating the morphology of restoration margins did not significantly influence the outcome of the results. The use of a rating scale with reference photographs decreased the operator error in rating the margin quality.

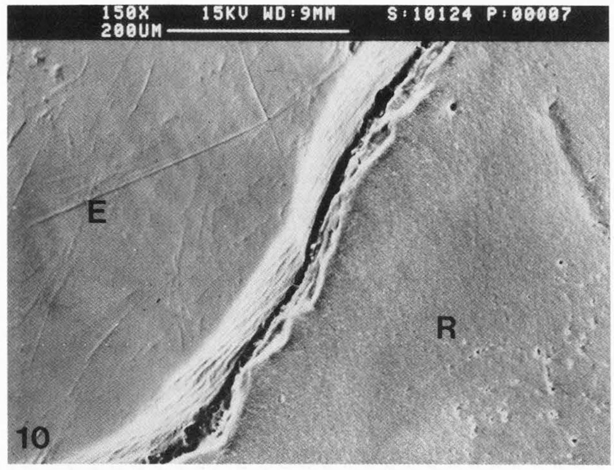
Examples of Applications

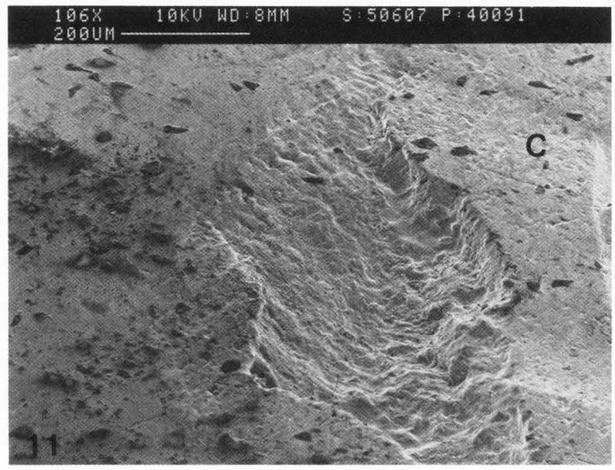
Figure 13 shows the results of an experiment which was designed to compare the margin quality of MOD composite restorations with the margin quality obtainable with adhesively luted composite inlays. It is clearly seen, that with the quantitative margin analysis it was possible to clearly show the superiority of the inlay technique at baseline and after 7 months in vivo. The inlays were able to maintain the high percentage of excellent margin, while the restorations underwent significant changes during use. Clinical investigation with mirror and explorer (USPHS criteria) showed no differences between the materials and techniques, since almost all restorations were rated alpha (Ryge 1981).

The results of another experiment designed to evaluate the influence of the cavity preparation design, the application technique and the composite construction on the marginal quality are shown in Figure 14. It is again









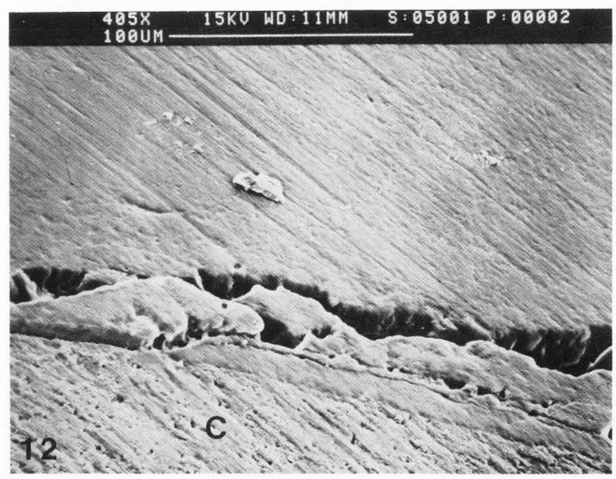


Fig. 7. "excellent margin" of composite resin restoration. (E = enamel, R = restoration)

Fig. 8. "marginal opening" of composite restoration. (E = enamel, R = restoration)

Fig. 9. "positive ledge" of composite restoration. (E = enamel, R = restoration)

Fig. 10. "negative ledge" composite restoration. (E = enamel, R - restoration)

Fig. 11. "restoration margin fracture" of composite restoration. (C = composite resin)

Fig. 12. "enamel margin fracture" of composite restoration. (C = composite resin)

Bars = 200 µm (Figs. 7-11), 100 µm (Fig. 12).

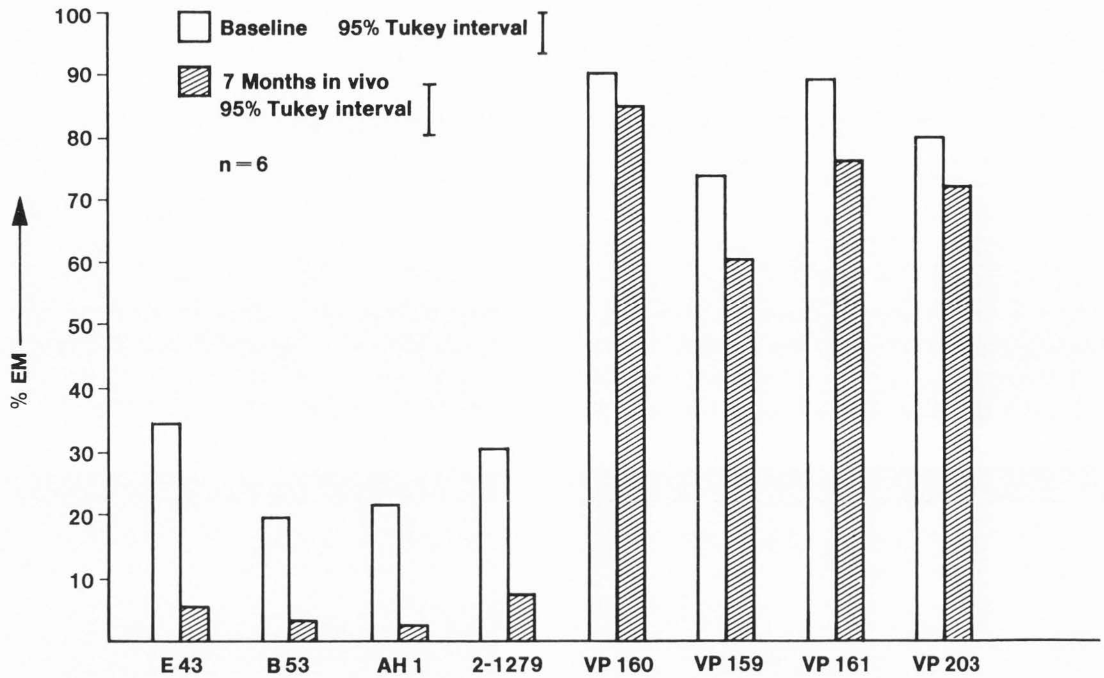


Fig. 13. % excellent margin of the occlusal margin of 4 composite fillings and 4 composite inlays (For inlays, only the enamel-luting composite interface is recorded). E 43 and B 53 are chemically cured hybrid composites, AH 1 and 2-1279 are light cured hybrid composites, all VP products are inlays made from heat cured microfilled composites. (Adapted from Roulet 1987)

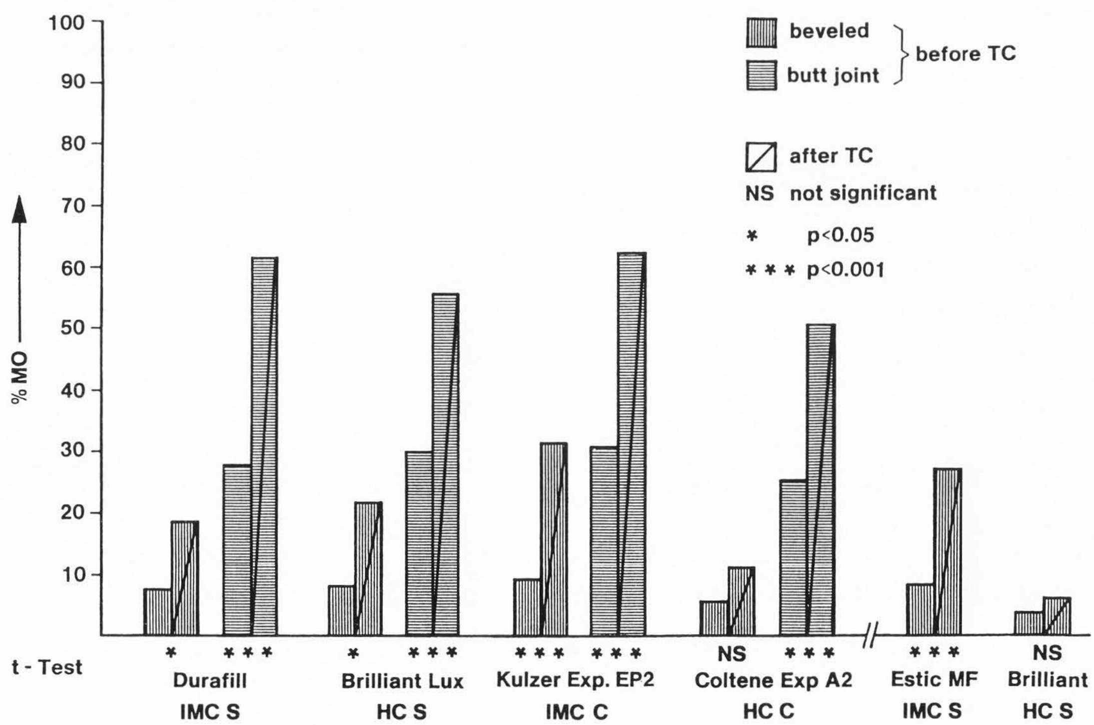


Fig. 14. % marginal opening before and after thermocycling of MOD composite restorations placed into bevelled and butt joint cavities.
TC = thermocycling, IMC = inhomogeneous microfilled composite, HC = hybrid composite. Condensable and syringable are related to the viscosity of the material and the dependent application techniques. (Adapted from Resch and Roulet 1986).

clearly demonstrated that the quantitative margin analysis is able to demonstrate differences. These can be enhanced if the restorations are subjected to thermocycling. These in vitro experiments have shown that the most influence in margin quality comes from the cavity design. The application technique (viscosity) of the material does not interfere with the margin quality and the hybrid composite is less affected by thermocycling.

The clinical behaviour of a hybrid composite (Lux-a-fill) and an inhomogeneous microfilled composite (Durafill) was monitored using the quantitative margin analysis. The results are shown in Figure 15. Both materials behaved equally well showing approximately 90 % "excellent margin". After use, both materials had a slight, but significant decrease in margin quality, which is clinically acceptable.

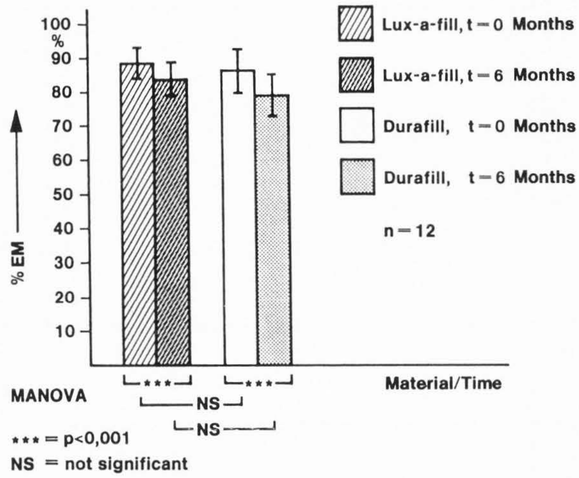


Fig. 15. % excellent margin of Class III composite restorations at baseline and after 6 months in vivo. (Adapted from Noack 1986)

Discussion

Compared to light microscopy, measuring techniques in the SEM are complex. Light microscopic pictures are always perpendicular to the optical axis and the object seen is only in one plane, due to the minimal depth of field. Therefore, measurements are possible in all directions on this plane. In contrast, in the SEM, there is an extremely large depth of field and the object is usually tilted. Thus the photograph is only a two dimensional projection of a three dimensional structure. Therefore, measurements on the photograph or screen are problematic because the relation to the true dimension is dependent on the projection given by the tilt and the true three dimensional orientation of the object. For example true measurements of crystal dimensions are only possible with stereoscopic techniques and require complicated calculations (Reimer and Pfefferkorn 1977). In designing the quantitative margin analysis we were well aware of these problems and we have tried to compensate for them as follows: our measurements are only relative, we calculate in digitizer units. The result is a % distribution. For repeated measurements we orient our samples identically in the SEM, resulting in equal distortions for both samples. We know that any deviation from a flat surface, e.g., if a restoration appears convex, will result in a shortened measurement of this portion, creating an error in the % distribution. Therefore we try not to measure restoration margins on strongly inclined surfaces. If restorations have different surfaces in different planes, the measurements are performed in different positions, e.g. a MOD restoration is observed from the mesial, then the sample is rotated and looked at from the distal and finally the occlusal surface is positioned to be approximately perpendicular to the "optical axis". Since we do not measure surface details, the three dimensional aspect of the structure (e.g., roughness of the surface, ledges at the margins, etc.) will not contribute to any measuring error. The surface characteristics are only used to rate the margin, not for measurements. The projection error given by the tilting of the sample is compensated for in two ways: (a) If possible we position the sample with tilt = 0, knowing that the gain in secondary electrons reaching the detector is poor in this situation and that it is difficult to distinguish between overhangs and underfilled sections. (b) If the sample has to be tilted, we operate the SEM without tilt correction and have the y-axis values corrected for the distortion in the computer. In all cases we have calibrated this value using grids with known interlattice distances. Thus we have different evaluation programs for the different tilts of the specimens. As a simplification we assume that the surface of the specimen is flat. The operator must decide prior to the evaluation of an experimental set which tilt is to be used and must select the corresponding evaluation program.

Since the SEM allows a high resolution, marginal defects are easily detected. Thus the rating "excellent margin", (defined as "the transition between the restorative material and the tooth hard tissue not clearly seen except from the different structures of the materials at the magnification selected") is only achieved with adhesive materials and techniques (e.g., composites with the enamel etching technique)(Lutz et al. 1976, Porte et al. 1984). For such materials and techniques this type of analysis works the best, because it is easy to define clear and reproducible criteria. Whenever the restorations are not able to produce an excellent margin morphology, due to contraction, the use of this method becomes quite problematic, because it is difficult to define different margin qualities. For example, in the case of amalgam, the morphology of the margins does not have the prime importance as in composites, because during use the obvious marginal gaps are filled with oxides (Passi et al. 1983, Going et al.1960, Grossmann et al. 1986). For the evaluation of materials with poorer adaptation it could be modified to also measure the width of gaps. Knowing the width and the length of gaps, material and procedure combinations (e.g., crowns or inlays) could be rated. However, when measuring gaps, the above mentioned projection problems become extremely important. Therefore the geometry of the sample mounting should be further investigated.

The high sensitivity of this method, due to the SEM's excellent detail reproduction is a great advantage for the clinical evaluation of new techniques or materials. With the quantitative margin analysis, minute changes in the restorations can be recognized after only a few months. The behaviour of the restorative material in the first 6 months can be used to predict the long term behaviour of the material. This is an important consideration for the approval of the clinical use of new materials. Actual procedures for the approval of composite resins are based on purely clinical investigations using the USPHS criteria (Roulet 1988). These criteria are much less sensitive than ours. The actual 3 year observation period for provisional acceptance and 5 years for definite acceptance (ADA 1986) could be significantly reduced if restorations would be evaluated with the above described method.

Since our method uses replicas, which are easily stored, the number of follow-up evaluations in a clinical study or in laboratory investigations is limited only by the amount of SEM time one is willing to invest. Experienced operators need aproximately 30 minutes per restoration depending on the size of the restoration and the quality of the margins. The better the quality, i.e., the more difficult it is to localize the margins, the more time consuming an analysis becomes.

Having a detailed protocol printed from every measuring procedure careful analysis of the single data is possible and provides additional information on the distribution of marginal defects. By marking specific landmarks on the protocol, e.g., corners of the proximal box, it is possible to find areas prone to failure in a restoration margin. This is especially important, if restoration techniques are investigated.

Conclusions

The quantitative margin analysis in the SEM is simple, non destructive and effective. With the appropriate interface it can be used with every SEM which offers a standard TV signal. Since the method is sensitive, it is especially well suited for all evaluations of high quality restoration margins. Therefore it should be used as a standard test for the in vitro and in vivo evaluation of composite resin restorations and all other composite adhesive applications (e.g., luting composite resins for inlays or resin bonded retainers).

References

American Dental Association (1986). Guidelines for composite resin materials for occlusal Class I and Class II restorations. ADA, Council on Dental Materials, Instruments and Equipment, Chicago IL.

Bergvall O, Brännström M (1971). Measurements of the space between composite resin fillings and the cavity walls. Swed Dent J 64: 217 - 226.

Brännström M (1984). Communication between the oral cavity and the dental pulp associated with restorative treatment. Operative Dentistry 9: 57 - 68.

Brännström M, Nyborg H (1971). The presence of bacteria in cavities filled with silicate cement and composite resin materials. Swed dent J 64: 149 - 155.

Brännström M, Nyborg H (1973). Cavity treatment with a microbicidial fluoride solution: Growth of bacteria and effect on the pulp. J Prosthet Dent 30: 303 - 310.

Brännström M, Nordenvall K J (1978). Bacterial penetration, pulpal reaction and the inner surface of Concise enamel bond. Composite fillings in unetched cavities. J Dent Res 57: 3 - 10.

Burt B A (1981). The epidemiology of dental caries. In: Silverstone L M, Johnson N W, Hardie J M, Williams R A D eds. Dental cares: Aetiology, pathology and prevention. MacMillan, London: 18 - 47.

Charbenau G T, Cartwright C B, Comstock F W, Kahler F W, Snyder D T, Dennison J B, Margeson R D, Ash M M, Avery J K, Ramfjord S P, Rowe N H (1981). Principles and practice of operative dentistry. Lea and Febiger, Philadelphia, PA. 2nd Ed. 474p.

Craig R G (Editor) (1980). Restorative dental materials. Mosby, St. Louis, MO. 6 th Ed. 478p.

Crim G A , Mattingly S L (1981). Evaluation of two methods for assessing marginal leakage. J Prosthet Dent 45: 160 - 163.

Crim G A , Chapman K W (1986). Effect of placement techniques on microleakage of a dentin-bonded composite resin. Quintessence Internat 17: 21 - 24.

Cvar J F , Ryge G (1971). Criteria for the clinical evaluation of dental restorative materials. US Dept of Health, Education and Wellfare, Public Health Service, Publ No 790-244, National Institute of Health, San Francisco, CA.

Dedmon H W (1982). Disparity in expert opinions on size of acceptable margin openings. Operative Dent 7: 97 - 101.

Derkson G D, Pashley D H, Derkson M E (1986). Microleakage measurement of selected restorative materials: A new in vitro method. J Prosthet Dent 56: 435 - 440.

De Trey E R (1976). Der Einfluss der Kavitätenform und des Versieglersystems auf die Adaptation und den Randschluss von approximalen Frontzahnfüllungen, in vivo (The influence of the cavity form and the bonding resin on the adaptation and the marginal seal on proximal anterior composite fillings in vivo). Thesis, Zürich.

Dumsha T, Biron G (1984). Inhibition of marginal leakage with a dentin bonding agent. J Dent Res 63: 1255 - 1257.

Flinn M (1978). Scanning electron microscope investigation of in vivo performance of eight composite resins. J Prosthet Dent 39: 529 - 532.

Fuks A B, Hirschfeld Z, Grajower R (1985). Marginal leakage of cervical resin restorations with a bonding agent. J Prosthet Dent 54: 654 - 657.

Going R E, Massler M, Dute H L (1960). Marginal penetrations of dental restorations as studied by crystal violet dye and 131 I. J Amer Dent Assoc 61: 285 - 300.

Goldberg J, Munster E, Rydinge E (1980). Experimental design in the clinical evaluation of amalgam restorations. J Biomed Mat Res 14: 777.

Gordon M, Plasschaert A J M, Saiku J M, Pelzner R B (1986). Microleakage of posterior composite resin materials and an experimental urethane restorative material, tested in vitro above and below the cementoenamel junction. Quintessence Internat 17: 11 - 15.

Grossmann E S, Witcomb M J, Jodaikin A (1986). Elements in marginal seals at amalgam-tooth interfaces. J Dent Res 65: 998 - 1000.

Hansen E K (1982). Visible light-cured composite resins: Polymerization contraction, contraction pattern and hygroscopic expansion. Scand J Dent Res 90: 32 9 - 335.

Hembree J H (1986). In vitro microleakage of a new dental adhesive system. J Prosthet Dent 55: 442 - 445.

Hembree J H, Andrews J T (1980). Microleakage evaluation of eight composite resins. J Prosthet Dent 44: 279 - 282.

Herder S, Roulet J.F. (1988). Marginale Adaptation von adhäsiv befestigten Dicor-Inlays in vivo. (Marginal adaptation of Dicor inlays luted with adhesive techniques in vivo). Deutsch Zahnärztl Z 43: 904 - 908.

Holmen L, Thylstrup A, Ogaard B, Kraggh F (1985a). A polarized light microscopic study of progressive stages of enamel in vivo. Caries Res 19: 348 - 354.

Holmen L, Thylstrup A, Ogaard B, Kraggh F (1985b). A scanning electron microscopic study of progressive stages of enamel in vivo. Caries Res 19: 355 - 367.

Kurosaki N, Kuroiwa S, Yokoyama H, Fusayama T (1974). Clinical tests of GK-101. J Japan Research Society of Dental Materials and Appliances 31: 221 - 232.

Lee H L, Swartz M L (1970). Scanning electron microscope study of composite restorative materials. J Dent Res 49: 149 - 158.

Leinfelder K F (1986). Die Seitenzahnfüllung mit Composites - Vom Material bis zur polierten Füllung (The posterior restoration with composite resins - From the material to the polished restoration). Internationales Composite Symposium, Bad Nauheim (Germany), 7./8. Nov. 1986. Quoted in: Butros R, Roulet J F (1987). Internationales Composite Symposium. Swiss Dent 8/3: 19 - 33.

Leinfelder K F, Sockwell C L, Sluder T B (1982). Two year clinical evaluation of Profile in posterior teeth. J Dent Res 61: 215, Abstr. No 327.

Leinfelder K F, Barkmeier W W, Goldberg S A (1983). Quantitative wear measurements of posterior composite resins. J Dent Res 62: 670, Abstr. No 194.

Lutz F (1980). Beiträge zur Entwicklung von Seitenzahnkomposits (Contributions to the development of posterior composites). KAR PAR PZM Zürich 1980.

Lutz F (1984). Zahn- und Zahnbetterkrankungen: Therapie - die Strategie der Wahl? Vorbeugen, heilen oder flicken? (Diseases of the teeth and the periodontium: Therapy - the strategy of choice? Prevent, cure, or repair?). Swiss Dent 5(3): 7 - 17.

Lutz F, Lüscher B, Ochsenbein H, Mühlemann H R (1976). Adhäsive Zahnheilkunde (Adhesive dentistry). KAR PAR PZM Zürich 259p.

Lutz F, Krejci I, Oldenburg T R (1986). Elimination of polymerization stresses at the margins of posterior composite resin restorations: A new restorative technique. Quintessence Internat. 17: 777 - 784.

Mahler D B (1979). Behaviour of three high-Cu-amalgams. J Biomed Mater Res 13: 693 - 699.

Mahler D B, Marantz R L (1979a). The effect of time on the marginal fracture behaviour of amalgam. J Oral Rehabil 6: 391 - 398.

Mahler D B, Marantz R L (1979b). The effect of the operator on the clinical performance of amalgam. J Am Dent Assn 99: 38 - 41.

McCune R J, Johnson B E, Cvar J F, Ryge G (1967). Clinical comparison for posterior restorative materials. J Dent Res 46 (special issue): 175, Abstr. No 546.

Michellod P Y (1984). Etude comparee au microscope electronique a balayage de deux methodes de dessication appliquees au composite (Scanning electron microscopical comparative study of two dehydration methods with composite resins). Thesis Bern 1984

Neter J, Wasserman W (1974). Applied linear statistical models. Irwin, Homewood, Ill., USA, 842p

Noack M J (1986). In vitro und in vivo-Vergleich eines experimentellen Hybrid-komposits mit einem inhomogenen Mikro-füller-komposit bei Klasse-III-Kavitäten (In vitro and in vivo comparison of an experimental hybride composite with an inhomogeneous microfilled composite placed in class III cavities). Thesis Berlin 1986

Osborne J W, Binon P P, Gale E N (1980a). Dental amalgam: clinical behaviour up to eight years. Operative Dent 5: 24 - 28.

Osborne J W, Leinfelder K F, Gale E N, Sluder T B (1980b). Two independent evaluations of ten amalgam alloys. J Prosthet Dent 43: 622 -626.

Passi P, Musajo F, Furlan C (1983). La chiusura marginale delle otturazioni in amalgama: Microanalisi dei detriti inorganici che concorrono a formare il sigillo (nota II)(The closure of the marginal gap of amalgam restorations: Microanalysis of the inorganic debris which compete to produce the marginal seal, 2nd notice). Dent Cadmos 51 (1): 37 - 48.

Phillips R W (1973). Skinner's science of dental materials. Saunders, Philadelphia, PA, 7th ed., 682p.

Porte A, Lutz F, Lund M R, Swartz M L, Cochran M A (1984). Cavity designs for composite resins. Oper Dent 9: 50 - 56.

Reimer L, Pfefferkorn G (1977). Rasterelektronenmikroskopie (Scanning electron microscopy). Springer Berlin 2nd ed., 282p.

Resch H, Roulet J F (1986). In vitro marginal integrity of MOD fillings with hybrid and microfilled composites. J Dent Res 65: 826 (Abstr. Nr. 902).

Roulet J F (1976). Untersuchung über den Randschluss von Komposits in vivo (Investigation of the marginal seal of composite resins in vivo). Schweiz Mschr Zahnheilk 86: 699 - 712.

Roulet J F (1978). Die Prüfung des Randschlusses sogenannt schnitzbarer Komposits (Assessment of the margin quality of so-called carvable composite resins). Schweiz Mschr Zahnheilk 88: 345 - 364.

Roulet J F (1987). Degradation of dental polymers. Karger Basel 228p

Roulet J F (1988). The problems associated with substituting composite resins for amalgam. J Dentistry 16: 101 - 113

Roulet J F, Michellod P Y (1984). La dessication d'obturations en composite pour l'etude au microscope electronique a ballayage - une etude methodologique (The desiccation of composite restorations for the investigation with the scanning electron microscope - a methodological study). Rev Mens Suisse Odonto-Stomatol 94: 1049 - 1060.

Ryge G (1981). Clinical criteria.Int Dent J 30: 347 - 358.

Schroeder A (1981). Endodontics - Science and practice. Quintessence, Chicago, IL, 286p.

Schutzbank S G, Galani J, Kronman J H, Golgman M, Clark R E (1978). A comparative in vitro study of GK-101 and GK-101 E in caries removal. J Dent Res 57: 861 - 864.

Thylstrup A, Fejerskov O (1986). Textbook of cariology. Munksgaard, Copenhagen, 392p.

Tsuchiya T, Zidan O, Gomerz-Marin O (1986). Marginal gaps in class V cavities: Comparative study of dentinal bonding agents and application techniques. J Dent Res 65: 765, Abstr. No 353.

WHO/FDI (1982). Aetiology and prevention of dental caries and periodontal diseases. Int Dent J 32: 78 - 88.

Wu W, Cobb E, Dermann K, Rupp N W (1983). Detecting margin leakage of dental composite restorations. J Biomed Mat Res 17: 37 - 43.

Discussion with Reviewers

A. Boyde: As a general question to the authors I should like to ask - "What evidence do they have that their classification of the goodness of cavity margins bears any relationship to the in vivo survival of restorations?"

Authors: The classification is purely descriptive and does not contain any judgement of the margin quality. The question is very fundamental for all researchers dealing with the assessment of the margin quality since there are almost no research data on correlations between margin quality and longevity of restorations. The only publications known to the authors are either based on few restorations or were done as a pilot study which only showed a trend. (Goldberg J et al. (1981): Cross sectional clinical evaluation of recurrent caries, restoration of marginal integrity and oral hygiene status. J Am Dent Assoc 102: 635 - 641. Jörgensen K D and Wakumoto S (1968). Occlusal amalgam fillings: Marginal defects and secondary caries. Odontol Tidskr 76: 43 - 54). The interpretation of margin quality and its influence on longevity is based on the concept that any site af plaque accumulation is a caries risk site. This is known for fissures and the proximal contact area of teeth. In addition it is known that fillings with proximal overhangs lead to a microbiological flora which has the same composition as the flora of an active periodontal pocket (Lang N P, Kiel R A, Anderhalden K (1983). Clinical and microbiological effects of subgingival restorations with overhanging or clinically perfect margins. J Clin Periodontol 10: 563 - 578). With composite resins, clinical experience has shown that composite fillings with marginal openings often have secondary caries. However, amalgam fillings with a very poor margin morphology may survive for years without secondary caries occurring. In adhesive dentistry the absence of marginal openings is proof of success (adhesion of the restorative material to the tooth).

The technique described is only a tool to precisely describe the margin quality in order to enable researchers to find any correlations between the survival rate and the margin morphology of restorations.

R. Elderton: The authors rather loosely list six categories of marginal adaptation of the restorative material to the tooth: excellent, marginal opening, positive ledge, negative ledge, restoration margin fracture and enamel margin fracture. These would seem to be very crude categories when such a sophisticated method of observation is being used, indeed the authors state that "the high sensitivity of this method due to the SEM's excellent detail reproduction, is a great advantage for the clinical evaluation of new techniques or materials." Would the authors not agree that the method is somewhat of an expensive overkill? - and if not why not? Could an optical stereomicroscope have served the purpose? Orientation errors of a few degrees would not seem to be very important - do the authors agree?

Authors: We definitely do not consider the method an overkill. Because crude work can be assessed with crude methods, fine work must be assessed with fine methods. Since the prime target of the method is to depict early changes, we definitely need the option of the SEM's high resolution. Our work with dentin adhesives has clearly shown that, with proper application techniques, the restorations look perfect when assessed with magnifying glasses, look good in the optical stereomicroscope, but show minute cracks visible only in the SEM at the restoration dentin interface, indicating a failure of the dentin adhesive system.

The classification of observations is a common research tool. The criteria listed are just an example. The system works independently of the selected criteria, which is an advantage, since the criteria can be adapted to the investigation. We agree that the criteria are somewhat crude, however if you ever had the opportunity to observe restoration margins in the SEM you would be impressed by the variety of the morphology. With our system the observer is an "integrator" of the variability. If you get stuck on details, you will never be able to assess the restoration as a whole. Let us use an analogy: if you only look at the damaged leaves, you will never notice that the forest as a whole is sick. Therefore, our criteria cover very crude findings e.g. enamel margin fractures, which occur with traumatic finishing techniques and very fine criteria e.g. marginal openings, which indicate debonding of the restorative material.

The use of an optical stereomicroscope would create other problems: 1.The optical microscope produces a virtual image which is not suitable for interfering with any measuring device. 2. If the image is projected on a screen, which is necessary for any image analyzing technique, the stereo effect is lost and therefore the morphology of the margins is very difficult to assess. 3. We would assume that the costs for transforming an optical microscope to do the same job with less convenience would be much higher than the price of the interface used to interfere with the SEM.

Orientation errors are not very important as long as flat surfaces are involved. The more a surface is inclined, the more orientation errors will become important. Since restorations usually have a complex surface morphology, we wanted to eliminate any orientation errors. It is possible, that the relevant changes occur in a very inclined part of the surface. If, due to orientation errors, the distortion would approach 10 %, the cumulation of this error with the operator error would make it impossible to depict the changes.

R. Elderton: The authors state "measuring techniques in the SEM are complex" yet they state "quantitative margin analysis in the SEM is simple". Perhaps we need a rather better yardstick against which to use the terms complex and simple; but could the authors please elaborate on this point?

Authors: The development of the interface was not simple, however, the resulting system is very simple. Therefore, our yardstick is perfectly OK. If the reviewer had read the appropriate portion of the quoted textbook (Reimer and Pfefferkorn 1977) he would not have had to ask this question. The measurements based on SEM images require stereoscopic techniques. This means that for any image, two exposures at defined angles are taken and that the tilt axis must be placed in the center of the image. Additionally the precise distance, focal center - specimen, must be known. The latter must be determined with a special test grid according to a formula. Then distances can be determined with a set of complicated formulas. Additionally the method is only applicable within restricted limits in the vertical dimension and requires a more or less perpendicular arrangement of the object to the electron beam. Wouldn't you call this rather complex? We do. Our method requires a simple length measurement according to the Pythagorean theorem, and an interface to connect a low cost computer to the SEM without changing any SEM hardware. The only thing the operator has to do is to mark the margins with the digitizer and decide which margin quality it was. If the restrictions for the specimen mounting procedure were respected, we can obtain data within an acceptable precision range. Woudn't you call this simple. We are convinced it is.