Relevance of In Vitro Tests of Adhesive and Composite Dental Materials

A Review in 3 Parts

Part 3: In vitro Tests of Adhesive Systems

Keywords: adhesives, bonding, shear bond strength, microtensile bond strength, dye penetration, marginal quality

Summary In the third part of this review of laboratory testing, methods of testing adhesive systems are evaluated. Test set-ups that are used to analyze the restorative material in combination with the adhesive system are presented. Currently, there is no standardized protocol available for the evaluation of adhesives. This complicates any direct comparisons of values between different testing institutes. Therefore, the statistically evaluated ranking of the different adhesives is more important than mean values. Depending on the testing institute, a correlation between bond strength measurements and clinical outcomes may exist. Qualitative analysis of adhesive/tooth interaction can help explain the functioning of a system, but the depth of penetration of the adhesive cannot predict bond strength. Indirect bond measurements or analyses of the interactions of adhesive and composite materials, such as dye penetration or marginal analysis, do not correlate or correlate only partially with clinical findings. Adhesive systems should be tested in vitro and compared to a well-known standard adhesive before they are used in the clinic. Water storage of specimens for several months before testing increases the predictability of the bonding performance of the tested adhesive.

Introduction

Numerous new adhesive systems are put on the market every year. Most of them claim that their application requires even less time and only one step, or that the antibacterial additive increases the longevity of the restoration. Because so many one-step systems are already available, the manufacturers have become more creative in terms of packaging: for instance, disposable packages and application pens are intended to make applying the adhesive more hygienic or simpler. The sales figures show that dentists are indeed interested in simplified systems. While sales of classic multi-step systems have stagnated or even declined in recent years, the market share of simplified systems has been increasing. But which in vitro tests provide information on an adhesive's long-term ability to bond the filling to the tooth and whether or not it does so without marginal discoloration or hypersensitivity occurring? The same thing applies here as was mentioned in Part 1: The
advertising brochures list the adhesive tests performed by various institutes, and the material being promoted is always the one with the best bond strength. However, it is easy to be deceived, as the results depend heavily on which other products the given material is compared with, whether the bond strength indicated is the immediate bond strength value, or if the specimens have undergone artificial aging prior to testing.

The bond strength of the adhesive system can be measured with different test protocols. Various direct test techniques are available, such as shear strength, macro- and microtensile, and push-out tests. In addition, the adhesive bond can be qualitatively evaluated, for instance, by examining the adhesive’s penetration into the hard tooth substance or analyzing the hybrid layer using transmission electron microscopy. The interaction of the composite restoration and adhesive system with the dental hard tissue can be examined with dye penetration measurements and microscopic analyses of the margins. Not all test protocols are validated; critical analyses of in vitro test methods often find little correlation with the clinical data.

This article presents the different test methods with their strengths and weaknesses and discusses possible correlations to clinical outcomes.

Materials and Methods

An article search of dental literature up to May 2010 in the databank PubMed using the keywords “bond strength” and “dentin” or “enamel” yielded 2286 publications about adhesive bond testing on dentin and 1360 about the same on enamel. In addition, 907 publications are listed in which the dye penetration test was used (search words “microleakage” and “dentin”). Selected articles were compared in terms of their test protocol and critically discussed in the context of the existing literature. A comparison of laboratory data with the results of clinical tests of adhesives will show the relevance of the in vitro test methods.

Testing the Adhesive Bond

Adhesive bonding tests are very widespread, as is evident from the large number of publications. However, this also shows that these methods are relatively easy to use and provide results quickly. To date, there is no standardized protocol for adhesive bond testing, so that the size of the specimens, type of testing jig, and testing machine settings differ between testing institutes. The adhesive bond itself is not a material coefficient – unlike the flexural strength of composite. A critical literature review comparing bond strength values was recently published (Tab. I). It was shown that the bond strength tests lead to a high variability of the data and that the stress distribution across different specimens is not even. Furthermore, many cohesive fractures (fracture in composite or dentin) occur with all test methods, which does not reflect the true bond strength (Scherrer et al. 2010).

Direct analyses of bond strength

Shear strength measurement

In shear strength tests, composite cylinders are adhered to flat-ground dentin or enamel (human or bovine) surfaces and then sheared off with a special testing machine (Fig. 1a, b). The force (in Newtons) required to debond the composite cylinder from the substrate is measured. Finally, this force is set in relation to the area of the bonding surface to yield the bond strength.
Microtensile test, in which the dentin/composite stick is attached to the jig with superglue.

In Megapascals (MPa = 1 N/mm²). The stress distribution is seen as a disadvantage in shear strength testing. For instance, enormous forces are exerted on the site at which the shearing blade contacts the specimen (DEHoff et al. 1995). The absolute bond strengths also depend on whether the shearing blade is flat or if it bears a notch which surrounds half of the specimen (Ultra- dent method) (Pecora et al. 2002). Using the Ultradent method, 3 adhesive systems were analyzed at 12 dental industry test centers by several operators, who always employed the same test protocol. Almost all test centers were able to distinguish between the 3 adhesive systems. It was found that the operator has a considerable influence on the test result. This method – “notch-edge shear (Ultradent)” – will probably be established as an ISO testing standard.

Macro- and microtensile tests
In a tensile test, composite cylinders are adhered to flat dentin or enamel surfaces and pulled off. Since the mid-90s, a test method has been promoted in which the cylinder is adhered to dentin, then sectioned into individual composite/dentin sticks (diameter 0.8–1 mm) and pulled apart (Fig. 2). This is called the microtensile test (Sano et al. 1994, Pashley et al. 1999). The advantages of this method are that only a few extracted teeth are needed, regional dentin differences can be evaluated, and it is easier to distinguish between different materials. However, the method is labor-intensive and technique-sensitive. Due to the small bonded area, spontaneous failures can occur in many specimens immediately after construction, depending on the adhesive system and method of specimen manufacture. Nevertheless, these “pre-test failures” must be correctly included in the statistical analysis, something which is often neglected (Scherrer et al. 2010). Furthermore, the values measured for sticks originating from the same tooth are not statistically independent of one another. If this dependency is not taken into account, it is entirely possible that erroneous product ranking will result (Eckert & Platt 2007).

The bond strength values obtained with the microtensile test are higher than those yielded by the “normal” tensile test, since smaller bonding areas generally exhibit higher bond strengths (Goracci et al. 2004). The mounting and fixation of the specimen in the testing machine also influence the bond strength values (Soares et al. 2008, Poitevin et al. 2008, Phrukkanon et al. 1998, Armstrong et al. 2003).

Usually, the adhesive bond is already measured 24 hours after luting the cylinder to the dental tissue. The specimens are only infrequently stored in water for longer time periods (3, 6, 12 months) in order to evaluate the influence of moisture on the adhesive bond. Depending on the adhesive system, a marked reduction in bond strength may be observed after water storage (Carrilho et al. 2005). Thus, 24-h values are not good predictors of a material’s long-term success.

It is possible to predict the clinical suitability of adhesive systems to only a limited extent based on the results of bond strength tests. Only systems with very low in vitro bond strength values clinically show increased retention loss in cervical restorations or marginal staining in posterior fillings (Van Meerbeek et al. 2010). Conversely, high in vitro values do not necessarily indicate good clinical performance. A comparative study showed a moderate correlation between the microtensile test results after 6-month water storage of specimens and the oc-

<table>
<thead>
<tr>
<th>OptiBond FL</th>
<th>Cleargel SE Bond</th>
<th>Adper Prompt L-Pop</th>
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<tr>
<td>MPa</td>
<td>Number of studies</td>
<td>MPa</td>
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<td>Macroshear test</td>
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<td>8</td>
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<tr>
<td>Microshear test</td>
<td>22.7*</td>
<td>1</td>
</tr>
<tr>
<td>Macrotensile test</td>
<td>18.7 (5.5)</td>
<td>3</td>
</tr>
<tr>
<td>Microtensile test</td>
<td>48.0 (13.7)</td>
<td>18</td>
</tr>
</tbody>
</table>

* The mean value is based on only 1 study; thus, the standard deviation is lacking.
correlation of marginal staining in cervical restorations; in contrast, there was no correlation with restoration loss (Heintze et al. 2010). The macro- and microtensile tests seem better correlated with retention loss of cervical restorations than do shear bond tests. Moreover, pooling of the data of different test institutes is better correlated with retention loss as it allows to better characterize the handling sensitivity of the adhesive system (Heintze & Rousson 2011).

Push-out test
This test is very commonly applied in the analysis of post adhesion in root canals (Fig. 3). Less often, the bond strengths of cements for ceramic systems are measured with this method. Only a few articles are available on the push-out testing of adhesive systems and composite materials, not least of all because the manufacture of the specimens is considerably more labor intensive than in the other test methods. This method was first described in 1970 (Roydhouse 1970). Standardized, conical preparations are made in teeth and filled with restorative material. Beginning at the pulpal axial wall, dental hard substance is removed up to the level of the restoration, which is then pushed out with the testing machine’s plunger. The advantages of this method are said to be the simultaneous testing of marginal seal and adhesive bond on the same specimen (Frankenberger et al. 1999). Specimen fabrication can be simplified by cutting dentin into disks and making conical preparations in them. The disk is then placed on a glass plate and the composite is inserted into the cavity. The chief advantage of this is that the time-consuming, technique-sensitive removal of the pulpal axial wall is omitted. A more clinically remote variation of this method is one in which the restorative material is placed in roughened metal molds.

Comparing the push-out with the microtensile test, it is evident that the bond strength values are significantly different. One advantage of the push-out test is that no pre-test failures occur at the bonded surface of the specimen; in addition, the coefficient of variation (see Part 1 of this review) of the test results was described as acceptable (Čeki-Nagas et al. 2008).

Discussion of bond strength tests
Because no internationally recognized standardized test protocol yet exists for the testing of adhesive systems, completely different bond strength values can be found and published for the same product, depending on the testing institute. If, however, a given testing institute has established a certain standardization in the lab and regularly checks the reproducibility of the results, then at least the ranking of products should be approximately the same from one testing laboratory to the next. The comparability of results would already be improved if the examiners kept to the technical specifications of the ISO, which roughly specify the essential parameters of adhesive bond tests (ISO 2005). Unfortunately, few research workers follow these recommendations (Leloup et al. 2001).

Adhesive bond tests can also be deceptive. For instance, most in vitro studies showed that the bond strength values of self-etching adhesive systems on prepared enamel are markedly lower than those of adhesive systems with a separate phosphoric-acid etching step (De Munck et al. 2005). Clinical studies, however, showed that most of the restorations done with self-etching systems were still clinically acceptable even after 8 and 10 years (Akimoto et al. 2007, Gordan et al. 2007). Nevertheless, these results cannot be generalized. The self-etching systems which use a hydrophobic adhesive in addition to the primer (2-step systems) perform decidedly better on both enamel and dentin than do the 1-step systems. This is true for both cervical (Peumans et al. 2005) and posterior restorations (Perdigão et al. 2009). Furthermore, it must be taken into account that in some self-adhesive bonding systems, the functional acidic monomers hydrolyze during storage, so that the bond strength decreases markedly during storage (Salz et al. 2005).

A laboratory trial attempted to simulate restoration loss in non-retentive cervical cavities (Heintze & Cavalleri 2006, 2010) by placing cervical fillings in extracted premolars using different adhesive systems and glass-ionomer cement, storing the teeth for $3 \times 6$ months in water, and subjecting them to various thermocycling and simulated mastication procedures. With none of the adhesive systems – not even in conjunction with glass-ionomer cement – did any filling fail out during the simulation trial (Heintze & Cavalleri 2010). Only when the conditioner (polyacrylic acid) was omitted for the fillings with glass-ionomer cement or the phosphoric acid etching of dentin and enamel for the fillings done with an etch-and-rinse system did restoration loss occur. Thus, it is apparently difficult to correctly simulate the clinical situation in the in vitro test.

Indirect analyses of bond strength
Evaluation of penetration into dental hard tissue
This examination method is predominantly performed with the confocal laser scanning microscope (CLSM). The adhesive...
system is marked with a fluorescent dye which is excited by the laser, and the depth to which the adhesive system penetrates into the dental hard tissue is observed under the microscope. However, because the dye does not chemically react with the adhesive system, the dye molecules can penetrate further into the dentin tubules than the adhesive system itself, thus distorting the results (MEYER-LÜCKEL & PARIS 2008, VAN MEERBEEK ET AL. 2000, WATSON 1997).

The penetration of adhesive systems into dental hard substance can also be evaluated with scanning electron microscopy (SEM). The restored tooth is longitudinally sectioned and partially or completely dissolved in hydrochloric acid. The composite resin remains and can be prepared for SEM analysis. The length of the resin tags can be measured and the thickness of the hybrid layer assessed. Adhesive systems which condition the dentin and/or enamel with phosphoric acid demonstrate greater microretention than self-etching systems (Fig. 4).

The penetration evaluation assumes that the depth of penetration is a measure of the adhesive bond. However, the penetration of the system’s resin tags into the dentin tubules constitutes only part of the adhesive bond (LOHBAUER ET AL. 2008). In contrast, penetration into the intertubular dentin and the collagen network is of greater importance for the adhesive bond. Nevertheless, a clinical study showed that complete removal of the collagen layer using 10% sodium hypochlorite did not negatively influence the retention (SABOIA ET AL. 2006). But in vitro studies on this yielded contradictory results.

Qualitative analysis of the hybrid layer
The penetration zone of the adhesive system into the dental hard substance (hybrid layer) is the critical area of the adhesive bond. Although the thickness of the hybrid layer does not influence the adhesive bond (GWINNETT ET AL. 1996, VARGAS ET AL. 1997), its analysis enables an assessment of the quality of the bond. Using silver nitrate staining, defects in the bond can be made visible. However, this test cannot determine how strong the bond actually is.

Both mechanical adhesive bond tests and microscopic evaluation of the bond overlook the clinical problems with the adhesive filling technique to a certain extent. The weakest point in terms of secondary caries formation is the cervical-gingival floor of Class II restorations. Caries occurs 5 times more frequently at this site than at the occlusal margin. Inadequate polymerization of the composite at this location has already been discussed in Part 1. There is also a structural biological problem: if the filling’s margin lies just above the cemento-enamel junction, the operator has to deal with enamel which becomes ever thinner towards the edge and breaks easily; furthermore, there are hardly any enamel prisms to etch at this site. In contrast, a filling’s margin below the cemento-enamel junction lies in an area of dentin which contains no dentinal tubules for a distance of ca 100 μm, and further towards the pulp their number is still quite low; thus, the preconditions necessary for a good bond are not met (FERRARI ET AL. 2001). Yet it is precisely in this region that a dentin adhesive must mediate a particularly strong bond if the formation of secondary caries is to be prevented.

Testing the Interaction of Adhesive System and Composite Material
Dye penetration
According to a dogma of restorative dentistry, the transition from the restorative material to the dental hard tissue must be smooth and continuous in order to increase the restoration’s survival probability. Poor marginal adaptation is said to lead to hypersensitivity, marginal staining, and ultimately to secondary caries as well (BERGENHOLTZ ET AL. 1982, BRÄNNSTRÖM 1992, BROWNING & DENNISON 1996).

With composite materials, the marginal adaptation is affected both by material-inherent properties such as shrinkage and shrinkage force (PEUTZFELDT & ASMUSSEN 2004) and operative techniques, for instance, adequate moisture isolation, cavity size, type and quality of bevel of the enamel prisms and dentin tubules depending on the location, type and quality of conditioning of the dental hard substance, layering technique, and polymerization protocol (ANUSAVICE 1989).

The method thought to most closely simulate clinical reality is the restoration of cavities in extracted teeth. The restorations are subjected to aging processes, such as mechanical loading in a chewing simulator (see Part 2 of this review article). After immersion in a dye or tracer solution, the teeth are sectioned and the dye’s penetration depth is evaluated with a microscope (microleakage) (Fig. 5). Hypothetically, a low dye penetration depth correlates with a high clinical survival rate of the restoration, and particularly with a low incidence of marginal discoloration and secondary caries.

Microleakage studies using the dye penetration method have shown that, independent of restoration material, all fillings exhibit microleakage to some extent. Usually, fillings with margins in dentin more frequently show dye penetration than those with margins in enamel (HEINTZE ET AL. 2008). However, a systematic study of this test also found that the results from different testing institutes could not be compared (RASKIN ET AL. 2001). The results are hardly reproducible (RASKIN ET AL. 2003). A correlation between dye penetration and the occurrence of marginal gaps is either not or only partially proven, and a dependency on the dye used and the location has been found (HEINTZE ET AL. 2008). Since this method of dye penetra-
tion has no clinical correlate with a valid threshold value (ROULET 1994). It does not make sense to use this elaborate, labor-intensive method in the laboratory (HEINTZE 2007).

**Automatic marginal gap detection**

Another, much faster method of evaluating marginal adaptation is the filling of cylindrical cavities (Ø 4 mm) in dentin. Automatic marginal gap detection using an optical sensor was developed to this end (HEINTZE ET AL. 2005b). After polishing the restorations, the margin of epoxy resin replicates of the fillings is evaluated with the optical sensor. The light beam moves radially from the center of the filling across the restoration margin and creates profiles. If a gap is present, it is recognized and its width and depth are automatically measured. Classifying the adhesive systems according to the number of application steps and according to whether they are self-etching or include a phosphoric-acid etching step, the classical multi-step adhesive systems exhibit the greatest proportion of gap-free margin and the self-etching one-step adhesives the least (HEINTZE ET AL. 2007). These results agree with the microtensile bond strength results of Leuven University, Belgium (DE MUNCK ET AL. 2005) and with the clinical results on retention loss in cervical restorations (PEUMANS ET AL. 2005).

**Microscopic marginal adaptation analysis and its clinical relevance**

In a different approach, restorations are placed in the cavities of extracted teeth, and both before and after various aging processes, the marginal adaptation is evaluated according to certain criteria using a light or scanning electron microscope (ROULET ET AL. 1989) (Fig. 6). Then the percentage of imperfect margin or marginal gap of the entire margin is calculated. The hypothesis states that a high proportion of perfect margin correlates with a high clinical survival rate of the restorations. This method is very dependent on the examiner who evaluates the margins. Despite calibration, the differences between two examiners can be up to 15% to 20% (HENISCH 1989). A further disadvantage of both dye penetration and marginal quality evaluation of fillings in extracted teeth is the high variability of the values. The coefficient of variation, that is, the quotient of the standard deviation and the mean value, ranges from 20% to 50%. If one wanted to statistically differentiate between two materials based on a 10% difference in the marginal quality or dye penetration, then 20 to 60 specimens would be necessary for each material group (HEINTZE ET AL. 2005a; HEINTZE ET AL. 2008). The effort involved would not be justifiable.

In the last 20 years, the Departments of Restorative Dentistry at the universities in Berlin (Charité), Germany, and Zurich, Switzerland, have examined adhesive systems and composites in terms of their marginal behavior in the cervical cavity (BESER ET AL. 2004, BLUNCK & ROULET 1999, BLUNCK & ZASLANSKY 2007). The cavity geometry of the two methods (Berlin and Zürich) is somewhat different (Fig. 6). Whereas in Berlin, the restorations are always placed and evaluated by the same operator, different operators and evaluators are involved in Zurich. In Berlin, the restorations are only subjected to thermocycling (2000 times, 5 °C and 55 °C), but in Zurich, they are also exposed to masticatory loads in a chewing simulator (1.2 million cycles). Systematically comparing the results of clinical studies on cervical filings with those of the two in vitro test methods for marginal adaptation, it becomes apparent that the correlation between the in vitro tests and the clinical results is weak, and exists only if not just the same adhesive system but also the same composite was used in the clinical and in vitro examinations (HEINTZE ET AL. 2009) (Fig. 7). In a different type of in vitro/in vivo analysis, in which only clinical studies were considered that examined at least 2 adhesive systems in one mouth (split-mouth design), the in vitro results agreed with the clinical results in only 20% of the studies of marginal integrity (HEINTZE 2007).

The marginal adaptation evaluation methods often yield false negative findings, which means that an adhesive system may be rated as poor, despite the fact that its performance in clinical testing was just as good as adhesive systems that did well in in vitro tests.

One may raise the objection that the clinical studies with which the in vitro results were compared usually only had an observation period of 2 or 3 years, and thus cannot be compared with testing methods which – in accordance with the Zürich method – simulate 5 years in vivo (KREICI & LUTZ 1990). Studies with longer observation periods are rare. One of these clinical studies published the results of 7 adhesive systems over a period of 13 years (VAN DUKEN ET AL. 2007). Although the loss of Class V (cervical) restorations increased markedly for some during this time, those adhesives which exhibited 80% loss after 10 years had already shown a high loss rate 1 to 2 years later.
after restoration placement. Only 2 of the 4 adhesives which had a low loss rate in the first 2 years demonstrated a relatively high loss rate at the 6-year recall.

An important disadvantage of comparing in vitro and clinical results can be found in the quality of the clinical studies, which do not meet the standards. In particular, the selection of subjects, number of restored teeth per subject, and number of fillings which can no longer be evaluated (drop outs) must be mentioned in this context. Moreover, the test criteria (USPHS, according to Ryge) (CWAR & RYGE 1971, RYGE & SNYDER 1973) which are most commonly used in clinical evaluations are too coarse to determine differences between today’s materials. A few years ago, a research group developed new, more precise criteria, which have also been accepted by the FDI (HICKEL et al. 2007).

In vivo and in situ studies have shown that microleakage per se or the presence of marginal gaps correlates neither with the occurrence of hypersensitivity nor with the formation of secondary caries (MJÖR & TOFFENETTI 2000, MJÖR 2005, OPDE et al. 1998a, b). Irregularities in the marginal seal correlate only to a limited extent with the formation of secondary caries. A clinical study in which the imperfect marginal areas of occlusal portions of composite fillings were drilled out in one piece and examined histologically showed that only frankly carious lesions at the clinical margins were associated with histological evidence of secondary caries (KIDD & BEIGHTON 1996). This was substantiated by the results of a 10-year prospective clinical study of Class II composite fillings, in which the marginal adaptation was clinically and microscopically (using impressions of a replica) evaluated annually (GÄNGLER et al. 2001, 2004). Although microscopic examination showed a discontinuous margin involving $\frac{1}{2}$ of the entire margin after 1 year in 90% of the restorations, marginal discoloration and secondary caries only occurred in 30% and 10% of the fillings, resp., and that after 3 to 5 years (Fig. 8). All of this speaks against the gap per se but instead for the width of the gap as being the problem. Furthermore, subjects with high caries activity exhibit more secondary caries than those with low caries activity (KÖHLER et al. 2000) (Fig. 9). In addition, sealing marginal defects with unfilled monomers can considerably increase the survival rate of posterior restorations, as shown in a recent clinical study (GORDAN et al. 2009).

**Conclusions**

In vitro tests are important for providing initial predictions for the success of a material. They by no means replace clinical tests in patients, because the clinical relevance of in vitro tests of adhesive systems is often not or only partially given (Tab. II). Microscopic examinations of the bonding layer are purely qualitative. Dye penetration measurements have no clinical relevance and are not to be relied on. Marginal gap analysis is clinically relevant only to a limited extent, and the results depend heavily on the examiner. Bond strength tests are useful as a screening test, but the specimens should be tested after 1 day and again after at least 3 months of water storage. The results
of well-researched standard adhesives should serve as the reference. Although the absolute values measured can differ between testing institutes, the ranking yielded by testing different adhesive systems should be the same.

Fig. 9 The marginal seal of this 20-year-old Cerec restoration is inadequate. The wide cement space has washed out over time. Nonetheless, no caries adjacent to the restoration is evident. The degraded cement space could be intraorally repaired with composite, if the patient so desires.

Résumé

La troisième partie de cet aperçu présente les tests d'évaluation des systèmes adhésifs en combinaison avec les matériaux composites. Actuellement, aucun test standardisé n'existe pour évaluer les adhésifs, ce qui rend difficile la comparaison directe des valeurs moyennes entre instituts. C'est pourquoi on retiendra principalement le classement («ranking») des matériaux établi par l'analyse statistique des résultats. Selon les instituts, une certaine corrélation a été trouvée entre un test d'adhésion in vitro et les résultats cliniques. L'analyse qualitative du mode d'action de l'adhésif permet de comprendre son fonctionnement. Par contre, la mesure de la profondeur de pénétration dans les tubulis dentinaires ne permettra pas de tirer des conclusions sur l'adhésion. De même, les mesures d'une pénétration par un colorant d'une interface composite-adhésif ou d'une marge dentinaire de restauration composite ne montre que peu ou pas de corrélation clinique. Les systèmes adhésifs devraient d'abord être testés au niveau de leur adhésion au laboratoire et comparés à un adhésif standard ayant démontré de bons résultats cliniques avant d'être utilisés en clinique. Leur stockage de plusieurs mois dans l'eau avant un test d'adhésion in vitro augmentera la prévision de leur comportement clinique.

Tab. II Overview of common in vitro methods for testing adhesive systems, and their clinical relevance

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<th>Test</th>
<th>Standardized test protocol</th>
<th>Test validated</th>
<th>Clinical relevance</th>
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<tr>
<td>Bond strength test</td>
<td>no</td>
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<td>slight</td>
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<tr>
<td>Marginal adaptation (microscopic evaluation of margin)</td>
<td>no</td>
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<td>slight</td>
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<tr>
<td>Marginal adaptation (dye penetration)</td>
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