Evaluation of a novel repetitive gas-enhanced permeation test for restoration leakage determination after thermo-mechanical loading

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Evaluation of a novel repetitive gas-enhanced permeation test for restoration leakage determination after thermo-mechanical loading

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Running title: Restoration tightness and adaptation testing

The manuscript contain 1 table and 4 Figures

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Abstract:

Objectives: To assess leakage of class-I restorations using a gas-enhanced permeation test (GEPT) as compared with conventional SEM or dye analysis. **Materials and methods**: Pressure differences over time and penetrating water volumes were measured simultaneously in a two-chamber system (GEPT) before and after class I cavity preparation in 30 molars. Ten teeth were restored with a composite restoration without bonding (A1), a composite restoration with bonding (A2) or a ceramic indirect restoration (B). Five intact teeth served as controls (C). Another GEPT measurement was performed, and impressions were taken. Teeth were subjected to thermodynamic loading (1'200'000 cycles), and final GEPT measurements and impressions were made. SEM evaluation of the marginal continuity was performed, and teeth were subjected to a Fuchsin dye penetration test. Spearman's rank test was used to compare results from different tests. **Results:** The GEPT and SEM values did not correlate before loading (0.359, $p=0.051$) but significantly correlated afterwards (0.662, $p<0.0001$). The correlations between the Fuchsin dye penetration test and GEPT and SEM surface marginal analysis were significant (0.777 and 0.534, p-values <0.0001 and 0.002, respectively). **Conclusions:** SEM marginal analysis was mainly limited in reflecting the surface restoration integrity. GEPT evaluation may therefore serve as a tool to non-destructively assess restoration subsurface integrity over time.

Clinical relevance: The current study provided proof, that restoration margin quality does not necessarily reflect its leakage behaviour.

Key words: thermodynamic loading, leakage, marginal analysis, restoration.

1. Introduction:

Restoration marginal integrity is crucial for predictable long-term clinical success, especially of adhesively placed restorations [1]. In this context, polymerization shrinkage reflects a major problem and may initiate early failures of the restoration–tooth interface, resulting in interfacial gaps [2] [3]. This might lead to a decreased marginal quality and consequently to microleakage, postoperative sensitivity, marginal discoloration and secondary caries [4]. Therefore, restoration quality improvement by material-related and technical means remains an important aspect in pre-clinical dental research.

Microleakage evaluation can be defined and classified according to the type of substrate used to study the penetration processes, e.g., air, bacterial, fluid, or molecular or ion penetration within the tooth-restoration interface [5]. In vitro air testing to assess restoration integrity was introduced in 1912 [6] when compressed air was forced through roots and bubble development at the tooth-restoration interface on the coronal side was observed with a microscope, which indicated restoration leakage. The same principle using compressing dyes was later implemented to assess sealability [7]. These methods were able to determine the quality of restorations by detecting the time point by which the leakage started.

To evaluate the performance of the surface margin morphology, replica techniques were also established to screen the complete marginal circumference of restorations under SEM in order to describe and quantify the quality of dental restoration margins [8]. This method, however, was limited to the evaluation of the surface conditions only. Overall, the marginal adaptation must be regarded as an important area to study surface and subsurface quality, which remain the main areas

for bacterial retention. Increased bacterial retention at defective interfaces can lead to secondary caries development [10].

Due to the limitation of the visual assessment of restoration marginal adaptation, dye penetration models were developed to assess the penetration depths of fluids within defective intra-coronal interfaces in order to score the extension of dye perfusion in dentinal tubules system within sectioned samples [9]. The later technique also bears some limitations, mainly the aggressive cutting of specimens and the ability to evaluate the margin only at a single time point, thus preventing understanding of the leakage process.

Unfortunately, analyses of the interactions among adhesive and resin composite materials, such as marginal analyses or dye penetration, do not necessarily correlate with clinical findings [12]. However, such in vitro tests are important to screen materials and techniques and to compare results before use in the clinic, taking into account the possible limitations of such set-ups.

It has been conceivably demonstrated that if leakage occurs, bacteria, along with their by-products and irritants, will follow the path of the dentinal tubules into the pulp, which could be one possible cause of hypersensitivity, pulpal inflammation or even pulpal death [14]. Recently, a gas-enhanced permeation test (GEPT) method allowing for fluid infiltration and pressure difference determination under standardized conditions was introduced for dentin fluid infiltration [15]. This system allows multiple non-destructive leakage measurements after different treatments, e.g., restoration placement, with high precision and reproducibility. To our knowledge, no study yet conducted has correlated surface marginal adaptation with leakage after thermodynamic loading using such an approach. Therefore, this study aimed to compare the outcomes of the three testing models, namely, the novel GEPT, SEM

surface marginal analysis and Fuchsin dye penetration tests, when applied to the same set of samples. For this purpose, standardized class-I restorations of different interface qualities were assessed, and correlations between the restoration surface quality of the margins and the leakage were established. It is hypothesized that a positive correlation exists between the amount of leakage (assessed by GEPT and Fuchsin dye penetration) and the surface marginal quality of the restoration assessed by SEM.

2. Materials and methods

Three test methods were used and compared with the same set of teeth without affecting the integrity of the sample embedding (Figure 1). For this purpose, thirty-five third molars were selected from the department collection of teeth of known age. The teeth were extracted from patients aged 18-21 years for reasons not related to the study and were stored in 0.2% thymol at a temperature of 5°C. To be included in this study, the teeth had to be free of caries and cracks and have incomplete root formation with a wide pulp chamber to ensure dentinal tubule patency. The teeth were randomly allocated into three test groups (n=10) and one control group using a randomization program (www.randomizer.org) (n=5).

2.1. Embedding procedures

Samples were embedded in brass rings with an outer diameter of 15 mm, an inner diameter of 10 mm and a thickness of 3 mm. The inner surface was sandblasted with aluminium oxide with a particle size of 50 µm (Benzer-Dental AG, Zurich, Switzerland). A conventional light-cured nail build-up material was used for fixation, primer, build up gel and glaze (Sina, Shenzhen Cyber Technology Ltd, Mainland, China) [13]. After primer application, samples were incubated for two minutes in a

light-curing unit (Spectramat, Ivoclar-Vivadent, Schaan, Liechtenstein). All parts then were assembled in a custom-made silicone putty carrier (Optosil, Heraeus Kulzer GmbH, Hanau, Germany). The nail build up gel material was applied to seal the space between the ring and the tooth sample. The gel material was extended to cover the root surface down to the last millimetre of the root tip. Samples were then light cured again for four minutes. A final layer of glaze material was applied to the top of the sample to improve the embedding and was light cured for another four minutes.

2.2. Cavity preparation

Thirty teeth were randomly assigned to one of three test groups (A1, A2, and B; detailed description below), and class-I preparations were prepared using a parallelometer on a XY table (Cendres & Metaux SA, Biel, Switzerland) with a diamond bur with a grit size of 80 μm (Bur 837 KR, 8614, Intensive SA, Grancia, Switzerland). The preparations were 6 mm long (in mesio-distal direction), 3 mm wide (in bucco-oral direction) and 2 mm deep as measured from the middle fissure level (Figure1, C). Five intact teeth without preparation served as negative controls (C).

2.3. Restorative treatments

2.3.1. Group A1: Composite restoration without bonding

Teeth in this group were restored with resin composite (Filtek Supreme, 3M ESPE, Seefeld, Germany) without any acid etching or bonding procedures, i.e., without any priming and bonding. The resin composite material was applied in three horizontal increments, which were polymerized for 20 s at 800 mW/cm^2 (Bluephase LED G2, Ivoclar, Vivadent, Schaan, Liechtenstein). Finishing of the restoration took

place using specially designed finishing burs (Intensiv SA, Grancia, Montagnola, Switzerland) and polishing discs (Sofflex discs, 3M ESPE D) under a stereomicroscope (Stemi 1000, Zeiss, Oberkochen, Germany).

2.3.2. Group A2: Composite restoration with bonding

Restorations were placed as they were in group A1 but with an etch and rinse approach using a 3-step adhesive system (Syntac Classic, Ivoclar, Vivadent, Schaan, Liechtenstein) prior to composite placement. The enamel was selectively etched for 60 s with 35% phosphoric acid (Ultra Etch, Ultradent, South Jordan, Utah-USA) followed by a 40-s wash with water spray. After drying with air, a self-conditioning maleic acid-containing primer (Syntac Primer, Ivoclar Vivadent) was applied for 15 s and gently air-dried before the application of the adhesive (Syntac Adhesive, Ivoclar Vivadent) for 20 s. After gentle air drying, an unfilled bonding resin (Heliobond, Ivoclar Vivadent) was applied for 20 s and light cured for 40 s (Bluephase LED G2). Resin composite (Filtek Supreme, 3M ESPE, Seefeld, Germany) was applied in three horizontal increments, which were polymerized for 20 s each. The samples were then finished and polished.

2.3.3.Group B: Ceramic indirect restoration (inlay)

Ceramic inlays were designed using a Cerec 4D program, milled with a CEREC MCXL milling unit (Sirona Dental GmbH, Salzburg, Austria) utilizing a glass-ceramic material (IPS Empress CAD Multi, Ivoclar, Vivadent, Schaan, Liechtenstein).

Teeth were conditioned as described above (2.3.2) using the same adhesive system (Syntac Classic, Ivoclar, Vivadent, Schaan, Liechtenstein). The ceramic

indirect restorations were acid-etched with hydrofluoric acid (Vita Ceramics Etch, Vita Zahn Fabrik, Bad Säckingen, Germany) for 60 s. After extensive water spray application, a silane was applied (Monobond Plus, Ivoclar Vivadent) for 60 s and the ceramic inlay was dried. Then, an unfilled bonding resin was applied (Heliobond, Ivoclar Vivadent) to the inlay base without light curing. Resin composite material (Filtek Supreme XT, 3M ESPE) was pre-warmed in an oven (AdDent Inc., Danbury, USA) to 37°C before application to the inlay and the cavity. The inlay was first positioned by finger pressure. Subsequently, ultrasound was applied (mini Piezon, EMS, Nyon, Switzerland) for 10 s to finalize the placement of the inlay and the excess material was carefully removed. Light polymerization was performed from 5 aspects for 60 s each from the occlusal, mesial, distal, buccal and oral directions, respectively.

2.4. The gas enhanced permeation test (GEPT)

Details of the device and its set-up were described in a previous validation study [15].

In brief, the apparatus consisted of a two-chamber system where a sample was placed in the middle separating the two chambers. The embedded sample was fixed between the two parts using a rubber O-ring with an outer diameter of 22 mm, an inner diameter of 15 mm and a thickness of 3.5 mm, which resulted in two fully separated and hermetically sealed chambers with the embedded sample in between.

The temperature was controlled and constantly held at 35°C in the inner chamber. This core system was installed in a second larger experimental box in which the temperature was stabilized at 31°C.

A pressure-difference measuring device (Testo 526, Testo AG, Lenzkirch, Germany) was connected to the upper and lower chambers. Readings were recorded with a computer-unit running a proprietary program (V 4.2 SP2, Testo AG, Germany). The O-ring was lubricated with a silicon grease (Molykote 111 compound, DOW Corning GMBH, Germany) and the sample was positioned in place in the lower part of the permeability/leakage device, and 2.5 ml of a pre-pressurized (N_2 gas 860 hPa) 0.9% saline solution was added on top. The upper part was repositioned and the three screws were tightened with a torque-controlled screwdriver. To achieve an effective pressure difference of 1030 hPa, the upper chamber was pressurized with N_2 gas to 860 hPa, whereas the lower chamber was negatively pressurized to -170 hPa. The pressure difference readings were initiated and continued over 40 min at a rate of 1 measurement/sec. The resulting data were plotted as the rate of pressure change expressed as a drop in pressure difference over time. The slope between pressure value differences at two fixed time points (1200 sec and 2400 sec) was defined to present the sample leakage status:

$$\text{Slope} = \frac{P2-P1}{T2-T1} \text{ hPa/min.}$$

P2: Pressure difference at time point 40 min.

P1: Pressure difference at time point 20 min.

T2: Time point 40 min.

T1: Time point 20 min.

In addition, the infiltrating physiological saline solution was collected and weighed to calculate the volume that permeated the specimen.

For all samples, measurements were carried out at the following time points:

a) At baseline, i.e., after embedding but before tooth preparation to assess tight sealing

b) After preparation, to determine the maximal leakage through the dentin wound

c) After restoration, to measure the restoration leakage value, which is expected to range between a) and b)

d) After thermodynamic loading

In general, higher GEPT values implied more leakage, whereas lower values indicated improved tightness.

2.5 Thermodynamic loading:

Samples were transferred to special carriers and embedded without interrupting the embedding disc mounting integrity (Figure 1). For this purpose, the stainless steel carriers had a separate cylindrical compartment (diameter of 11 mm and a depth of 12.5 mm), which was filled with heavy body impression material (3M ESPE Pentamix 2, 3M Deutschland GmbH, Seefeld, Germany). To maintain a space between disc and carrier, a rubber separator 1 mm in height was placed between the embedding disc and the carrier and was later removed. Thereby, any luxation of the disc was avoided and stress was transported only to the root ensuring no effect on the mounting integrity.

Antagonists were fabricated with resin composite material for each sample individually to allow full occlusal contact (Filtek Supreme XT, 3M ESPE).

The samples and their antagonists were mounted in a computer-controlled masticator and subjected to thermodynamic loading; 1,200,000 loadings at 20N/cm^2 and 3,000 temperature cycles, 5°C/50°C [14].

2.6 SEM surface marginal analysis

The quality of restoration margins was studied before and after the thermodynamic loading. After the restoration placement, occlusal surfaces were cleaned with alcohol, rinsed with water spray and dried with air. Impressions were made using low viscosity silicon impression material (President plus jet light body, Coltene, Altstätten, Switzerland).

After 24 hours, epoxy resin was poured into the impressions (Stycast 1266, Emerson & Cuming, Henkel Eleotronlo Materials, Westerlo, Belguim), and 24 hours later, the casts were trimmed and mounted on SEM holders (SCD 030, Balzer Union AG, Balzer-FL). The mounted samples were dried for another 24 h. With the aid of a sputtering device (Oerlikon Balzers Coating AG, Balzer, Liechtenstein), casts were coated with a 90-nm gold layer under 0.08 mbar and current of 45 mA for 3 minutes. The replicas were then analysed at a 200-fold magnification for gap presentation. A gap was defined as a pronounced defect in the continuity between the tooth and restoration surfaces, where the floor of the defect was non-detectable (Figure 2, B). The total margin of the restoration was analysed in steps using a scanning electron microscope (SEM; Carl Zeiss Supra 50 VP FESEM, Carl Zeiss, Oberkochen, Germany). The total visual quality of the restoration margin was presented for each sample as a percentage of discontinuity, i.e., the percentage of defective restoration margin [8]. The surface marginal analysis was performed by one blinded and calibrated operator. The repeatability of identical samples at different time intervals (2 weeks) was 91%. The criteria for the marginal assessment were as follows:

1. Perfect margin: No visible interruption of the interface continuity, i.e., no different levels visible.
2. Marginal gap: the interface showed discontinuity, e.g., cracks or gaps.

3. Non-assessable areas were defined as any deviation from the above-mentioned criteria. Non-assessable areas were mainly the result of impression inaccuracies and were mainly derived from bubbles, excess material, debris or contaminations at the restoration-tooth interface, which hampered the clear visualization and judgement of the respective margins.

All restorations were assessed before and after thermodynamic loading.

2.7. Fuchsin dye penetration test:

The Fuchsin dye penetration test was completed at the very final stage of the evaluation series described above because it required sectioning of the samples. Teeth were carefully demounted from the embedding medium for this purpose and were circumferentially sealed up to 1 mm from the restoration margin with nail varnish (Cover Girl, Nail slicks, Procter and Gamble, OXP, UK), and the samples were immersed in 0.5% basic Fuchsin dye solution for 20 h.

Under kerosene cooling, teeth were then sliced in the bucco-lingual direction utilizing a slow speed diamond saw (0.4 mm, Strures GmbH, Zweigniederlassung, Switzerland). In total, four sections were prepared for evaluation, which were photographed at a 25-fold magnification and digitized. Samples were dichotomously categorized as "non-leaking" (=0) when the dye did not reach the pulp chamber or "leaking" (=1) when the dye reached the pulp chamber (Figure 2, C). All sections were independently evaluated by two blinded investigators. In cases of disagreement, sections were reassessed and discussed until an agreement was reached.

2.8. Statistical analysis

Descriptive statistical analyses were completed separately for the three restorative treatments for the GEPT test (before and after the thermodynamic loading), the SEM surface marginal analysis (before and after the thermodynamic loading) and the Fuchsin dye penetration test (only after the thermodynamic loading). For the negative control, a descriptive analysis of the GEPT test and the Fuchsin dye penetration test (after the thermodynamic loading) was applied (Table 1). The following tests were applied to assess different statistical outcomes. The Kolmogorov-Smirnov test was applied to assess normality in the data distribution. To compare all test outcomes within the same treatment group before and after thermodynamic loading, the Wilcoxon signed rank test was applied. The Kruskal-Wallis test was used to compare different tests for before and after thermodynamic loading outcomes between different treatment groups. In order to further assess where differences appear, the Mann-Whitney U test was applied. Finally, for all respective correlations, a Spearman's rank correlation test was applied. A significance level (probability for type I error) of 0.05 was used with the two-sided p-values.

3. Results

For the GEPT results, the assumption of a normal distribution was rejected for all groups before (p-values 0.003, 0.027, 0.013 for groups A1, A2 and B) and nearly all groups after the thermodynamic loading (p-values 0.009, 0.200, 0.000, 0.026 for groups A1, A2 and B and negative control) by a Kolmogorov-Smirnov test. For the SEM surface marginal analysis, normality was never rejected. Nevertheless, due to the small group sizes, only nonparametric tests were used.

A comparison of the values before and after thermodynamic loading (separately within each group) revealed that only the GEPT results in group A1 were

significantly different (Wilcoxon signed rank test, p-value 0.016) and were improved after thermodynamic loading. For all other groups (A2, p-value 0.084 and B, p-value 0.129) as well as for the SEM surface marginal analysis (A1 p-value 0.114, A2 p-value 0.139, B p-value 0.169), no significant changes were observed. For the negative control, this comparison was not meaningful.

A further examination showed that for GEPT and SEM surface marginal analysis (before and after the thermodynamic loading), the results among groups A1, A2 and B (and negative control for GEPT after thermodynamic loading) differed significantly (Kruskal-Wallis tests). To see, where the differences appeared between groups, pairwise group comparisons were made using Mann-Whitney U tests. The GEPT results between groups A1 and A2 were significant before or after loading or by SEM surface marginal analysis after thermodynamic loading. For groups A1 and B, all tests showed significantly different outcomes. For A2 and B, only SEM surface marginal analysis after thermodynamic loading showed significant differences.

The Fuchsin dye penetration test results were significantly different among the four groups (Fisher's exact test, p-value < 0.0001). This finding was mainly due to the data from group A1 where a high number of leakage scores were observed (8 out of 10).

Finally, we looked at the correlation among the tests (globally over all groups). Spearman's rank correlation was used to correlate the different test results. For GEPT and SEM surface marginal analysis before thermodynamic loading, the correlation was only moderate (0.359) and not significant (p-value 0.051), while it was significant after thermodynamic loading (0.662, p-value < 0.0001). Also, the correlations between the Fuchsin dye penetration test and GEPT and SEM surface marginal analysis (after loading) were significant (0.777 and 0.534, p-values <0.0001

and 0.002), with a higher level of significance observed for the GEPT evaluation technique.

4. Discussion

This study aimed to evaluate a new gas-enhanced leakage measurement system in comparison to a traditional SEM surface marginal analysis and a subsurface Fuchsin dye penetration test. It was hypothesized that leaking restorations - as measured by the GEPT method – should therefore also display poorer marginal adaptation and an increase in their dye penetration profiles. This could be corroborated by the findings of the present study, where a significant correlation was found between the Fuchsin dye penetration test - considered as a gold standard in displaying the liquid penetration leakage pathways [17] - and the GEPT and the SEM. Although this correlation was significant, the SEM, which stands for the surface marginal and maybe subsurface analysis, was shown not necessarily to represent the true performance of the restoration, especially before loading, which coincides with other studies where this evaluation method was judged to have controversial clinical relevance [11] [12] [13] and may resulted in false negative conclusions with regard to tracer penetration and maybe caries formation [17]. Heintze and co-workers compared SEM quantitative marginal analysis data with the penetration depth of the three most commonly used tracers for microleakage in Class II fillings in vitro, i.e. fuchsin, silver nitrate and methylene blue [18] . In their study, teeth were subjected to occlusal loading and simultaneous thermodynamic loading in a comparable protocol as in the present study and the percentage of continuous margin of the cervical dentin and enamel was evaluated on replicas using SEM. They concluded that tracer penetration showed a moderate correlation with SEM quantitative marginal analysis at dentinal

margins, but not at enamel margins. It must be highlighted at this point that a class I defect was prepared in the present study with all margins located in the enamel. Therefore it may not be surprising that the correlation in our study was not significant before loading and became only significant after loading, but with a still rather low correlation. Nevertheless, despite the fact that the dye penetration method displays a high detection limit, it cannot assess and compare the exact effect of thermodynamic loading on restorations as this technique allows for a single time point measurement only because it requires sectioning of the samples.

In this context, the GEPT method may be a valuable measuring tool, as it displays a high sensitivity for detecting leakage without destroying the sample and also shows a low detection limit of 0.002 hPa/min for the pressure slope and 0.0225 µl/min for the fluid infiltration, respectively [15]. This was confirmed by the highly significant correlation with the Fuchsin dye penetration test. Unlike previous setups where the dye and fluid infiltration was applied in a reverse direction [7] [19] [20], the current study applied the normal possible direction of leakage (out-in) and therefore simulated the clinical situation more accurately where leakage is expected to occur from the oral cavity towards the pulp. This was also highlighted in a recent publication, which assessed the combined effect of cyclic loading and bacterial exposure on bacterial penetration at the interface between dentin and resin composite restorative material using a novel bioreactor system [21]. The study showed that gaps, which were as small as 15–30 µm, were enough to allow bacterial leakage to the full depth under thermodynamic loading. This fact highlighted the necessity of assessing leakage and also provided a new model, which was able to detect bacterial penetration and demineralization at the same time. However, this method also presented a limitation in its capability to allow multiple evaluation methods to compare and

correlate results because this would require sectioning of the samples again. GEPT tries to overcome this problem, but other options for evaluation remain open for scrutiny.

When focusing on the different restorative treatments, the unbonded composite restorations showed higher GEPT values than did the bonded composite restorations and the adhesively placed ceramic indirect restorations. Surprisingly, the GEPT values improved after thermodynamic loading, which could be explained - in part - by some occlusion of the dentinal tubules by a dynamic frictional smear layer production. Another possibility could lie in the hygroscopic effects after fluid uptake [22].

As expected, both bonded restoration groups performed significantly better in contrast to the non-bonded group at each evaluation stage, which underlines the importance of adequate bonding to ensure a tight and durable restoration interface. Overall, the results of the marginal quality evaluation corresponded to those in previously published studies that assessed marginal quality after loading with comparable evaluation techniques, i.e., SEM surface marginal analysis and dye penetration test [23] [24]

The excellent performance of the negative control group before and after loading proved the adequate sample embedding procedures under thermodynamic loading conditions. A previous study using the same set-up, but under static conditions, also showed that repetitive measurements of identical samples resulted in reproducible readings [15].

5. Conclusion:

- Restorations with visually detectable deteriorated margins do not necessarily present higher subsurface leakage than do restorations with visually well-adapted margins.

- While SEM is a suitable method to judge surface marginal adaptation, it does not necessarily display the real leakage status of a restoration.

- The described GEPT method seems to be a suitable non-destructive approach to study the leakage behaviour of restorations and may therefore display interesting insights into leakage status.

- Bonding quality remains a determinant factor in the ability of a restoration to prevent leakage.

6. Acknowledgements:

The authors declare that they have no conflicts of interest.

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8. Figures legends:

Figure 1:

Overview of the different testing phases: after mounting of the samples (A), GEPT measurements were taken (B) and preparations were drilled (C). GEPT was re-assessed (D) and restorations were placed (E). Leakage was determined by SEM (F) and GEPT (G). Thermodynamic loading was performed in a loading chamber (H, I), and the final evaluation was made with SEM surface marginal analysis (J), GEPT (K) or Fuchsin dye penetration testing (L).

Figure 2:

Illustration of the results of the three test methods (left: "non-leaking", right: "leaking"): GEPT evaluation with representative baseline pressure curves (A; blue = baseline, red = after preparation and green = after restoration); (B) SEM surface marginal analysis; (C) Fuchsin dye penetration test.

9. Tables:

Table 1:

Table 1- Results of the different test methods with regard to the respective treatment groups

Group	Before Thermodynamic Loading		After Thermodynamic Loading		
	GEPT hPa/min	SEM marginal defect analysis (%)	GEPT hPa/min	SEM marginal defect analysis (%)	Fuchsin (% of samples with dye reaching pulp chamber)
Group A1 (Composite restoration without bonding)	0.431 ± 0.449 ^{A}	18.9 ± 9.2 ^{a}	0.131± 0.076 ^{A*}	26.7 ± 11.0 ^{a}	80.0 ^{A}
Group A2 (Composite restoration with bonding)	0.074 ±0.020 ^{B}	15.2 ± 9.8 ^{ab}	0.065 ± 0.014 ^{B}	11.2 ± 6.5 ^{b}	10.0 ^{B}
Group B (Ceramic indirect restoration)	0.065 ± 0.010 ^{B}	3.6 ± 4.3 ^{b}	0.060 ± 0.008 ^{B}	5.7 ± 4.4 ^{c}	0.0 ^{C}
Group C (Negative Control)	0.062 ± 0.005 ^{B}	-	0.064 ± 0.005 ^{B}	-	0.0 ^{C}

Test results are presented as mean values and standard deviations when applicable.
Different superscript capitals represent statistically significant differences in GEPT measurement/Fuchsin dye penetration, between the different treatment groups ($p < 0.05$; read vertically). Different superscript lower case letters represent statistically significant differences in SEM assessment between the different treatment groups ($p < 0.05$; read vertically). Asterisks indicate statistically significant change in the measured after thermodynamic loading value compared to the before thermodynamic loading measured value of a respective treatment group ($p < 0.05$; read horizontally).

10. Figures:

Figure 1:

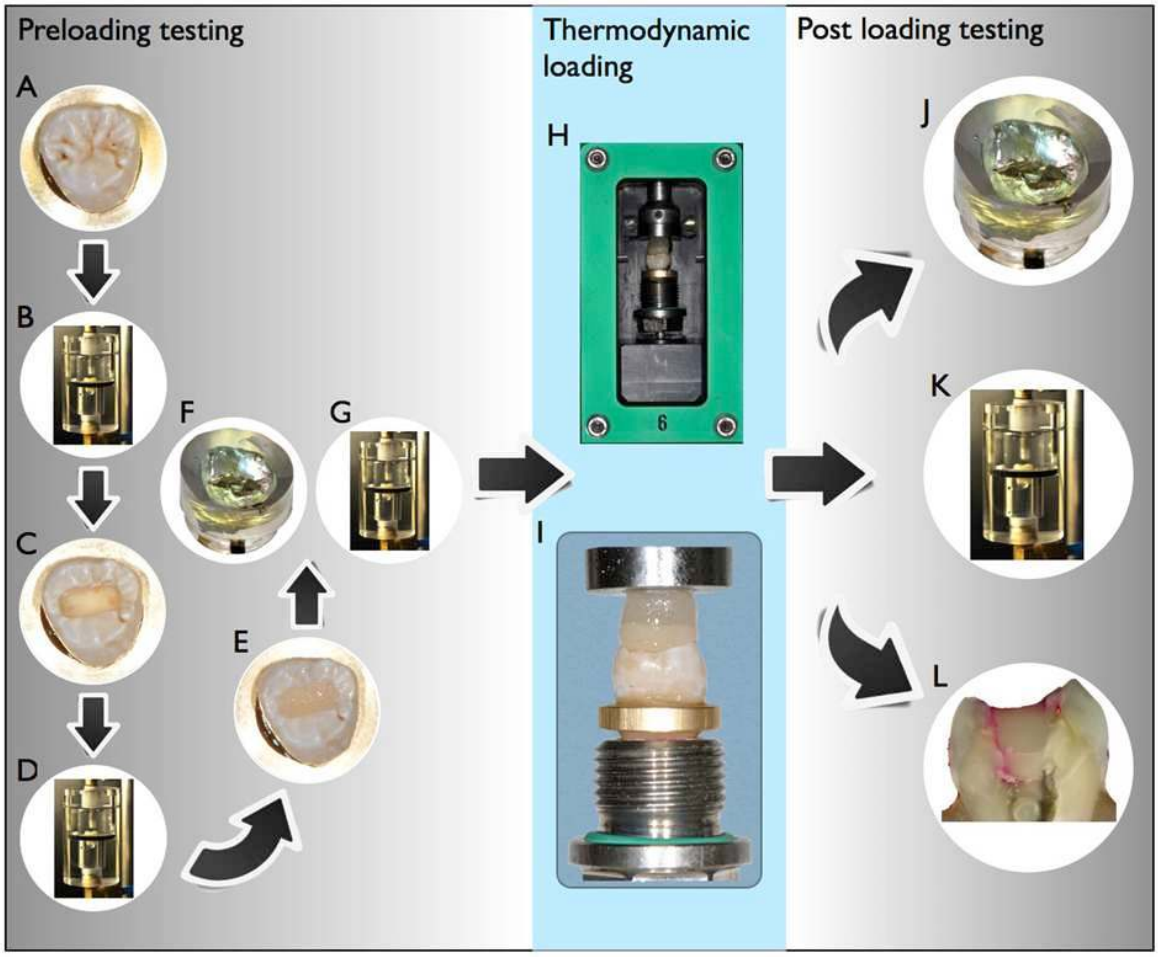
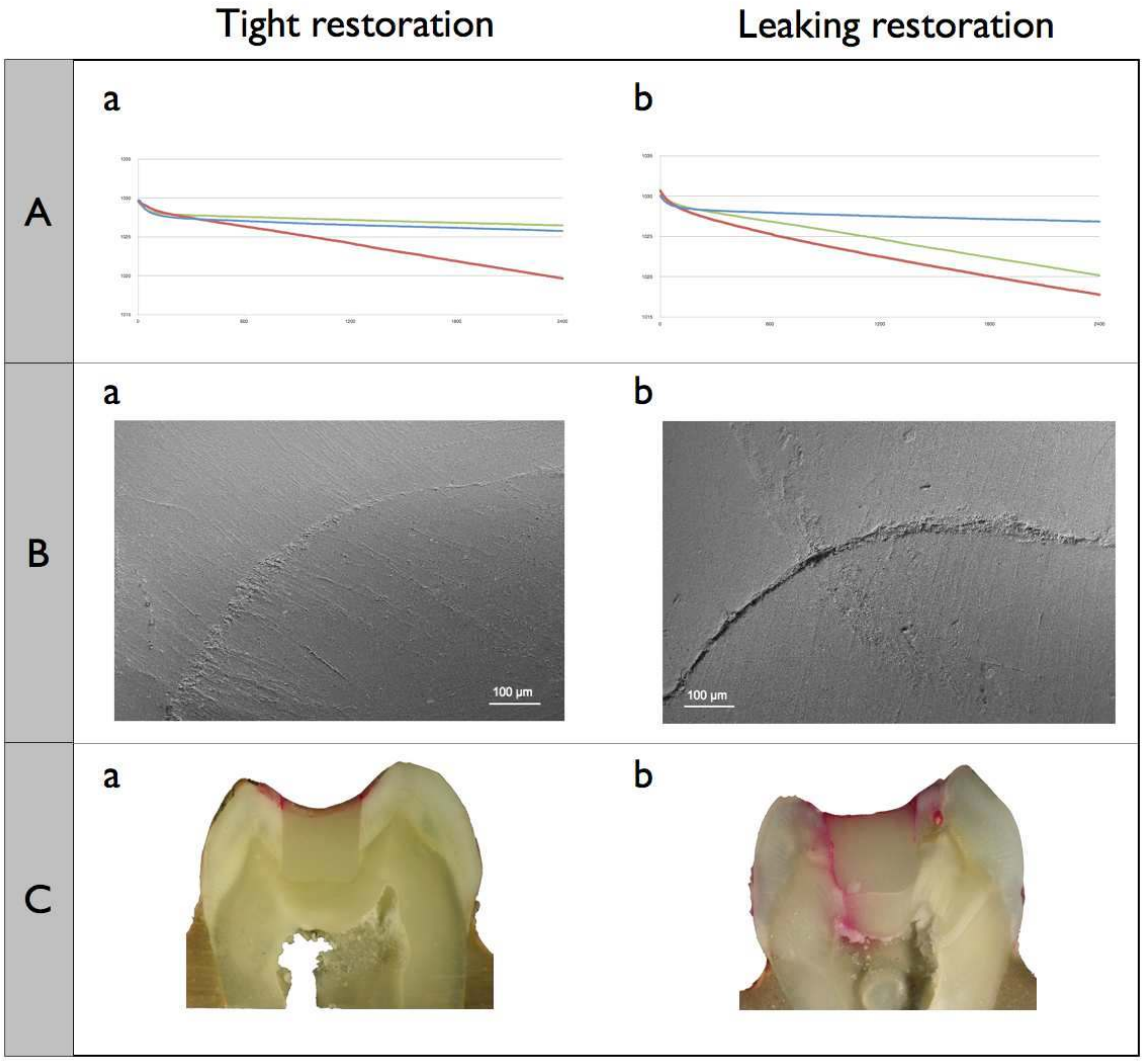


Figure 2:


Tight restoration
Leaking restoration
A
a
b
B
a
b
100 µm
100 µm
C
a
b