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# **Correlation between marginal adaptation *in vitro* and clinical outcome of Class V restorations**

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## **ABSTRACT**

Microscopic evaluation of the margins of restorations placed in extracted teeth is a common method to predict the clinical performance of dental adhesive systems. We examined the correlation between the quantitative margin analysis of two different laboratory test methods (Berlin, Zurich) and the clinical outcome in Class V restorations. For the correlation approach, prospective clinical studies with an observation period of at least 18 months were searched in the literature, for which laboratory data were also available. The clinical outcome variables were retention loss, marginal discoloration, detectable margins and secondary caries. Forty-four clinical studies matched the inclusion criteria, including 34 adhesive systems (AS) for which laboratory data were also present. For both laboratory test methods and the clinical studies, an index was formulated to better compare the *in vitro* and *in vivo* results. Linear mixed models which included a random study effect were calculated. As most clinical data were available for 12 and 24 months, the main analysis was restricted to these recall intervals.

The comparative analysis revealed a weak correlation between the clinical index and both *in vitro* indices. The correlation was more significant with the Berlin method than with the Zurich method and only present if studies were compared which used the same composite in the *in vitro* and *in vivo* study. When defining specific cut-off values, the prognosis for the good clinical performance of an AS based on *in vitro* results was 78 % (Berlin method) or 100% (Zurich method). For poor performance it was 67% or 60%, respectively. No correlation was found between both *in vitro* methods.

**Keywords:** adhesive system, marginal adaptation, *in vitro*, *in vivo*, Class V.

## INTRODUCTION

In restorative dentistry, the laboratory evaluation of adhesive systems is mainly based on bond strength tests as well as tracer penetration and the marginal analysis of restorations placed in extracted teeth (human or bovine). These methods are also part of the ISO Technical specification on the adhesion to tooth structure (ISO, 2003). The concept of bond strength testing is based on the assumption that low values correspond with early retention loss of cervical restorations and poor marginal adaptation. Likewise, poor marginal adaptation or the deep penetration of tracers in restorations placed in extracted teeth is indicative of the poor clinical performance of an adhesive system. There is a vast body of published studies that have applied one of these methods. According to a review published in 2007, almost 1,500 publications are cited in MEDLINE which apply some sort of bond strength test, while in 877 publications the evaluation is based on tracer penetration (microleakage), and about 90 publications use the microscopic evaluation of marginal gaps (Heintze, 2007). A systematic review of the correlation between bond strength tests and either tracer penetration or the microscopic evaluation of the marginal seal revealed that about 80% of the selected studies showed no correlation (Heintze, 2007). Furthermore, a poor or no correlation was found between tracer penetration and SEM analysis of restorative margins (Dietrich *et al.*, 2000; Heintze *et al.*, 2007a; Schuckar and Geurtsen, 1997).

Laboratory testing should not become an end in itself but allow a prognosis of the clinical performance of restorative materials and/or operative techniques. Therefore, a significant correlation between laboratory test methods and the clinical outcome is indispensable and should be part of each validation process. In a review which involved a limited number of cases, the researchers tried to correlate the *in vitro* data of Class V fillings published by one dental institute with the outcome of clinical trials with the same adhesive system (Heintze, 2007). In the study, only clinical trials were taken into consideration which evaluated at least 2 adhesive systems (in the same study), mostly using a split-mouth design. It was assumed that the variability of placement and evaluation of the restorations was lower within the same study than in studies of different research institutes. In 9 of the selected 11 studies, the laboratory data did not match the clinical data and could therefore not predict the clinical outcome (Heintze, 2007). Contrary to Class II restorations, Class V restorations are

especially useful for *in vitro/in vivo* comparison due to the easy access of the restoration margins for clinical inspection and evaluation. Furthermore, the operational technique for the placement of Class V restorations presents less variability compared to that of Class II restorations, taking into account the cavity size, application of the adhesive, layering technique, curing protocol, matrix technique, removal of excess, etc.

In the same study, the researchers also tried to correlate tracer penetration with the clinical outcome, but it was not possible to select an adequate number of adhesive systems tested at the same research institute with the same test method (Heintze, 2007). The broad variability of the tracer penetration methodology with regard to test parameters has been confirmed by a review study that evaluated the reliability of this specific test method (Raskin *et al.*, 2001). But even if the same methodology was applied in conjunction with the same material, multicenter studies yielded different results for Class V fillings (Raskin *et al.*, 2003) and fissure sealants (Raskin *et al.*, 2005).

Likewise, due to the microscopic evaluation of restorative margins, the methodology differs quite a lot between one institute and the other. Another influencing factor that contributes to the bias of the laboratory method is that the quantitative analysis outcome of the margin may differ up to 20%, which has been confirmed in a study where different operators evaluated the same margins (Henisch, 1989). A recently published new approach which uses an optical device that automatically measures the restorative margins eliminates the operator bias but can only be applied in cylindrical cavities (Heintze *et al.*, 2005; Heintze *et al.*, 2007b).

As up to now no comprehensive systematic research has been carried out to correlate the *in vitro* findings in regard to the marginal adaptation of restorations with the clinical outcome, the goal of the present study was to elucidate the *in vitro/in vivo* relationship by selecting clinical outcome variables (retention, marginal discoloration, marginal integrity) of prospective clinical trials on Class V restorations and relate them to laboratory data of the same materials in Class V restorations coming from two research institutes (Berlin, Zurich). This created a vast body of marginal adaptation data.

The following hypotheses were examined:

1. There is no correlation between *in vitro* and *in vivo* findings.

2. The correlation is independent of whether the same composite material has been used for the *in vivo* and *in vitro* study.
3. The correlation is independent of whether the cavity in the clinical trial has been prepared or not or whether the enamel has been bevelled or not.
4. There is no correlation between both laboratory test methods.

## **MATERIALS & METHODS**

### **Evaluation of Restorative Margins *In Vitro***

Two research institutes (University of Berlin, University of Zurich) have established standardized test methods to evaluate adhesive systems in extracted teeth in the laboratory. Since the late 80ties, adhesive systems are routinely tested at both institutes. Therefore, they possess a multitude of data on the different adhesive systems; most of the data have not been published in dental journals. Both test methods involve the quantitative analysis of the restorative margin of Class V cervical fillings using SEM. at high magnification. However, both test methods differ with regard to the type of tooth, cavity preparation, artificial ageing of restorations and evaluation criteria.

The test method developed at the University of Zurich is described in detail elsewhere (Krejci *et al.*, 1993; Peters *et al.*, 2000). This method was developed to evaluate *in vitro* the suitability of restorative materials to meet the Swiss quality guidelines for restorative dentistry. In these guidelines, a clinical service time of more than five years has to be assured (SSO 1999). In the following, a short description is given. In extracted premolars, six Class V cavities are prepared, per material group, placing them both at the lingual and buccal side. Since 1998 eight restorations are placed per group. One day before tooth preparation, the teeth are connected to a dentinal fluid simulation device, which contains horse serum diluted with Ringer solution. Thus, a hydrostatic pressure of about 25 mm Hg is established within the tooth. Two Class V wedge-shaped cavities are prepared at the cemento-enamel junction under a stereo microscope at x12 magnification, one on the lingual and one on the buccal surface. Half of the cavity is located in dentin, the other half in enamel. It features the following dimensions: mesial-distal 3-3.5 mm, apical-coronal 2.5-3 mm, depth 1.5 mm. A 1 mm-wide bevel is placed at the enamel margin and rounded with flexible aluminium oxide discs. The adhesives are applied

according to the manufacturer's instructions and light-cured. Finally, two layers of the respective composite material are applied and light-cured separately, the first layer covering the dentinal margin with a thickness of 1.3-1.5 mm and the second covering the enamel margin. Excess is immediately removed using flexible aluminium oxide discs of decreasing roughness. After having stored the specimens in distilled water for 7 days at 37°C, all the teeth are subjected to 3,000 thermal cycles (5°C-55°C-5°C, i.e. 6,000 temperature changes) and 1,200,000 load cycles at 49 N with a frequency of 1.7 Hz in a chewing simulator with integrated dentinal fluid simulation; the chewing simulator is described elsewhere (Krejci *et al.*, 1990). For the SEM analysis, impressions of the restorations for the replicas are made before and after thermomechanical loading. The replicas are analyzed with a SEM at x200 magnification by using a computerized analysis programme to determine the percentage of continuous margin of the entire dentinal and enamel margin (modified NIH Image, National Institute of health, USA). A continuous margin is defined as having no gaps, irregularities or fractures. The dentinal and enamel part of the restorative margin is reported on separately.

At the University of Berlin, extracted upper central incisors are used to prepare a Class V cavity on the labial surface. Eight fillings are placed per material group. On the apical side, a 90° angle is prepared with the margin in dentin, whereas on the coronal side the enamel is bevelled with a finishing diamond bur (Blunck and Roulet, 1999). The cavity dimensions are as follows: height approx. 4 mm, width 3 mm, and depth 1.5 mm. One half of the margin length is located in dentin. The adhesive system is applied according to the manufacturer's instructions and light-cured. The composite material is applied in two layers starting at the cervical margin and light-cured separately. After completion of the restoration the excess is removed with flexible discs, the restoration is polished and the teeth are stored in water at room temperature for 3 weeks prior to thermocycling (2,000x 5°C/55°C, i.e. ,4,000 temperature changes). Before and after thermocycling, the restorative margin is quantitatively assessed using SEM (x200 magnification), whereby 4 different criteria are allocated to each part of the margin:

Criterion 1: Margin not or hardly visible, no gap.

Criterion 2: No gap but severe irregularities such as porosities or marginal fractures.

Criterion 3: Visible gap up to 2  $\mu\text{m}$ .

Criterion 4: Severe gap exceeding 2  $\mu\text{m}$ .

For each criterion, the percentage related to the entire margin length is calculated with the help of a software programme. For the present study, only data after thermocycling were used (time 1= T1).

When the University of Berlin started investigating adhesive systems that involved etching of the enamel, only the dentinal margin was evaluated as the enamel margin was always close to perfect. However, an inferior quality of the enamel margins was observed in conjunction with self-etching adhesive systems and in most material groups, the enamel margin was evaluated separately (for these materials) using the same criteria.

The specimens fabricated with some AS were subjected to prolonged storage in water and additional thermocycling:

T2 = after 1 year water storage

T3 = after a second phase of thermocycling (x2000)

T4 = after 2 additional years of water storage (total of 3 years' water storage)

T5 = after a third phase of thermocycling (x2000).

### **Selection of Clinical Trials on Class V Restorations**

For the selection of clinical trials, prospective clinical studies on Class V restorations were searched in MEDLINE and the IADR abstract database. Additionally, a hand search of the German dental journal "Deutsche Zahnärztliche Zeitschrift", which is not available in MEDLINE, was carried out. The search words were "Class V" and "clinical", the search period was June 2007. The inclusion criteria were as follows:

1. Prospective clinical trial involving at least 1 adhesive system in cervical Class V cavities.
2. Minimal duration of 18 months.
3. The following criteria had to be investigated for: retention, marginal discoloration, marginal integrity, and secondary caries. These criteria were defined as "outcome variables".

Studies that involved different cavity classes (Class V and Class III) were excluded.

For each clinical trial, the following data had been included in the data file:

- type of composite material per adhesive system.



- number of restorations per material at baseline and each follow-up/recall
- number of subjects per material at baseline and each follow-up/recall
- preparation of dentin yes/no
- bevelling of enamel yes/no
- the outcome variables per material at each recall visit.

If a clinical trial investigated the effect of etching the enamel by comparing the results with those of etch&rinse adhesives, only the data of the etching group were selected.

### **Statistical Analysis**

The following clinical outcome variables were defined and correlated with the results of the *in vitro* measurements:

100 - % of retention loss (in what follows 100-R).

100 - % of marginal discoloration (in what follows 100-MD).

100 - % of detectable margins (in what follows 100-MI).

In order to summarize the *in vivo* performance, we combined these three clinical outcomes into one single clinical index (IND) using the following weighted average:

*In vivo* index IND =  $(4 \times (100-R) + 2 \times (100-MD) + 1 \times (100-MI)) / 7$ .

This index was calculated at 12, 18, 24 and 36 months, obtaining hence IND12, IND18, IND24 and IND36.

For the Berlin data, dentin, respectively dentin and enamel, were recorded at five or three different time points, respectively (T1-T5, see above). *In vitro* data at T1 on dentin were available for 32 AS, for which also data of *in vivo* studies were available; the number of *in vivo* experiments in relation to the number of AS was 76, as many studies evaluated more than one AS. As data for dentin at T2, T3, T4 and T5 were only available for 11 AS, we restricted our attention to dentin on T1 (after thermocycling). Original data were expressed as a distribution on a four-point ordinal scale (for each experiment, we had thus 4 percentages corresponding to the values of 1-4). To combine this information into a single index, we considered the average of the

Berlin index =  $(1 \times \% \text{ of } 1 + 2 \times \% \text{ of } 2 + 3 \times \% \text{ of } 3 + 4 \times \% \text{ of } 4) / 100$ .

Thus an index of 1 corresponds to the best possible distribution (100% of 1, 0% of 2, 0% of 3, 0% of 4), whereas an index of 4 corresponds to the worst possible distribution (0% of 1, 0% of 2, 0% of 3, 100% of 4). In the analysis

below, we also considered 100 - % of 1 as a second indicator of the *in vitro* performance in Berlin.

In conjunction with the Zurich method, dentin or dentin and enamel data (both before and after thermomechanical loading TML) were available for 28 AS. *In vivo data* were also available for these systems. The number of *in vivo* experiments in relation to the number of AS was 70. Two different indices were defined to characterize the *in vitro* performance:

Delta D = dentin before - dentin after thermomechanical loading TML

Delta DE = dentin and enamel before - dentin and enamel after thermomechanical loading TML.

Note that all the indices characterizing the *in vivo* and *in vitro* performance were defined in such a way that larger values corresponded to a worse performance of the AS. Therefore, positive correlations between these indices would indicate a positive association between them (meaning, for example, that *in vivo* and *in vitro* measurements would partly agree).

One goal of the present study was to explore at which recall and with which outcome variable of the *in vivo* measurements the *in vitro* measurements were the most correlated. For this purpose, we calculated a Spearman correlation coefficient *rho* between each *in vitro* index and each *in vivo* measurement/index, and plotted the data using scatter diagrams. The numbers in these diagrams refer to the number of the AS (see Table 1).

We could not test the statistical significance of these correlations since the experiments were not conducted independently (many studies involved more than one AS which were partly applied in the same subjects). To test the significance of the relationship between *in vivo* and *in vitro* indices, while accounting for this dependence, we used a linear mixed model which included a random study effect:

$in\ vivo\ index = a + b \times in\ vitro\ index + study\ effect + random\ error.$

Since each study involved a different number of patients (sample sizes varied from 11 to 81), and thus the results did not show the same degree of precision, we weighted the different experiments by their sample sizes (this step, however, not being a decisive one, our results were pretty identical with or without using

these weights). The p-value for the slope b above was calculated using SPSS (release 13.0) and based on a "pseudo degree of freedom" (indicator of the real sample size available).

Predictive values had been calculated based on cut-off values. For the clinic index IND24, the cut-off value was set at 10% after 2 years. For the Berlin index, a cut-off value of 1.2 and for the Zurich Delta index a cut-off value of 10% was defined.

A predictive value for poor performance of the AS was defined as follows:

$$pV = a/a+b,$$

where a=number of experiments with IND24>10% and Berlin index>1.2, respectively Zurich Index>10%,

b=number of experiments with IND24<10% and Berlin index<1.2, respectively Zurich Index<10%.

A predictive value for good performance of the AS was defined as follows:

$$pV = d/c+d,$$

whereby d=number of experiments with IND24<10% and Berlin index<1.2, respectively Zurich Index<10%,

c= number of experiments with IND24<10% and Berlin index>1.2, respectively Zurich Index>10%.

## RESULTS

Sixteen clinical studies were identified with an observation period of 18 months, 31 with an observation period of 24 months 18 with an observation period of 36 months and only two studies with an even longer observation period (60 months and 84 months, respectively). Of these studies, 28 also reported results after 12 months.

As most clinical data were available for 12 and 24 months, the main analysis was limited to these recall intervals. As regards the *in vivo* data, 158 out of the 170 percentages of secondary caries (SC) across the different experiments and the different time points were equal to zero. This is why we did not consider SC when characterizing the "*in vivo* performance" (it did not vary enough from experiment to experiment and was thus not useful to discriminate among the materials). On the other hand, the average percentages of retention (R), marginal discoloration (MD) and marginal integrity (MI) across studies and time points were 8%, 13% and 16%, respectively.

Clinical data were available for 31 AS that could be compared to the Berlin data, and for 28 AS that could be compared to the Zurich data. For the Berlin data, we could find 19 AS for which the composite material was the same as in the clinical study, whereas this was only the case for 13 AS investigated with the Zurich method. For three AS which had been tested clinically (No 14, 16 and 36), no *in vitro* data were available neither in Berlin nor in Zurich. Therefore, these AS do not appear in the scatter plots below (Figures 2-5) which thus contain a maximum of 34 AS. As far as the clinical studies are concerned, the majority of them (19 studies) only evaluated one AS, 18 studies evaluated two AS, 5 studies three AS and two studies as many as four AS. In 14 of the 44 studies, the dentin was prepared and in 19 studies the enamel was bevelled; however, in only 7 studies the dentin was prepared and the enamel bevelled .. Figure 1 shows the clinical index for the respective AS at the 2-year recall (IND 24). One can see a large variability, even among the studies using the same AS. For instance the results for the AS no. 30 (Single Bond) varied between 5% and 20% and those for the AS no. 31 (Scotchbond Multipurpose) between 0% and 27% . The results were not very different when only those studies were considered for which the same composite had been used.

The scatter diagrams in Figures 2-5 show the correlation between the *in vitro* indices (the Berlin index in Figures 2 and 3 and the Zurich Delta D index in Figures 4 and 5) and the *in vivo* measurements for the different recall intervals (12, 18, 24, 36 months), the different outcome variables (R, MD, MI) and the summarized clinical index IND. In all the figures, plots of each experiment are shown indicating the number of the adhesive used (see Table 1). Most of the correlations in Figures 2 and 4 (which involved all the experiments) were pretty low, with a few exceptions, such as the correlation  $\rho=0.61$  between MD after 18 months and the Zurich Delta D index, which could be a chance finding. Similar low correlations were obtained using the percentage of 1 in Berlin or the Zurich Delta DE index.

Interestingly, these correlations increased when restricting our attention to those experiments which used the same composite (*in vivo* and *in vitro*), especially in conjunction with the Berlin data where we had several correlations larger than 0.5 (Figure 3), and to some lesser extent in conjunction with the Zurich data (Figure 5). Note that only a few experiments with the same composite were

available for the Zurich index at the 12 and 18 months recall, so that the corresponding correlations were not meaningful.

As indicated in the statistical section, it was not possible to directly test the significance of these correlations, so that we used linear mixed models instead. As it is not a good statistical practice to perform too many tests, we restricted our attention to the *in vivo* indices after 12 and 24 months (IND12 and IND24), which were summary indices without too many missing values.

Table 3 contains the estimates of the regression slopes from these linear mixed models, together with standard errors, t-statistics, pseudo degrees of freedom and p-values, for different subsets of experiments. Note that one convergence problem occurred because of the small sample size available (reported as NA in Table 3). The slope indicates the average increase of the *in vivo* index when the *in vitro* index increases by one unit. For instance, a slope of 11.8 for the Berlin index means that the increase of 1 point on this *in vitro* scale leads to an average increase of 11.8 on the clinical scale.

No significant result could be found when considering all experiments. The only clearly significant results ( $p=0.005$  or smaller) could be found for the Berlin index (as well as for the percentage of 1 in Berlin) when considering only those experiments which used the same composite, with the significance being even higher after 24 months than after 12 months. For the Zurich index of dentinal margins, the corresponding result was almost significant after 24 months ( $p=0.061$ ), but not at all significant after 12 months. When taking the whole margin (dentin and enamel) into account, results were not at all significant, the slope being even negative after 12 months. When considering only clinical studies for which the dentin had been prepared or for which the enamel had been bevelled, no significant positive result could be found, neither in conjunction with the Berlin data nor in conjunction with the Zurich data. For the Zurich Delta DE index at 12 months, a slightly significant negative slope could be found when considering bevelled enamel only ( $p=0.035$ ).

Cut-off values for characterizing the good and poor performance of an AS can be defined, and it can be checked whether the *in vitro* performance correctly predicts *in vivo* performance. If the cut-off value for the clinical index after 2 years was set at 10%, the cut-off value for the Berlin index at 1.2 and the cut-off value for the Zurich Delta D index at 10%, 30%-40% of the AS were predicted to perform poorly (using either *in vitro* index) when indeed the clinical studies

showed the contrary. The result was similar when the same composite was used in the *in vitro* and *in vivo* experiments.

Figure 6 illustrates the correlation between the Berlin and Zurich *in vitro* indices. This correlation was rather weak ( $\rho=0.12$ ) and slightly increased ( $\rho=0.36$ ) when considering only experiments conducted with the Berlin and Zurich method using the same composite.

## **DISCUSSION**

This is the first study which systematically evaluated the relationship between laboratory data on marginal integrity and the clinical outcome in Class V restorations. As laboratory methods are claimed to have clinical significance without providing scientific proof, carrying out a systematic study of this type seemed overdue. In the laboratory, the development of marginal discoloration or secondary caries (caries adjacent to restorations) cannot be simulated. Therefore, the established laboratory methods use surrogate parameters, namely the percentage of continuous margin in relation to the entire margin, to predict the clinical performance of a certain adhesive system or a combination of adhesive system and overlying composite. A low percentage of continuous margin should be correlated with a high percentage of discoloured margin, secondary caries and also retention loss in Class V restorations. The hypothesis is that even small marginal defects or gaps of 1-3  $\mu\text{m}$  width allow the penetration of cariogenic bacteria and/or the retention of pigments that lead to marginal discoloration. An increased influx or percolation of liquids and saliva eventually leads to debonding and retention loss in Class V fillings. A recently published study tried to simulate the clinical findings of retention loss by means of restorations placed in cervical defects of extracted premolars without prior preparation using a one-step self-etching adhesive system, which showed high retention loss in clinical trials (> 20% after 2 years). The specimens were subjected to 1,200,000 cycles of thermomechanical centric loading followed by 1,200,000 cycles of eccentric loading on the lingual cusp (Heintze and Cavalleri, 2006). No single retention loss was observed throughout the whole *in vitro* procedure, which indicates that factors other than gaps and water influx contribute to debonding and loss of retention. Even water storage of the restorations made with 8 self-etching adhesive systems for up to 12 months, , multiple thermocycling intervals (up to 30,000 cycles) and mechanical loading

did not result in any retention loss as preliminary results showed (*unpublished data*). Another indication of the fact that laboratory tests do not simulate what happens intraorally is that no loss of restoration was observed with any of the tested adhesive systems neither in Berlin nor in Zurich, even after 1,200,000 cycles of thermomechanical loading (Zurich) or prolonged water storage (Berlin; (Blunck and Zaslansky, 2007). In contrast, the clinical data showed that 20-50% of the restorations were lost within a period of only 2-3 years when the same materials were used. In addition to thermomechanical loading, the Zurich method involves the simulation of dentinal fluid flow during the application of the adhesive system as well as during the chewing simulation. It has been proven that the simulation of dentinal fluid flow further decreases the amount of continuous margins in conjunction with some adhesive systems, but does not lead to any retention loss (Krejci *et al.*, 1993).

However, the present study has several shortcomings. (1) The cavity design and preparation technique used for the clinical trials differ from those of the laboratory study and also from clinical study to clinical study. (2) The parameters that are measured both in the clinical trial and with the laboratory method are different. (3) The artificial ageing of the specimens (thermocycling, mechanical loading) do not necessarily reflect the clinical situation. (4) The clinical trials show a variability for the same materials with regard to the outcome variables, thus making comparisons difficult to interpret.

Especially the inconsistent clinical results obtained with the same materials and material combinations are worth mentioning and further complicate the correlation between *in vitro* and *in vivo* data. This inconsistency can be attributed to various reasons and is subject to speculation. No systematic studies have been carried out to elucidate the possible reasons. Factors that may play a role are different operators (no calibration), different operative techniques (no standardization), different patients (no stratified randomization), samples size (low number of subjects), different evaluators (no calibration), different outcome variables or other factors. Therefore, a standardization of clinical trial design is required. Recently, a group of research workers came up with recommendations for the design of studies and criteria to evaluate restorations. (Hickel *et al.*, 2007).

As far as the selection of studies is concerned, also studies (9 of 44) were included for which only an IADR abstract was available. This was done to back

up the comparative analysis by the broadest possible range of data and to also include newer AS for which only IADR abstracts are available so far. IADR abstracts have also been included in other systematic studies like the one conducted by Peumans *et al.*, which evaluated the retention loss rate of contemporary AS (Peumans *et al.*, 2005b). Some studies selected for the present study were first published as IADR abstract before being published in peer-reviewed journals, e.g. (Kubo *et al.*, 2006; Perdigão *et al.*, 2005; Peumans *et al.*, 2005c).

The rationale for creating a clinical index is that a better statistical analysis can be carried out by correlating one *in vivo* and one *in vitro* index. The weighing of the three outcome variables attributing retention loss a four-fold weighing, marginal discoloration a two-fold weighing and marginal integrity a one-fold weighing was based on the following considerations: (1) The retention loss in a Class V cavity is the most obvious sign of failure of an adhesive system and is also the most reliable diagnostic evaluation criterion with little variability between different evaluators. (2) Marginal discoloration and especially marginal integrity are outcome variables which may show a greater variability between different evaluators.

For the *in vitro* results, an index was created too. For the Berlin index, all four marginal adaptation criteria were included in one index, which provided a better correlation with the clinical index than when only criterion 1 (percentage of continuous margin) was used. In regard to the Zurich index, better correlation with the clinical index was achieved when using the difference between continuous margin after thermomechanical loading and before than when only using the percentage of continuous margin after loading, which is normally done in publications applying this methodology. The results of the latter criterion are not shown in the present publication.

One may argue that the observation period of 2-3 years is too short to detect any differences between the materials, especially when one assumes that e.g. the Zurich *in vitro* data should simulate 5 years *in vivo* (Krejci and Lutz, 1990). However, most of the studies on Class V restorations have a duration of only 3 years. The reason for the short observation period may be that the American Dental Association's acceptance period for an adhesive system to be evaluated in Class V cavities is 18 months only. There were only two studies which followed up the restorations for 5 and 7 years, respectively (Kubo *et al.*, 2006;



Peumans *et al.*, 2005a). Recently, a study was published showing the performance of 7 adhesive systems over a period of 13 years (Van Dijken *et al.*, 2006); but only retention loss was reported. This study has shown that Class V restorations placed with adhesive systems that demonstrate a retention loss of around 10-20% within the first five years of service may exhibit a sharp increase in retention loss in the years thereafter (up to 50-60%).

The correlation between clinical and laboratory data on adhesive systems was weak and only present if studies were taken into account that evaluated the same composite material. It came as a surprise that the composite material played a crucial role in the comparative analysis, as the general view has always been that in Class V cervical fillings the adhesive system is more decisive than the overlying composite. Perhaps the influence of the composite resin on the longevity of Class V fillings has been underestimated in the past. However, no systematic analysis was performed that used the same adhesive system but various composite resins were used in the same patient. No correlation between *in vitro* and *in vivo* data was found between clinical studies in which bevelling of the enamel and preparation of the dentin was performed. One might assume a better correlation, as bevelling the enamel margin and preparation of the dentin was carried out in the laboratory study as well. As the number of cases was too small for the group "preparation + bevelling + same composite", no correlation coefficient was calculated for this combination. The results of the present study suggest, that the composite material plays a more important role for the correlation between laboratory and clinical indices than dentin preparation or bevelling of the enamel. Time could be another important factor. The artificial ageing procedure including thermocycling and mechanical loading may not reflect what is happening clinically as the degradation of the adhesive/tooth substance interface is a long-term process. There are no Class V restorations that are lost as early as during the first month after placement. In order to obtain meaningful bond strength data, 3-6 months' storage of the specimens in water prior to testing them is recommended (De Munck *et al.*, 2005; ISO, 2003). The data obtained for some adhesive systems after prolonged storage in water using the Berlin method indicated a rapid deterioration of the marginal adaptation in conjunction with some adhesive systems (Blunck and Zaslansky, 2007). But as the number of adhesive systems

for which prolonged water storage data were available was very limited, calculating correlations for these groups was not deemed reasonable.

When defining cut-off values for good or bad *in vivo* and *in vitro* performance as it was done in the present study, the predictive value for bad and good performance was 67 % and 78% for the Berlin index and 60% and 100% for the Zurich index; for the latter, however, only 3 cases were included. So, in 20-40% of the cases a false prediction may be given, mostly because a bad performance is ascribed to an adhesive system due to the laboratory test results, when indeed its clinical performance may be equal to that of an adhesive system with good *in vitro* results. However, this discrepancy may partly be due to the simulated clinical service *in vitro* of 5 years, which is much longer than the relatively short-term clinical data which we have available for comparison.

Due to the fact that laboratory studies can be conducted under standardized, optimal conditions, clinical observations may show a different picture and not reproduce data from laboratory studies. Restorations with microscopically sound margins cannot be produced under clinical conditions. In a study, in which Class II restorations were placed in premolars and extracted after 4 to 6 months due to orthodontic reasons, the SEM analysis showed that 43% of the cavities had been overfilled and 25% had been insufficiently filled (Opdam *et al.*, 1998). This result was achieved independent of the adhesive system, the layering technique and the matrix system used, or the experience of the operator. In a prospective study on Class II composite restorations conducted over a period of ten years, in which the marginal adaptation was examined clinically and on replicas by means of SEM every year, showed that already at the one-year recall more than 90% of the restorations had margins which were not perfect on more than two thirds of their length, as SEM analysis of the replicas revealed (Gaengler *et al.*, 2001; Gaengler *et al.*, 2004). Clinically, less than 5% of the restorations had detectable margins at a length of less than one third. At the three-year recall stained margins were observed in about 25% of the restorations, whereas more than 90% of them had imperfect margins on the replicas. The marginal imperfections identified, either those detected with SEM on the replicas or those observed clinically, did not influence the survival rate of the restorations or the development of secondary caries.

In a study on Class I molar restorations with five different adhesive systems, where the *in vitro* results were compared to the clinical results by the same researchers, the percentage of gap-free margins was similar *in vitro* and *in vivo*. In contrast to the present study, a high degree of standardization for placement and evaluation was given (Frankenberger *et al.*, 2007). However, the study did not answer the question about what are the clinical implications of open margins. In another article it was assumed that poor marginal quality might decrease clinical longevity due to the misdiagnosis of secondary caries, which leads to overtreatment as the restoration is replaced prematurely (Sarrett, 2007).

As there is only a weak correlation between the presence of gaps and the clinical outcome, one may assume that not the percentage of margins with gaps, but rather the width and depth of the marginal gap could be a more significant factor. However, no clinical studies exist, which have systematically examined the relationship between the width of the marginal gap and the occurrence of secondary caries and studied the factors of caries activity, location in the mouth and the type of restorative material used. Nevertheless, there are indications that the width of the marginal gap *per se* is not a prognostic indicator for the occurrence of secondary caries. In one study on amalgam restorations, parts of the occlusal margin of composite restorations with imperfections, discoloration and marginal gaps were completely removed (tooth structure + restoration) and histologically examined (Kidd and Beighton, 1996). Marginal gaps were considered to be closely linked to secondary caries only if the needle of a periodontal probe whose tip had a diameter of 400  $\mu\text{m}$  was able to penetrate into the gap. In areas such as the proximal gingival floor of Class II restorations, which are more difficult to reach with oral hygiene measures and self-cleaning mechanisms, the marginal gap width in association with secondary caries is most probably smaller. As systematic studies are lacking, this can only be assumed. The proximal margin of Class II restorations, however, is the site where 80% of the secondary carious lesions occur (Mjör, 1998).

In summary, the systematic analysis of the correlation between laboratory data of marginal adaptation and the outcome of clinical trials of Class V restorations revealed that the correlation was weak and only present if studies were compared which used the same composite for the *in vitro* and *in vivo*

evaluation. Therefore, hypothesis 1 and 2 had to be partly rejected and hypothesis 3 was accepted. However, one laboratory method (Berlin) showed a more consistent and more significant correlation with the *in vivo* findings than the other method (Zurich), where the specimens were stressed more heavily and for a longer period of time. No correlation was found between both *in vitro* methods. The variable outcome of different clinical studies of Class V restorations involving the same materials may be caused by the lack of a standardized study design and evaluation criteria as well as calibrated operators and evaluators.

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## Figures

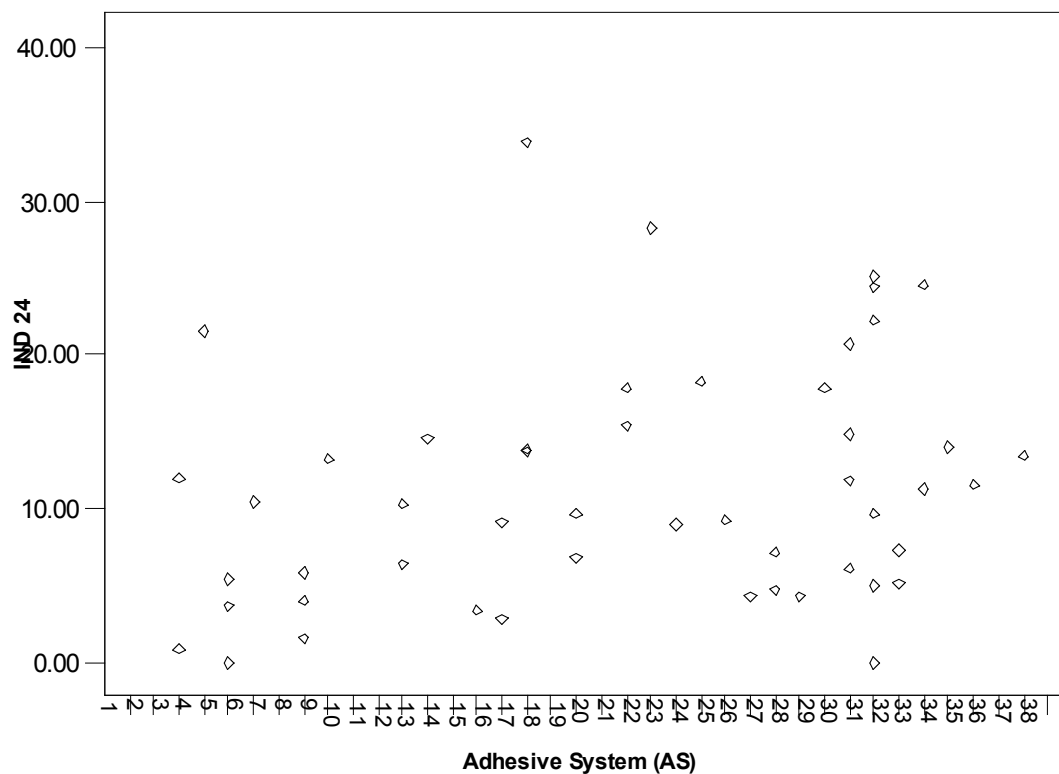


Figure 1: Clinical results in relation to the adhesive system AS (1-37) at the 2-year recall (Clinical Index IND24).

Note: For some of the AS no clinical result was available after 2 years but for other recall intervals.

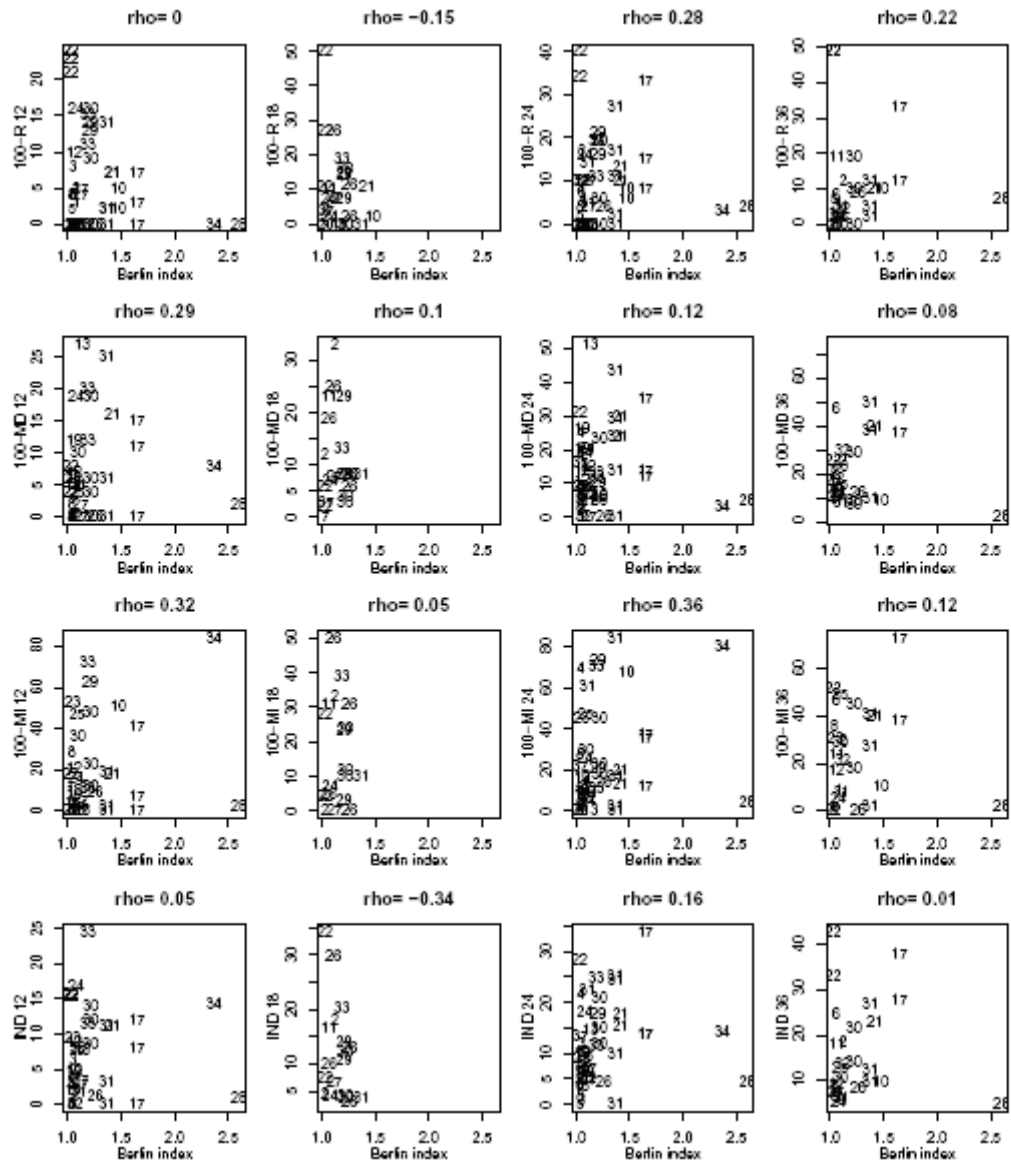


Figure 2: Correlations with Spearman correlation coefficient  $\rho$  between *in vivo* measurements and the Berlin *in vitro* index.

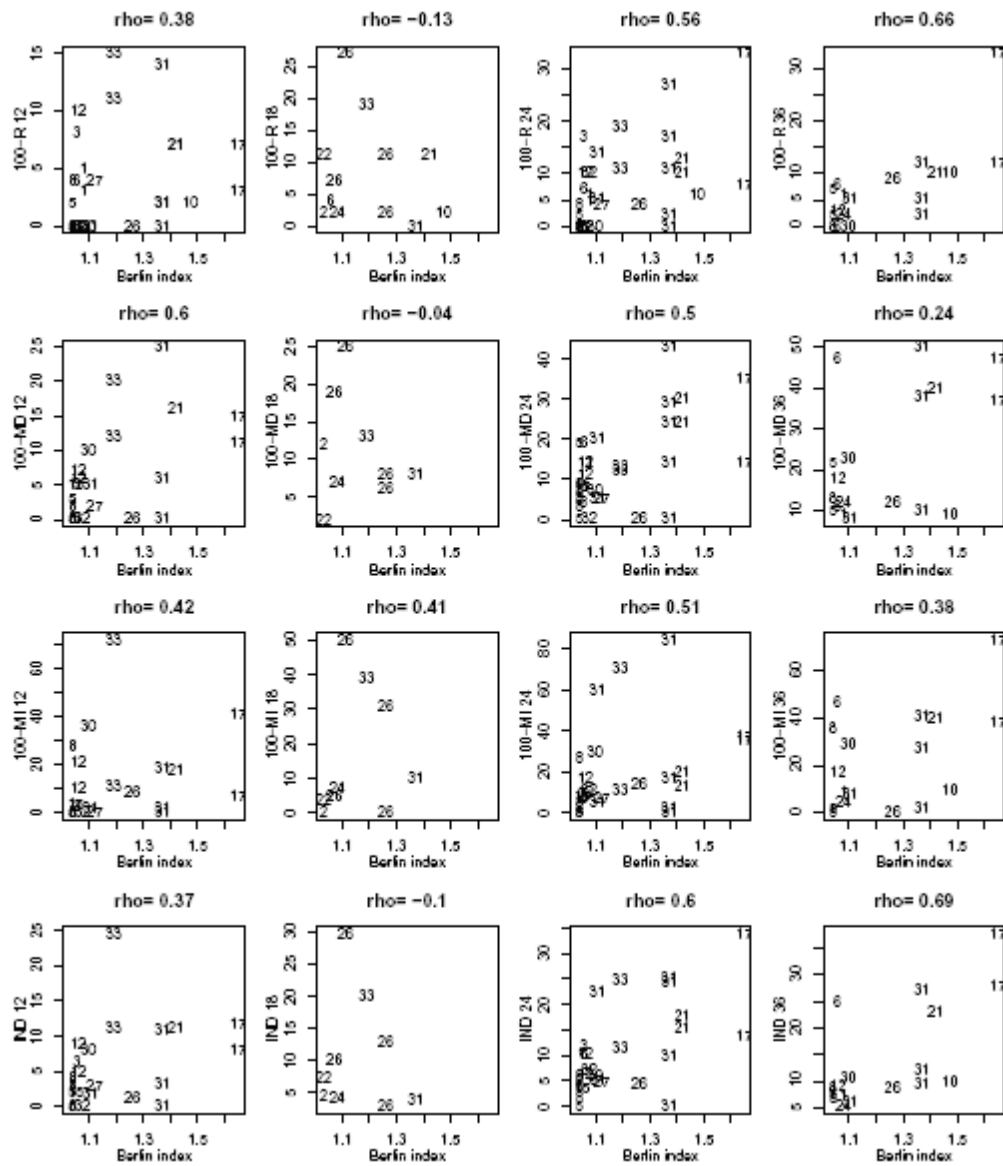


Figure 3: Correlations with Spearman correlation coefficient  $\rho$  between *in vivo* measurements and the Berlin *in vitro* index when using the same composite..

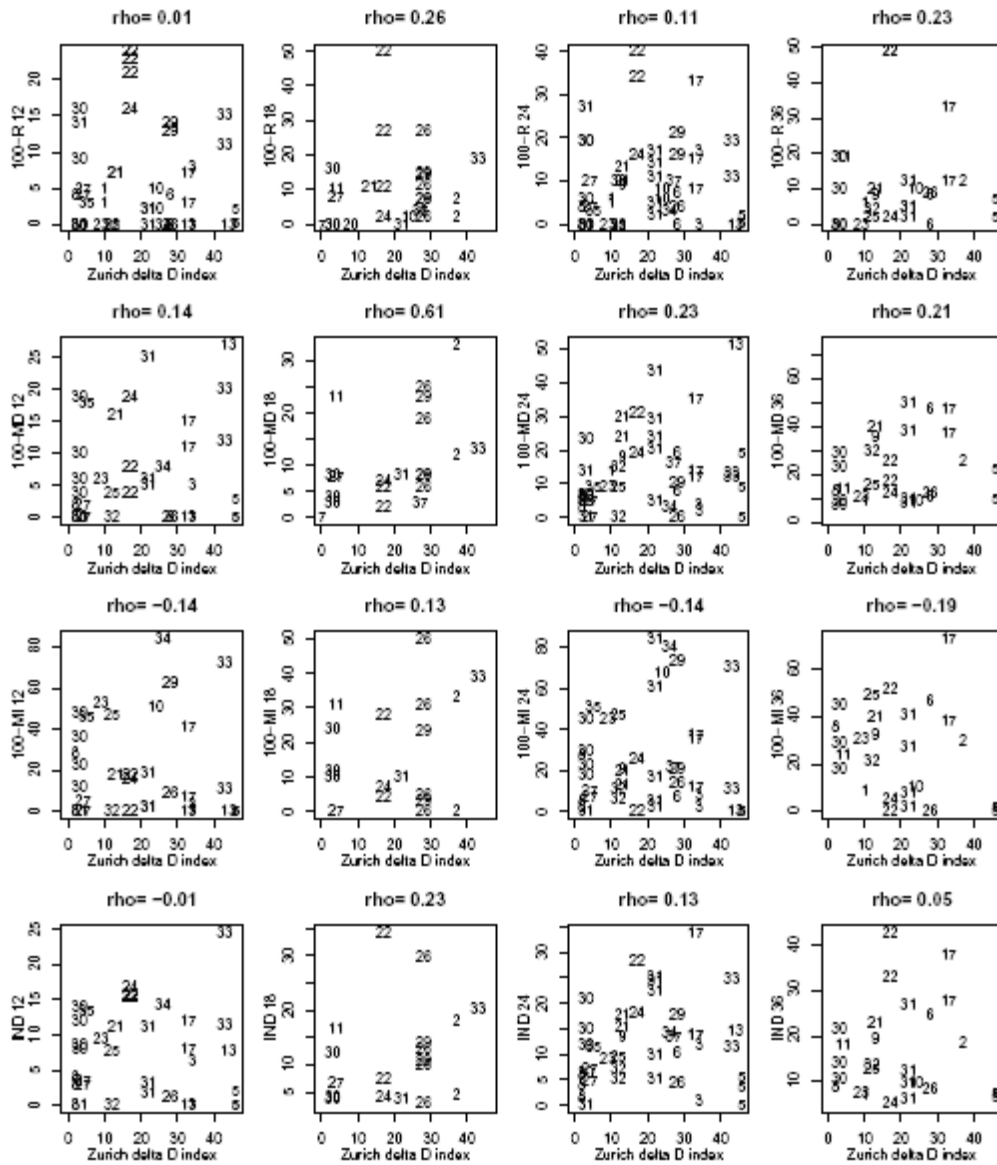


Figure 4: Correlations with Spearman correlation coefficient  $\rho$  between *in vivo* measurements and the Zurich *in vitro* index ( $\Delta D$ ).

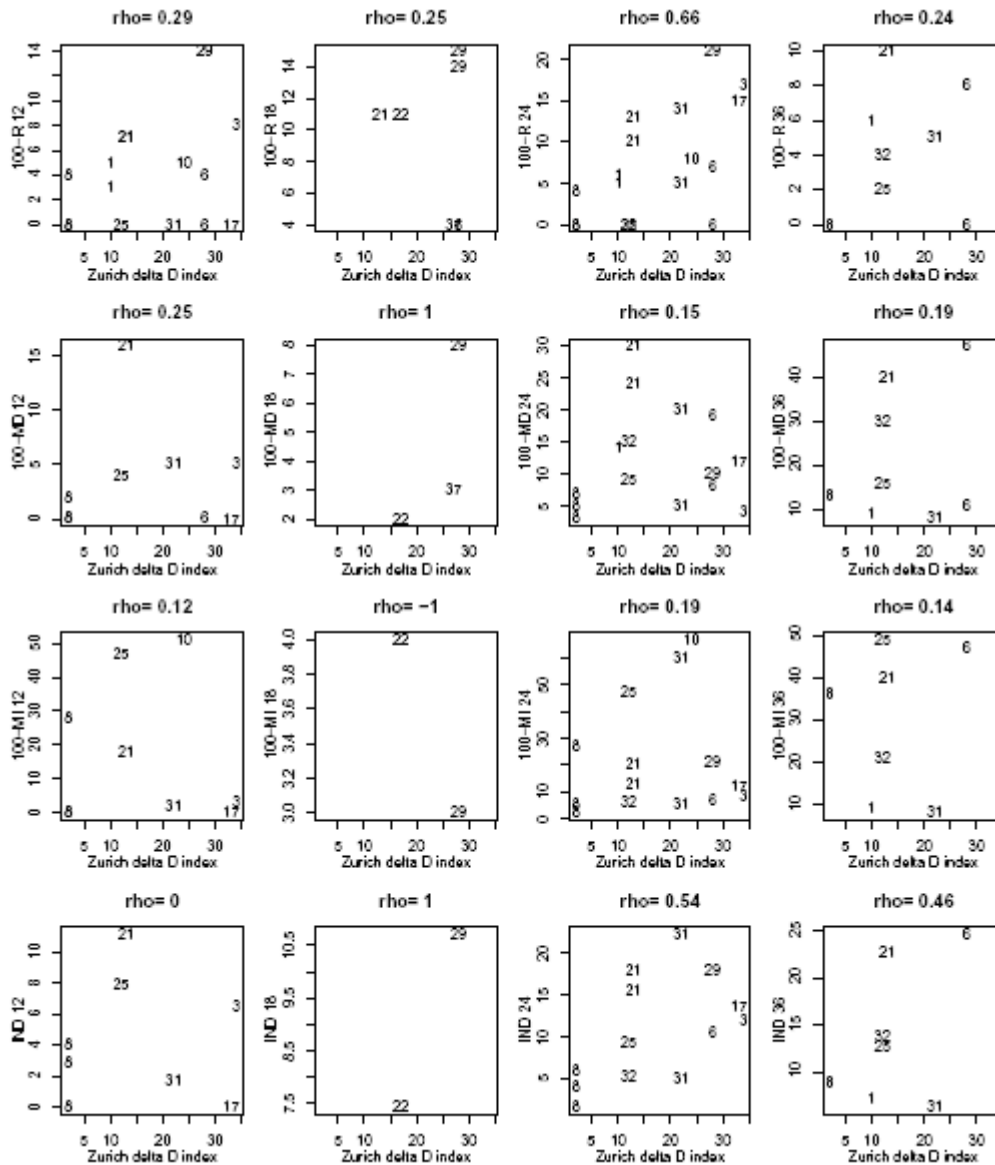


Figure 5: Correlations with Spearman correlation coefficient  $\rho$  between *in vivo* measurements and the Zurich *in vitro* index ( $\Delta D$ ) when using the same composite.

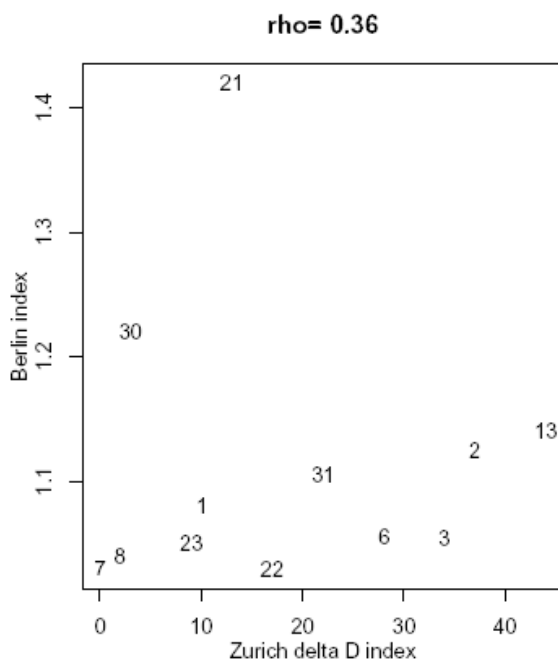
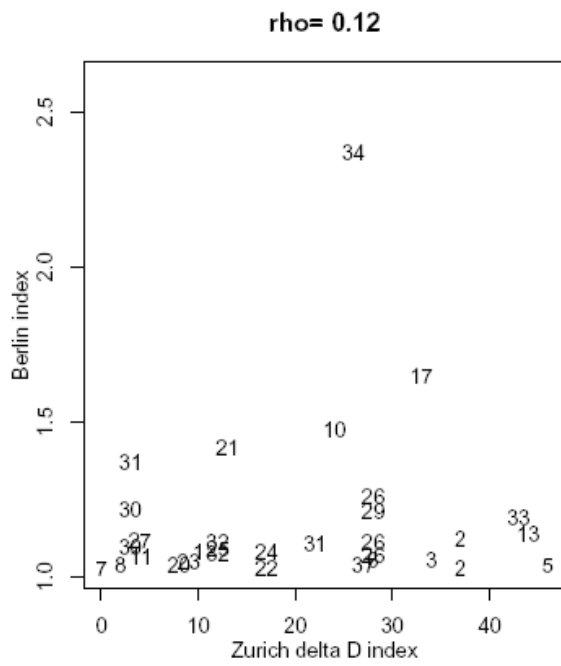


Figure 6: Correlations between Berlin and Zurich delta D index: Above: all cases, below only those for which the same composite was used.

1	A.R.T. Bond
2	AdheSE
3	Admira Bond
4	All Bond 2
5	Clearfil Liner Bond
6	Clearfil Liner Bond 2
7	Clearfil Protect Bond
8	Clearfil SE Bond
9	Dyract PSA
10	EBS
11	Excite
12	F2000 SEP
13	Fuji Bond LC
14	Fuji II LC
15	Futurabond NR
16	GC Conditioner
17	Gluma 2000
18	Gluma Solid Bond
19	Hybrid Bond
20	iBond
21	One Coat Bond
22	One Step
23	OptiBond FL
24	OptiBond Solo
25	Permaquick
26	Prime & Bond 2.1
27	Prime & Bond NT
28	Prisma U Bond 3
29	Prompt-L-Pop
30	Single Bond
31	Scotchbond Multipurpose
32	Syntac
33	Syntac Single Component
34	Tenure
35	Tripton
36	Tyrian
37	Xeno III

Table 1: Adhesives systems and their allocated number (AS) evaluated in 44 clinical trials.



In vitro experiment	slope	SE	<i>t</i> -stat	df	<i>p</i> -val
Berlin index (all cases)	2.8	2.6	1.07	35.8	0.29
Berlin index (same composite)	11.8	2.1	5.49	7.5	0.001
Berlin index (with preparation)	-3.2	5.2	-0.62	8.6	0.55
Berlin note 1 (all cases)	0.08	0.06	1.45	35.8	0.16
Berlin note 1 (same composite)	0.23	0.06	3.86	8.0	0.005
Berlin note 1 (with preparation)	-0.02	0.12	-0.20	7.7	0.84
Zurich dentin	-0.01	0.05	-0.17	19.5	0.87
Zurich dentin (same composite)	0.03	0.12	0.23	6.0	0.82
Zurich dentin (with preparation)	NA	NA	NA	NA	NA
Zurich dentin+enamel	-0.03	0.05	-0.68	18.5	0.50
Zurich dentin+enamel (same composite)	-0.03	0.19	-0.18	6.0	0.86
Zurich dentin+enamel (with bevelling)	-0.11	0.05	-2.35	13.5	0.035

In vitro experiment	slope	SE	<i>t</i> -stat	df	<i>p</i> -val
Berlin index (all cases)	4.5	3.7	1.21	38.0	0.24
Berlin index (same composite)	31.0	6.6	4.72	25.0	< 0.0001
Berlin index (with preparation)	9.4	12.6	0.75	6.8	0.48
Berlin note 1 (all cases)	0.10	0.08	1.23	40.5	0.23
Berlin note 1 (same composite)	0.53	0.13	4.12	25.0	< 0.0001
Berlin note 1 (with preparation)	0.27	0.29	0.93	6.5	0.39
Zurich dentin	0.04	0.09	0.49	39.0	0.63
Zurich dentin (same composite)	0.28	0.13	2.11	9.9	0.061
Zurich dentin (with preparation)	0.01	0.09	0.15	9.9	0.88
Zurich dentin+enamel	-0.04	0.09	-0.43	38.6	0.67
Zurich dentin+enamel (same composite)	0.05	0.18	0.30	7.6	0.77
Zurich dentin+enamel (with bevelling)	-0.08	0.12	-0.66	22.0	0.52

Table 3: Estimates of regression slopes and associated standard errors, *t*-statistics, pseudo degrees of freedom and *p*-values from linear mixed model with random study-effects to predict an in-vivo index for 12 months (IND12) (above) and 24 months (IND24) (below) from two *in vitro* indices where experiments are weighted by the sample size.

Method <i>in vitro</i>	Prognosis for good performance of AS	Prognosis for bad performance of AS
Berlin – all AS	60 %	72 %
Berlin – same composite	78 %	67 %
Zurich – all AS	67 %	64 %
Zurich– same composite	100 %	60 %

Table 4: Prognosis of the good and bad performance of an AS according to *in vitro* test results based on defined cut-off values (s. text).