# Cytotoxicity and shear bond strength of four orthodontic adhesive systems

# Erwin Jonke\*, Alexander Franz\*, Josef Freudenthaler\*, Franz König\*\*, Hans-Peter Bantleon\* and Andreas Schedle\*

\*Department of Orthodontics and Central Research Unit, Bernhard Gottlieb University Clinic of Dentistry and \*\*Core Unit for Medical Statistics and Informatics, Medical University of Vienna, Austria

SUMMARY The objective of this study was to compare the cytotoxicity of four orthodontic bonding systems, Light Bond<sup>™</sup>, Enlight<sup>™</sup>, Concise<sup>™</sup>, and Transbond<sup>™</sup>, and to evaluate their shear bond strength (SBS). These orthodontic bonding materials were applied to metal brackets (Mini Diamond<sup>™</sup>). Glass specimens were used as controls in all experiments. Only Concise<sup>™</sup> was a chemically cured system, the other systems were light cured. The specimens were added to L-929 fibroblast cultures immediately after fabrication or after pre-incubation for 7 days. The incubation time was 72 hours and the cells were counted by flow cytometry. One hundred and fifty-seven freshly extracted human third molars were used for testing the SBS in a universal testing machine. Statistical significance was determined using analysis of variance followed by *post hoc* comparisons for multiple-level alpha control.

Pairwise comparisons showed a significant difference only between Light Bond<sup>TM</sup> and Concise<sup>TM</sup> (P = 0.0126). The highest SBS was obtained with Light Bond<sup>TM</sup> ( $23.23 \pm 1.53$  MPa) followed by Transbond<sup>TM</sup> ( $20.39 \pm 1.18$  MPa) and Enlight<sup>TM</sup>, ( $20.32 \pm 1.06$  MPa). Concise<sup>TM</sup> ( $17.87 \pm 1.04$  MPa) showed the lowest SBS. The cytotoxicity of all light-cured systems for fresh specimens was comparable, whereas the chemically cured system, Concise<sup>TM</sup>, was significantly more cytotoxic. After 7 days of pre-incubation, all systems were significantly less cyotoxic than fresh specimens (P < 0.001). Brackets alone were not cytotoxic. All bonding systems showed a clinically satisfactory bond strength higher than 10 MPa, with the chemically cured system showing the lowest SBS.

# Introduction

Direct bonding of orthodontic attachments has become an established procedure for better aesthetics, patient comfort, and improved oral hygiene. Buonocore (1955) introduced acid etching of enamel for increasing the adhesion of acrylic filling materials to enamel. Newman (1965, 1973) utilized this technique for direct bonding of brackets.

Cytotoxicity of dental resins is a well-known phenomenon (Schedle et al., 1998; Franz et al., 2003, 2007). However, a review of the literature shows a disparity between reports on the cytotoxicity of restorative and orthodontic resin adhesives, with greater attention given to the former. Furthermore, the physical requirements differ in different dental specialties. Restorative dentists focus mainly on bond strength to dentine and orthodontists on bond strength to enamel. Orthodontists are interested in shear bond strength (SBS), rapid curing, and ease of handling, whereas restorative dentists are also interested in durability, leaching, and colour stability. There are a number of studies on the bond strength of brackets to enamel (e.g. Moin and Dogon, 1978; Lopez, 1980). Some of them have described the impact of different acid-etching techniques on the tooth surface as well as the bond strength of the brackets (Surmont et al., 1992; Glasspoole et al., 2001). The bond strength of different orthodontic composites for metal, plastic, and ceramic brackets has been compared in several studies (e.g. Schulz et al., 1985; Gwinnett, 1988;

Joseph and Rossouw, 1990; Chaconas *et al.*, 1991), while only few reports are available on their cytotoxicity (Davidson *et al.*, 1982; Terhune *et al.*, 1983; Tell *et al.*, 1988; Tang *et al.*, 1999; Gioka *et al.*, 2005).

As new orthodontic resin adhesives continue to be marketed, rapid and sensitive tests for examining their toxic effects at the 'cell and tissue level' are needed because patient safety has been identified as a legal concept (Zentner *et al.*, 1994; Eliades *et al.*, 2004).

The present investigation was intended to compare the cytotoxicity of a self-etching system [self-etching primer (SEP) and composite] with that of three conventional composites (three-step systems with etching gel, bonding, and composite) and to determine the SBS of these four orthodontic bonding systems. The following null hypotheses were formulated: (I) all bonding materials tested show the same SBS, (II) the three- and two-step systems show the same cytotoxicity, and (III) the pre-incubation time of test specimens has no influence on cytotoxicity.

#### Materials and methods

# Collection and storage of teeth

One hundred and fifty-seven freshly extracted human third molars were collected at the Department of Oral Surgery

(Bernhard Gottlieb University Clinic of Dentistry, Vienna, Austria) and stored in a solution of 0.5 per cent chloramine T trihydrate. Tooth extraction was unrelated to the objective of this study. Ethical approval was received from the ethics commission of the Medical University of Vienna (No. 559/2007). Informed consent was obtained from all patients. The teeth selected had to have intact buccal enamel without pre-treatment with chemical agents, fillings, or caries. Adherent soft tissue was removed manually using a scaler, and the teeth were cleaned in an ultrasonic water bath containing a 1 per cent sodium hypochlorite solution. After cleaning, they were stored in 0.5 per cent chloramine T trihydrate solution at 4°C until use. All teeth were polished with a pumice slurry for 10 seconds and then washed with water immediately before use.

#### Bonding procedures

The materials used in this study are listed in Table 1. The manufacturers' instructions were followed as documented in Table 2. For systems 1 (Light Bond<sup>TM</sup>), 2 (Enlight<sup>TM</sup>), and 4 (Concise<sup>TM</sup>), a 37 per cent phosphoric acid gel (Gel Etching Agent<sup>TM</sup>) was applied to the buccal surface of each tooth for 30 seconds. The teeth were rinsed with water for 30 seconds and dried with an oil-free air source for 30 seconds until the buccal surfaces of the etched teeth were chalky white. For system 3 (Transbond<sup>TM</sup>), a SEP (Transbond Plus<sup>TM</sup>) was used.

# Light Bond

Light-cure resin sealant was applied to the etched surface, dried, and light cured for 30 seconds. Light-cure adhesive paste was lightly pressed onto the metal bracket base (Mini Diamond<sup>TM</sup>, Ormco) with a Heidemann spatula. Excess bonding paste was removed with the spatula. The bracket was then light cured from its mesial, distal, and occlusal aspects for 20 seconds each. Thirty minutes after light curing, the teeth were stored in water.

### Enlight

Enlight<sup>TM</sup> was applied as described above except for the application of the bonding materials (no light curing required). Ortho Solo<sup>TM</sup> Bond Enhancer was applied to the etched surface. Enlight Adhesive Paste<sup>TM</sup> was lightly pressed onto the bracket base with a spatula. After placement of the brackets, the standard protocol was followed.

# Transbond

Transbond Plus<sup>™</sup> SEP was applied as recommended by the manufacturers and dried. Transbond XT<sup>™</sup> paste was lightly pressed onto the bracket base with a spatula. The procedure after placing the brackets was similar to that for Light Bond<sup>™</sup> and Enlight<sup>™</sup>.

#### Concise (resin paste)

Equal portions of orthodontic bonding pastes A and B were placed on a mixing pad and processed following the manufacturer's instructions. The paste was lightly pressed onto the bracket base with a plastic spatula. The procedure was then similar to the other systems.

#### Light-curing unit

All materials were light cured with an Optilux 501 curing device (Kerr Co., Orange, California, USA). The light intensity was 890 mW/cm<sup>2</sup>.

# Bracket

A total of 561 upper second metal incisor brackets (Mini Diamond<sup>TM</sup>) were used. The average surface area of the bracket base was 10.5 mm<sup>2</sup>, based on the measured dimensions of five brackets.

#### SBS testing

The teeth were embedded in type III dental stone gypsum (Moldano blue<sup>TM</sup>, Heraues Kulzer, Hanau, Germany), so that the labial surface was parallel to the force applied during shear testing. Three hours after initial bonding, an

**Table 1**Adhesive systems tested in the investigation.

| Material  | Manufacturer   | Lot no.   |
|---|--|-----------|
| Light Bond™ light-cure sealant resin                | Reliance Orthodontic Products, Itasca, Illinois, USA | 050067    |
| Light Bond <sup>™</sup> adhesive paste              | Reliance Orthodontic Products                        | 048116    |
| Enlight <sup>™</sup> light-cure adhesive            | Ormco, Glendora, California, USA                     | 007058    |
| Ortho Solo Bond Enhancer™                           | Ormco  | 416281    |
| Transbond <sup>™</sup> Plus self-etching primer     | 3M Unitek, Monrovia, California, USA                 | 194156-L3 |
| Transbond XT <sup>™</sup> light-cure adhesive paste | 3M Unitek  | 4MB       |
| Concise <sup>™</sup> paste A, paste B               | 3M Unitek  | 4BP, 4BW  |
| Concise Enamel Bond System Resin A, Resin B         | 3M Unitek  | 4AN, 4BF  |
| Gel etching agent                                   | Reliance Orthodontic Products                        | 052279    |
| Positive control PVC strips                         | Portex Ltd, Hythe, Kent, UK                          | 30375     |

| Brand name<br>Individual production steps           | Reliance Light Bond™<br>Time recommended<br>in seconds | Ormco Enlight™<br>Time recommended<br>in seconds | 3M Transbond™<br>Time recommended<br>in seconds | 3M Concise™<br>Time recommended<br>in seconds |
|---|--|--|---|---|
|   |  |  |   |   |
| Enamel etching                                      | 30   | 30   | _   | 30  |
| Rinsing   | 30   | 30   | _   | 30  |
| Air-drying  | 30   | 30   | _   | 30  |
| Application of adhesive                             | 10   | 10   |   | 15  |
| Air-drying  | 5  | _  |   | _   |
| Etching and bonding in one step                     | _  | _  | 10  | _   |
| Light curing of adhesive                            | 30   | _  | _   | _   |
| Application of composite and positioning of bracket | 10   | 10   | 10  | 25  |
| Light curing of composite                           | 60   | 60   | 60  | _   |
| Total time  | 215  | 180  | 90  | 140   |

Table 2 Minimum time required per bracket for the four different bonding procedures according to the manufacturers' instructions.

occlusogingival load was applied to each bracket to generate a shear force at the bracket-tooth interface. This was accomplished using the flattened end of a steel rod attached to the cross head of a Zwick Universal Testing Machine<sup>TM</sup> Z 010 (Zwick GmbH & Co., Ulm, Germany).

The tests were performed with testXpert V11.0 (Zwick GmbH & Co.). The results of each test in megapascals were computerized. SBSs were measured at a crosshead speed of 1 mm/minute.

# Cytotoxicity test

Fabrication of specimens. Brackets were tested alone, with composite materials, or with composite materials and bonding materials. Bonding was applied to a Hostaphan polyester foil (Mitsubishi Polyester Film, Wiesbaden, Germany) in one layer. For system 1 (Light Bond<sup>™</sup>), the bonding was dried and light cured for 30 seconds. For system 3 (3M Transbond<sup>TM</sup>), only the bonding was dried. Subsequently, brackets with the composite materials were placed on top of the bonding materials. Systems 1 (Light Bond<sup>TM</sup>), 2 (Enlight<sup>TM</sup>), and 3 (Transbond<sup>TM</sup>) were light cured for 60 seconds. After light or chemical curing (system 4. Concise<sup>TM</sup>), adherence at the base was confirmed visually. Specimens were light cured with an Optilux 501 curing light (Kerr Co., light intensity 890 mW/cm<sup>2</sup>). All specimens were sterilized by ultraviolet radiation for 40 minutes on each side. Glass specimens, resembling those of the composite specimens in diameter and height, were used as negative controls.

*Pre-incubation of specimens.* The specimens were either used immediately after fabrication (fresh specimens) or preincubated in cell culture medium [one specimen in 10 ml of Dulbecco's modified Eagle medium (DMEM; Sigma-Aldrich, Schnelldorf, Germany)] at 37°C, pH 7.2, for 7 days. The culture medium was then removed and the specimens were used for the experiments. Culture of L-929 fibroblasts. The murine fibroblast cell line L-929 was obtained from the American Type Culture Collection (Rockville, Maryland, USA). L-929 cells were cultivated in Costar 162 cm<sup>2</sup> flasks (Costar, Cambridge, Massachusetts, USA) in DMEM supplemented with 10 per cent foetal calf serum (PAA Laboratories GmbH, Pasching, Austria), 1 per cent glutamine, and 1 per cent penicillin/ streptomycin at 37°C in a fully humidified air atmosphere containing 5 per cent CO<sub>2</sub> and were passaged by trypsinization. Fibroblasts were exposed to freshly prepared specimens (added to the cultures immediately after fabrication) or pre-incubated specimens (see above) in polystyrene six-well tissue culture plates (Costar) at 37°C/5 per cent CO<sub>2</sub> for 72 hours. Two specimens were added to each well in order to cover approximately 2 per cent of the cell layer surface as in previous experiments with dental composite materials (Franz et al., 2006). Cells were then harvested with trypsin (2.5 per cent in Ca<sup>2+</sup>- and Mg<sup>2+</sup>-free Hanks balanced salt solution; JRH Biosciences, Lenexa, Kansas, USA), centrifuged, and re-suspended in 500 µl DMEM.

# Flow cytometry

Cells were counted in a volume of 500  $\mu$ l DMEM for a fixed time of 30 seconds with a flow cytometer (FACSCalibur, Becton Dickinson, San Jose, California, USA) equipped with an argon laser tuned to 488 nm. Cell counts after exposure to test specimens were compared with the controls.

*Positive control.* PVC strips for 10993-5 cytotoxicity testing (International Organization for Standardization, 1999) were used as positive controls.

# Statistical evaluation

*SBS testing*. Analysis of variance (ANOVA) was performed to detect differences between the materials. *Post hoc* comparisons were made using Tukey's method for multiple-level alpha control.

*Cytotoxicity testing.* Cell numbers were standardized by calculating the ratio between test cell counts and negative-control cell counts expressed as

standardized cell number of material X (in % of control)

 $= \frac{\text{cell number of material X}}{\text{cell number of glass control}} \cdot 100\%.$ 

If the mean standardized cell number of a specific material equals 100 per cent, this can be interpreted that this material has no toxicity because the cell number is equal to the cell number of the glass control.

An adaptive two-stage design was used to define the sample size in order to ensure that the two-sided confidence interval (CI) for the mean standardized cell percentage numbers did not exceed a maximum of 15. In the first stage of the experiment, 18 observations per material (orthodontic bonding system) and pre-incubation time were performed. A second stage had to be run with fresh specimens of each individual bonding system, if the standard deviation (SD) was >15 for at least one of the fresh specimens. The second-stage sample size was determined by the maximum SD found. This two-stage test yielded the means and the corresponding 95 per cent CI for all fresh substances. In addition, the means and 95 per cent CI for specimens pre-incubated for 7 days were recorded.

#### Further analysis

Subsequently, all data of the first and second stage of all individually analysed orthodontic bonding systems were pooled. To explain the effects of the four adhesive systems, the substances (bracket, bracket + composite versus bracket + composite + bonding), and the ageing time (fresh versus 7 days) on cell counts, an ANOVA was run. In this model, the systems, the substances, and the ageing time were included as fixed factors. Interactions between the system versus substance, system versus ageing, and substance versus ageing were also considered in the model.

For ranking the toxicity of the four systems, a *post hoc* comparison was performed (Ryan, 1959, 1960; Einot and Gabriel, 1975; Welsch, 1977). The standardized cell number constituted the dependent variable. When calculating the ANOVA, the data of the positive control were disregarded. Continuous variables are presented as mean  $\pm$  standard error of the mean. A two-sided *P*-value <0.05 was considered to indicate statistical significance. All calculations were performed with SAS<sup>©</sup> Release 8.2 (SAS Institute Inc., Cary, North Carolina, USA).

#### Results

SBS test

ANOVA showed significant differences between the materials (P=0.025; null hypothesis I was rejected). Pairwise comparisons



**Figure 1** Effects of four different bonding systems (Light Bond<sup>TM</sup>, Transbond<sup>TM</sup>, Enlight<sup>TM</sup>, and Concise<sup>TM</sup>) on shear bond strength (SBS). (Vertical lines show the means ± the standard error of the means.) SBS was tested 1 hour after application of the bonding systems and brackets.

showed a significant difference only between Light Bond<sup>TM</sup> and Concise<sup>TM</sup> (P = 0.0126; Figure 1). The highest SBS was obtained with Light Bond<sup>TM</sup> (23.23 ± 1.53 MPa). For Transbond<sup>TM</sup> (20.39 ± 1.18 MPa) and Enlight<sup>TM</sup> (20.32 ± 1.06 MPa), the SBS was lower than that of Light Bond<sup>TM</sup>. Concise<sup>TM</sup> (17.87 ± 1.04 MPa) showed the lowest SBS.

#### Cytotoxicity test

*Two-stage test.* The cell culture test with Transbond<sup>TM</sup> was determined after the first stage (n=18). For all other systems, a second stage had to be performed resulting in a total sample size of n=36 for Light Bond<sup>TM</sup>, n=21 for Enlight<sup>TM</sup>, and n=40 for Concise<sup>TM</sup>. The means and 95 per cent CI of this analysis are shown in Figures 2a–d for each system. The effects of a bracket base without composite, Transbond XT<sup>TM</sup> with bonding, and Concise<sup>TM</sup> with bonding on cell numbers of L-929 fibroblasts are depicted in Figures 3a–c.

*Further analysis.* The cytotoxicity of all orthodontic bonding systems tested differed significantly (ANOVA, P < 0.001, Figures 2; null hypothesis II was rejected). Specimen ageing had a significant effect on cytotoxicity (P < 0.001). All freshly prepared materials showed lower cell counts than the controls (Figures 2a–d). The factor substance, was statistically significant (P < 0.001): specimens consisting of a bracket, bonding substance, and composite (Br + C + Bd) were more cytotoxic than specimens consisting of a bracket and composite (Br + C), whereas brackets alone were devoid of cytotoxicity (Figures 2a–d). The cytotoxicity of all substances diminished after 7 days of pre-incubation (P < 0.001, null hypothesis III was rejected), with Concise<sup>TM</sup> still being the material with the highest cytotoxicity level (Figures 2a–d).



**Figure 2** Effects of four different bonding systems [Light Bond<sup>TM</sup> (a), Enlight<sup>TM</sup> (b), Transbond XT<sup>TM</sup> (c), and Concise<sup>TM</sup> (d)] on cell numbers of L-929 fibroblasts. Specimens were added to the cultures immediately after production (fresh) or after pre-incubation for 7 days in cell culture medium at  $37^{\circ}$ C, pH 7.4. Two specimens (length: 3.5 mm; breadth: 3 mm; height: 1.8 mm) per well were incubated with L-929 fibroblasts in six-well culture dishes for 72 hours and cell counts were determined by flow cytometry. Standardized cell numbers were expressed as the percentage of controls (cultures with glass specimens). The means of fresh specimens and specimens pre-incubated for 7 days are connected for brackets (solid line), bracket + composite (dotted line), bracket + composite + bonding (dashed line), and positive controls (dashed–dotted line). The vertical bars are the corresponding 95 per cent confidence intervals for the means.

Ranking by cytotoxicity levels, based on all data available, showed Enlight<sup>TM</sup> to be the least cytotoxic followed by Light Bond<sup>TM</sup> and Transbond<sup>TM</sup>. Concise<sup>TM</sup> was the material with the highest cytotoxicity. The difference versus all other systems was statistically significant. The same results were obtained with data of fresh specimens. The cell numbers of Concise<sup>TM</sup> (66.80 ± 20.19 per cent) after pre-incubation for 7 days were still lower than those for freshly prepared Enlight<sup>TM</sup> (78.26 ± 13.42 per cent).

#### Discussion

Two important features of orthodontic bonding materials, cytotoxicity and SBS, were tested together for the first time in this *in vitro* study. Exactly the same brands of materials were used consecutively, as both aspects are essential for a

satisfactory clinical outcome. The investigation clearly showed that the self-etching system, Transbond<sup>TM</sup>, a material of the most recent generation of orthodontic resins, was less toxic than Concise<sup>TM</sup>, an established chemically cured system. In addition, Transbond<sup>TM</sup> had a higher SBS (20.39 MPa) than Concise<sup>TM</sup> (Vicente *et al.*, 2006). Compared with conventional three-step systems, the SBS of Transbond<sup>TM</sup> was similar to that of Enlight<sup>TM</sup>, but slightly lower than that of Light Bond<sup>TM</sup>.

Light-cured resins offer a dual advantage over chemically cured resins for bracket placement: since the chemical reaction does not start before light curing, several brackets can be placed consecutively and there is sufficient time to place them accurately (Forsten, 1984; Watts *et al.*, 1984; Trimpeneers *et al.*, 1996; Thind *et al.*, 2006). Furthermore, the light-curing system, Transbond<sup>TM</sup>, is a one-step



Figure 3 Effects of three different specimens on L-929 mouse fibroblasts.
(a) Bracket specimen without composite. Arrow 1: intact monolayer of L-929 mouse fibroblasts. (b) Transbond XT<sup>TM</sup>. Arrow 1: reduced cell number of L-929 mouse fibroblasts. Arrow 2: bracket base with Transbond XT<sup>TM</sup>.
(c) Concise<sup>TM</sup>. Arrow 1: highly significantly reduced cell number of L-929 mouse fibroblasts. Arrow 2: bracket base with Concise<sup>TM</sup>. Bar = 50 μm.

procedure, which is less technique sensitive and requires less chair time (Table 2), because etching and priming are combined in one step. The use of bonding systems with a SEP results in saving of at least 50 seconds of chair time per tooth (Table 2; Bishara *et al.*, 2006). This reduces the overall treatment time by approximately 8 minutes per jaw.

In the present study, the SBS of the light-curing adhesive systems were significantly higher than that of the chemically cured orthodontic system. The bond strength of all four bonding systems was in the range needed for a sufficiently strong bond between orthodontic attachments and enamel. Various studies suggest bond strengths between 2.8 and 10 MPa to be clinically adequate (Miura *et al.*, 1971; Reynolds, 1975; Keizer *et al.*, 1976; Moin and Dogon, 1978).

Previously, the tissue compatibility of orthodontic bonding agents was studied in animal experiments (William

et al., 1982). Ethical considerations, poor reproducibility, and small sample sizes resulted in the development of *in* vitro cytotoxicity tests and their standardization. The experiments in this study were performed according to the International Organization for Standardization (ISO) 1999, which leaves some flexibility in specimen fabrication. However, two aspects require further standardization for more precise and reliable evaluation of the biological effects of dental materials. Partly addressed in ISO (2008), these are the ratio of the specimen surface/cell culture medium and the specimen fabrication. The brackets covered approximately 2 per cent of the cell laver surface. This proved to be sufficient for distinguishing materials with different cytotoxicity (Schedle et al., 1998; Franz et al., 2003, 2006). The orthodontic adhesives were applied to the brackets and then tested in cell cultures to simulate the clinical setting as closely as possible. L-929 fibroblasts and gingival fibroblasts have previously been shown to have similar cytotoxicity levels. Consequently, L-929 fibroblasts make a useful screening model for in vitro toxicity testing of dental materials (Schedle et al., 1995). Because of its excellent reproducibility, the L-929 cell line was preferred to primary gingival fibroblasts. Different orthodontic resin adhesives were found to have varying toxicity levels in vitro and some were shown to lose their toxicity more rapidly than others. Chemically cured liquid-paste materials were more cytotoxic than light- or chemically cured two-paste materials (Tang et al., 1999). A study of dental composites found a chemically cured composite to be significantly more cytotoxic than a light-cured composite of similar composition (Schedle et al., 1998). Dental composite cytotoxicity has previously been shown to decrease significantly after 7 days of pre-incubation (Schedle et al., 1998; Franz et al., 2003, 2006). Therefore, both fresh specimens and specimens pre-incubated for 7 days were used in this study and the significant decrease in cytotoxicity was confirmed for orthodontic bonding resins. Figure 2 shows, for all materials tested, that brackets alone were not cytotoxic and that brackets in combination with composites and bondings were more cytotoxic than brackets with composite only. This suggests that resin composites and adhesives rather than the brackets themselves contribute to the overall toxicity of the orthodontic bonding systems.

Many bond strength studies lack comparability because of the difference in the test methods used (McSherry, 1996). General problems with bond testing protocols include load location, test mode (shear, tensile, torsional), and differences in enamel preparation (Katona, 1997). This study followed recognized protocols using the universal testing machine as the gold standard (McSherry, 1996; Pickett *et al.*, 2001; Klocke and Kahl-Nieke, 2005; Vicente *et al.*, 2006). The results suggest that an adequate bond strength to enamel was achieved with all test materials. This agrees well with other studies (Miura *et al.*, 1971; Reynolds, 1975; Keizer *et al.*, 1976; Vicente *et al.*, 2006). Clinically non-significant differences may be due to a larger bracket base area and physiological differences between bovine and human enamel (Klocke and Kahl-Nieke, 2005).

The results of the present research are consistent with several *in vitro* studies showing that most direct bonding adhesives, including self-etching systems, provide acceptable bond strength (Surmont *et al.*, 1992; Zeppieri *et al.*, 2003; Donmez *et al.*, 2006; Vicente *et al.*, 2006). The present findings are also supported by a randomized clinical trial showing that the overall bond failure per patient for a three- and a two-step bonding system (Transbond<sup>TM</sup>) was neither statistically nor clinically significant after 6 months and 1 year (Aljubouri *et al.*, 2004).

In the present study, shear force was measured, as this best simulates the clinical situation. Other authors tested tensile force and torque with resultant different force values (Bishara *et al.*, 2006). In one study, shear force, tensile force, and torque were compared for the same materials leading to different results (Eliades *et al.*, 2004). This underlines the necessity for further systematic studies of this phenomenon with other bonding materials and methods.

The new generation of orthodontic self-etching bonding systems combines etching and bonding in one step thus eliminating etching with 37 per cent phosphoric acid (Gel Etching Agent<sup>TM</sup>). SEPs were developed as bonding agents to dentine (Asgari et al., 2002). Many studies have examined the effect of SEPs on the adhesion of composite to dentine, but only few considered enamel (Dorminey et al., 2003). Transbond<sup>TM</sup> shortens the chair time for the patient by reducing the clinical bonding procedure, eliminates cross-contamination of the etchant and primer, and minimizes the risk of phosphoric acid injury (Asgari et al., 2002). Moreover, it helps to avoid the more extensive enamel loss to be expected with 37 per cent phosphoric acid in the conventional three-step bonding procedure (Ireland et al., 2005). Surprisingly, the use of light-cured products is not as widespread in orthodontics as in restorative dentistry (Thind et al., 2006). Orthodontists still tend to use chemically cured resins with inherent shortcomings such as higher cytotoxicity, lower bond strength, reduced time for placing the brackets, and increased chair time per patient (Graber et al., 2004).

The minimum bond strength of 6-8 MPa suggested by Reynolds (1975) was criticized for not being based on evidence (Larmour *et al.*, 2006). Furthermore, these data were related to tensile testing. This means that the debonding force acted on the enamel surface at 90 degrees. No data exist on the minimum debonding force in shear mode, which would be an equivalent to the force during mastication (Thind *et al.*, 2006).

In summary, tests for SBS and cytotoxic properties of orthodontic materials urgently need further standardization. Cytotoxicity assessment of orthodontic bonding materials is essential because the materials remain in the oral environment for at least 2 years and the total bonding area of a fixed appliance is approximately 250 mm<sup>2</sup>, i.e. about four times that of a Class II molar cavity restoration.

#### Conclusions

- 1. The chemically cured orthodontic adhesive showed a high cytotoxic potential and a low bond strength.
- 2. The light-curing bonding systems had a cell toxicity potential at a level significantly lower than that of the chemically curing system.
- 3. The two-step bonding system with a SEP showed a moderate degree of cytotoxicity comparable with a three-step light-curing system. The bond strength of the two-step bonding system was comparable with the three-step light-curing systems. Since etching liquid is unnecessary, application of the two-step bonding system is safer for the patient.
- 4. Further investigations should be based on a standardized experimental protocol to characterize the cytotoxic potential of a variety of orthodontic bonding materials and their SBS.

#### Address for correspondence

Andreas Schedle Bernhard Gottlieb University Clinic of Dentistry Währingerstrasse 25a A-1090 Vienna Austria E-mail:andreas.schedle@meduniwien.ac.at

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