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The influence of various light curing units on the cytotoxicity of dental adhesives

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ABSTRACT

Objectives. The objective of this study was to test the hypothesis that various light curing units (LCUs) have an influence on the cytotoxic action of adhesive systems.
Methods. Samples of the dental adhesives (Syntac®, iBondTM, Clearfil™ Protect Bond, Prime & Bond™ NT, Adper™ Prompt™ L-Pop™) were prepared in microwell plates, making use of the LCUs Voco Polofil Lux (VPL), EMS Swiss Master Light® (SML) and the LED prototype developed by the IMT of Jena University. To obtain extracts, the samples were topped with cell culture medium, which was changed daily on the 1st to 7th days and then on the 14th, 21st and 28th day, and stored for further use at −20 °C. Human gingival fibroblasts (HGFs) were cultivated in the extract-containing medium for 48 h. The viability of the HGFs was determined by the neutral red (NR) uptake test. The statistical test was performed by one-way ANOVA according to Bonferroni.
Results. During the first few days, reduction of the viability rates of the HGFs by 85–90% were observed in all adhesives. A rise up to a plateau phase was observed at different times depending on the materials. The influence of the LCUs on the cytotoxic action of the dental adhesives was clearly evident for the adhesives Syntac® and Clearfil™ Protect Bond. In case of the Syntac® extracts, cytotoxicity after polymerization with the VPL was statistically significant reduced compared to the other LCUs used ($p < 0.001$). A comparison between all the adhesives used proved that Adper™ Prompt™ L-Pop™ and Prime & Bond NT® had the lowest overall cytotoxicities.
Significance. In practice, one should use combinations of dental adhesive and LCU in which the material has the least toxic influences.

1. Introduction

The connection between the light curing unit used and the degree of polymerization of the composites and dental adhesives is currently being discussed in the literature [1–4]. The issue of interest is the influence of light curing on the toxic action of composites and dental adhesive systems [5,6]. Recently our group reported on the influence of different light curing units on the cytotoxicity of various dental composites [7]. We were able to prove that the combination of a high power LCU with various composites caused the lowest cell toxicity [7].

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In a clinical situation, however, a direct contact to the dentin–pulp complex is made not via the composite, as a rule, but via the dental adhesive. Several studies have demonstrated toxic effects of adhesive system components on the dentin–pulp complex [6,8–11]. For this reason, a possible connection between the application of composite fillings and post-operative irritations of the pulp is discussed [12]. It has been shown that the clinical application of an adhesive system can be associated with intense irritation of pulp in deep cavities [12]. The question arises whether there is a possible chemical toxicity of adhesive system components. Most dental adhesive systems require the application of acid conditioners to remove the smear layer from cavity walls and to promote the superficial demineralization of the dentin substrate. These products can alter the physical and chemical properties of dentin, facilitating its penetration through the dentin tubules [13]. Investigations into the penetration capability of cytotoxic constituents of dental filling materials by means of dentin barrier tests established that these constituents can penetrate the dentin and act on pulpal tissue there [14,15]. In particular, it may be assumed that insufficient light curing leads to incomplete polymerization and, in the end, to a higher residual monomer content with a greater total share of soluble, potentially toxic constituents [16].

Dental adhesives consist of methacrylates, dimethacrylates, phosphorized pentacrylates, acrylamides, aldehydes and organic acids [17]. Water, acetone or alcohol is added as solvent. Some adhesive systems also contain fillers. In particular, the unpolymerized monomers are primarily harmful to health; if they go into solution, they can get into the human body via the dentin and the pulp. For these reasons, in particular, it is important to achieve optimum polymerization of the dental adhesive. A current investigation shows that, after insufficient polymerization of the dental adhesive system, the release of IL 6 by macrophages was stimulated significantly [18]. Recently it has also been shown that the permeability of an incompletely polymerized adhesive layer is higher [19], which makes it possible for cytotoxic substances from the composite to penetrate the hybrid layer. Hence, the cytotoxicity of dental adhesives and especially the influence of the polymerization quality is increasingly at the focus of interest [9,10,20].

Polymerization parameters, such as light spectrum, light intensity, polymerization time and light control directly influence the degree of polymerization [5,6,21,22]; this indirectly also influences the residual share of non-polymerized toxic constituents [21,23].

The present study was focused on the examination of potential toxic influences of dental adhesives as a function of light curing. Its objective was to investigate, by way of an *in vitro* model, the possible cytotoxicity of different adhesive–LCU combinations.

2. Materials and methods

2.1. Sample preparation

In the present study, we investigated the cytotoxicity of five currently used dental adhesives of various types (Table 1). Polymerization was performed with two halogen-based light curing units of different light outputs and an LED unit (Table 2). The cytotoxicity of the sample extracts was investigated *in vitro*.

Samples of each dental adhesive were prepared in black 96-well microplates under aseptic conditions. Five samples were made of each dental adhesive–LCU combination. The respective adhesive system or its individual components were pipetted into the wells, so that every well contained a total volume of 10 μL of the material. The ratio of sample surface area to the volume of the solution was adjusted to approximately 4.32 cm^2/mL, which is within the 0.5–6.0 cm^2/mL range recommended by ISO [24]. The samples were then further processed in accordance with the manufacturers' instructions.

The polymerization time for the Polofil Lux halogen light curing unit (LCU) and the LED LCU was 10 s, while that for the SML-LCU was 4 s as instructed by the manufacturer.

To get extracts from the various dental adhesives, the samples were topped with 200 μL cell culture medium (DMEM – Dulbecco's modified eagle medium, 10% fetal calf serum, 0.1% antibiotic antimycotic solution) immediately after their preparation.

The cell culture medium was removed at 24-h intervals from the 1st to the 7th day, and then on the 14th, 21st and 28th day. After every removal of the medium, the samples were rinsed with 200 μL PBS (phosphate buffered balanced salt solution) and then again topped with 200 μL cell culture medium. For extraction, the samples were placed in an incubator at 37 °C and a carbon dioxide partial pressure of 5%. Until further processing, the extracts obtained were kept in sterile Eppendorf vessels at −20 °C.

Table 1 – Adhesives.

Name, manufacturer	Class	System	Generation	Period of application (s)
iBond™, Heraeus	All-in-one	One bottle	7th generation	20 s
Adper™ Prompt™ L-Pop™, 3M Espe	All-in-one	Two bottles: Fluid 1; Fluid 2	7th generation	10 s
Prime & Bond NT®, Dentsply	Self-priming	One bottle	5th generation	10 s
Clearfil™ Protect Bond, Kuraray Medical Inc.	Self-etching primer, antibacterial	Two bottles: Primer; Adhesive	6th generation	10 s
Syntac®, Ivoclar Vivadent AG	Three-component adhesive	Three bottles: Primer; Adhesive; Heliobond	4th generation	10 s

Table 2 – Light-curing units.

LCU	Voco Polofil Lux (halogen LCU)	Swiss Master Light® (SML)	LED LCU
Manufacturer	Voco GmbH, Cuxhaven, Germany	Electro Medical Systems, EMS, Switzerland	Prototype, Institute of Material Science and Technology (IMT), Jena University
Light source	1 halogen lamp, 75 W	1 halogen lamp, 360 W	1 LED, 5 W
Fiber-optic light guide [mm]	8.0	11.0	8.0
Power density [mW/cm^2]	500	3000	600
Period of application [s]	40	4	40
Emission spectrum [nm]	400–500	390–520	425–500

2.2. *Cell culture*

After approval (1881-10/06) from the Ethics Committee of the Friedrich Schiller University Jena, human gingival fibroblasts (HGFs) were obtained from a gingival biopsy of a healthy female patient aged 42 by the explant method [25]. The HGFs were cultivated by several passages in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal calf serum (FCS) and 0.1% antibiotic antimycotic solution (AAS) at 37 °C and 5% CO_2. Only cells of the sixth to eighth passage were used to minimize the differences in cell cycle.

2.3. *Cytotoxicity tests*

The neutral red uptake test was used to investigate the cytotoxicity of the extracts of the different dental adhesives.

The cells of the HGF culture were pipetted into 96-well microplates with a cell density of approximately 10,000 cells per well. To increase the sensitivity of our test setup, we immediately covered the cell layer with 100 μL adhesive extract per well. In this way, five parallel test batches were obtained from each adhesive–LCU combination, plus 10 control batches with fresh DMEM. Incubation of the cell cultures with the test substances was for 24 h at 37 °C and 5% CO_2.

The quantitative neutral red (NR) uptake test is based on the fact that only live cells are able to take up the NR dye and accumulate it in the lysosomes. The test can thus determine the share of live cells in a culture. The test was performed with the Biochrom-Gamma kit of Seromed®. After the dye was extracted from the cells, the optical density was measured at a wavelength of 540 nm by means of a microplate reader.

Cell viability in the neutral red uptake test was calculated by the following formula:

$$\text{Cell viability (\%)} = 100 \times \left[\frac{\text{OD}_{\text{mean}}\ \text{test group}}{\text{OD}_{\text{mean}}\ \text{control group}}\right]$$

2.4. *Statistics*

The data were analyzed with the SPSS 14.0 statistics program for Windows. Significance was tested with one-way ANOVA, and correction according to Bonferroni was applied in multiple comparisons. The differences of the measured data are significant at a level of $p < 0.01$. The number of samples was 5 for each adhesive–LCU combination.

3. Results

Basically, the cytotoxicity curve for the extracts of all dental adhesive–LCU combinations tested in this study shows three phases. The curve starts with an initial phase of high toxicity, followed by a phase of rise in cell viability, which finally turns into a plateau phase without any major change in cell viability (Fig. 1). In the analysis of the entire observation time, though, each material showed its own specific toxicity curve (Fig. 2). They differed especially in the length of the initial high-toxicity phase, the length and gradient of the subsequent viability rise, and the level of the plateau phase.

On the 1st day of the experiment, the NR tests of all extracts of the dental adhesives revealed a high toxic action on the HGFs. In the further course of the experiment, the adhesives iBond™, Prime & Bond NT® and Adper™ Prompt™ L-Pop™ were observed to have similar cytotoxicity curves (Fig. 2),

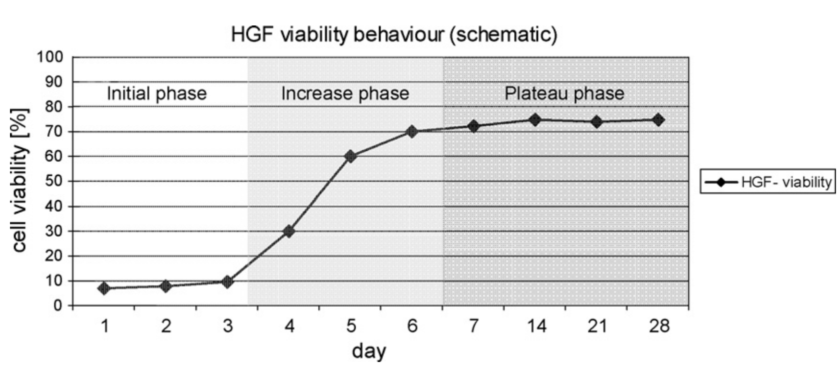

Fig. 1 – Viability behavior of the HGFs after exposure to the adhesive extracts (schematic).

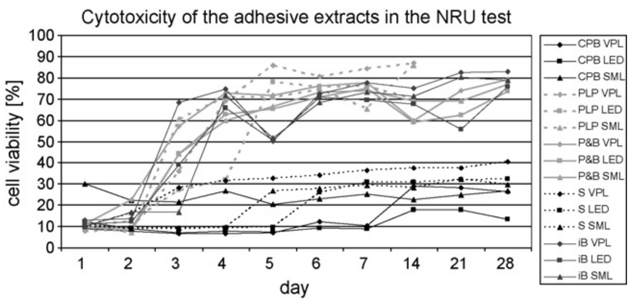

Fig. 2 – HGF cell viability behavior in the NRU test after exposure to adhesive extracts (CPB-Clearfil™ Protect Bond, PLP-Adper™ Prompt™ L-Pop™, P&B-Prime & Bond NT®, S-Syntac®, iB-iBond™) throughout the observation period.

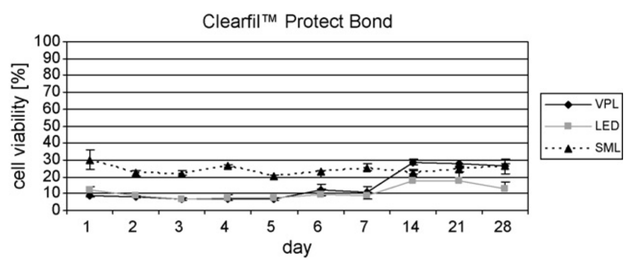

Fig. 3 – HGF cell viability behavior in the NRU test after exposure to the extracts from Clearfil™ Protect Bond.

whereas in case of Clearfil™ Protect Bond and Syntac® the curves of cytotoxic action of the HGFs were completely different (Fig. 2). The initial phase of Clearfil™ Protect Bond lasted until the 7th day (Fig. 2). The subsequent viability rise was substantially poorer than that of iBond™, Prime & Bond NT® and Adper™ Prompt™ L-Pop™, and the level of the plateau phase was markedly lower (Fig. 2). The extracts of Syntac® showed a long high-toxicity phase, too, which in most cases lasted until the 5th day (Fig. 4). Here again, the subsequent HGF viability rise was much poorer compared to iBond™, Prime & Bond NT® and Adper™ Prompt™ L-Pop™, and the plateau level was lower (Fig. 2). The dental adhesives iBond™, Prime & Bond NT® and Adper™ Prompt™ L-Pop™ showed a distinct viability rise of the HGFs already on the 2nd day. The increase in cell viability in this group was significantly greater than that of the extracts of Syntac® and Clearfil™ Protect Bond. Also, for iBond™, Prime & Bond NT® and Adper™ Prompt™ L-Pop™, the transition to the plateau phase was observed considerably earlier, and the level of cell viability was significantly higher, than in case of Syntac® and Clearfil™ Protect Bond (Fig. 2).

Generally, and for all materials tested, though, polymerization with the various LCUs has less influence on the cell viability curve than the material itself. Regarding the influence of the various light curing units on cytotoxicity, none of the dental adhesive extracts showed any difference in cell viability on the 1st day of the experiment (initial phase). Regarding the increase in cell viability after the high-toxicity phase, the combinations of the VPL with the adhesives iBond™, Prime & Bond NT® and Adper™ Prompt™ L-Pop™ yielded significantly better values than the combinations with the LED and SML (Figs. 3–7). Nevertheless, the combinations of these adhesives with the SML showed a more distinct viability rise of the HGFs than their combinations with the LED LCU. Clearfil™ Protect Bond in combination with the VPL and the LED showed a highly toxic phase of 7 days (Fig. 3). During this time, the viability after incubation with the extracts of the SML polymerisates was significantly higher than the viability of the other extracts. The combinations of Syntac® with the SML and the LED also exhibited a long high-toxicity phase, which lasted until the 4th or 5th day, respectively (Fig. 4). The combinations of iBond™, Adper™ Prompt™ L-Pop™ and Prime & Bond NT® with the various light curing units generally reached the

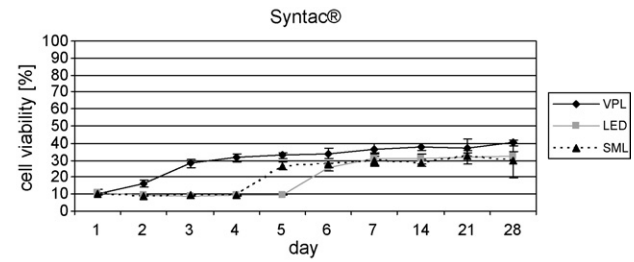

Fig. 4 – HGF cell viability behavior in the NRU test after exposure to the extracts from Syntac®.

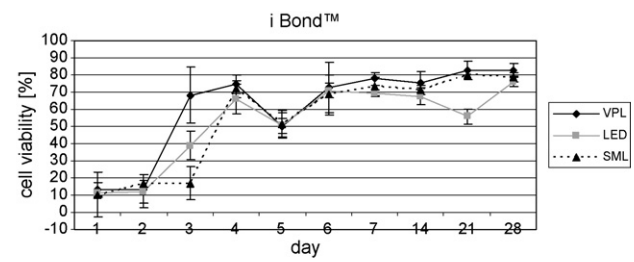

Fig. 5 – HGF cell viability behavior in the NRU test after exposure to the extracts from iBond™.

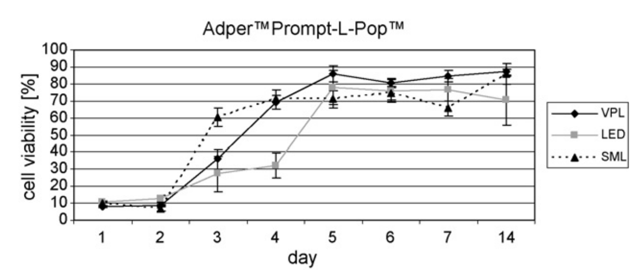

Fig. 6 – HGF cell viability behavior in the NRU test after exposure to the extracts from Prompt™ L-Pop™.

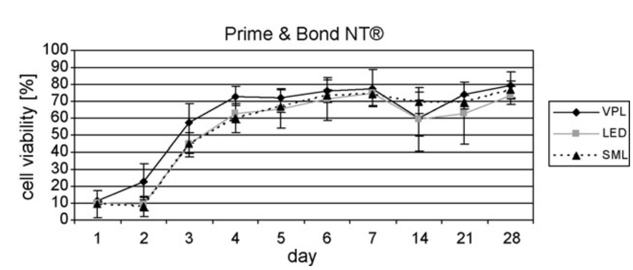

Fig. 7 – HGF cell viability behavior in the NRU test after exposure to the extracts from Prime & Bond NT®.

plateau phase on the 4th day, with no significant differences between the materials (Figs. 5–7). This situation continued right to the end of the experiment on the 28th day. It was only for the combination of Prime & Bond NT® with the VPL that significantly higher cell viability compared to the LED was found (Fig. 7). For the adhesives Adper™ Prompt™ L-Pop™ and iBond™ in combination with the VPL, the highest cell viability levels (Adper™ Prompt™ L-Pop™ = 88%, iBond™ = 83%) were found on the 28th day. For the extracts of Clearfil™ Protect Bond, too, in combination with the VPL, significantly higher cell viability levels were detected, compared to the extracts after polymerization with the SML and the LED LCU (Fig. 3). Thus, significant differences in cell viability between all Clearfil™ Protect Bond samples were found on the 28th day of the experiment. The measured values of the VPL group (26%) and of the LED-group (13%) were significantly below those of the SML group (33%). In case of the extracts of Syntac® from polymerization with the VPL, cell viabilities on the 3rd day were significantly higher than those measured for the polymerization of the SML and the LED–LCU combination (Fig. 4). These extracts reached the plateau phase already on the 4th day. On the last day of the period observed we measured viability levels of 40% in the VPL group, which were significantly higher than the levels measured in the SML (30%) and LED (32%) groups.

4. Discussion

The present study investigated the cytotoxicity of currently used adhesive–LCU combinations. The results show that the extracts of some dental adhesives have a toxic action on the viability of human gingival fibroblasts, which varies with the LCUs used. In the initial high-toxicity phase, which, depending on the adhesive–LCU combination, extended over a period of 1–7 days, a significant decrease in cell viability was observed in all groups examined. It can be assumed, therefore, that a major part of the toxic substances is released to the elution medium during the first hours or days after polymerization. A similar phenomenon was recently described by our group for several composite–LCU combinations [7]. Increased toxicity of composites immediately after polymerization was found by other authors, too [26–29]. It is known that the complete polymerization of the superficial layer of the composite is inhibited by oxygen contact [29]. With the experimental setup used, it can be assumed that the toxicity determined on the 1st day is partly influenced by the oxygen inhibition layer; the share of this, however, should be distinctly reduced already on the 2nd day due to the change of medium. But even the subsequent topping of the adhesive, and, thus, this inhibition layer, with composite will not guarantee in a clinical situation that no residual monomers will be released from this region.

Previous studies which report especially that the overall resin toxicity is primarily material-dependent make it plain that the influence of the oxygen inhibition layer may play a part immediately after polymerization, whereas the long-term overall toxicity is finally determined by the material as such [30,31]. For this reason, too, we especially observed in the present study a typical behavior over 28 days of cell viability for all adhesives investigated. For all materials we found that the initial, high-toxicity phase was followed by an increase in cell viability. This was observed between the 1st and the 7th day, depending on the dental adhesive. Generally there follows a plateau phase. The length of the phases and the viability levels measured differ with the materials, though. Comparable results with regard to the decrease in the cytotoxicity of composites and dental adhesives with increasing observation time have been reported by other authors as well [7,24,32,33]. On the other hand, there are reports of cases in which no connection was observed between the observation time and the toxicity of the materials [10,34].

In the dental adhesives investigated in the present study, cytotoxicity was found to vary with the materials. The extracts of the all-in-one adhesives Adper™ Prompt™ L-Pop™ and iBond™ and the self-priming adhesive Prime & Bond NT® had the least toxic effect on HGFs. With these materials, the initial phase lasted for a maximum of 2 days only, compared to Clearfil™ Protect Bond and Syntac®. The plateau phase was reached on the 5th day at the latest. Even at the end of the observation period, these adhesives were found to have a lower toxic action on the HGFs than Clearfil™ Protect Bond and Syntac®. The last-named two materials exhibited a rather long initial phase, also in combination with all light curing units tested. It can be assumed, therefore, that these two dental adhesives contain a larger proportion of non-linked substances after polymerization, which can then be released to the culture medium over a prolonged period. Also, the viability rise and the subsequent plateau phase of these two materials were distinctly lower. In other studies, too, an increased toxicity of Syntac® was documented, compared to other adhesives of the fourth and fifth generations [35–38]. In one study on the cytotoxicity of dental adhesives, though, a lower toxicity was found for the Syntac® adhesive system [39]; the authors could, for Syntac®, not detect the toxicity increase known in some dental adhesives of the fourth generation, which is caused by the mixing of primer and adhesive during the application procedure. Another reason for the high toxicity of Syntac® could be the approximately 5% share of glutaraldehyde contained in it, which has a very high toxic potential [36]. Glutaraldehyde reacts with the collagen fibers, which get exposed in the dentin by the etching process, and serves for stabilizing the collagen fiber network. In this way, most of the free glutaraldehyde is irreversibly bonded; i.e. tissue damage in vivo is largely excluded. Whether all glutaraldehyde molecules are bonded in a clinical situation cannot be judged with sufficient certainty, though. Other studies also found a stronger cellular reaction in glutaraldehyde-containing adhesive systems than in glutaraldehyde-free ones [40]. Another glutaraldehyde-containing dental adhesive investigated in the present study (iBond™), however, showed a significantly lower toxicity compared to Syntac®. Possibly, the concentration of this substance used in the respective adhesive plays a significant part.

Regarding the cytotoxicity as a function of the light curing unit employed, we observed significantly lower toxicity levels for the adhesives Clearfil™ Protect Bond in combination with the Swiss Master Light® and for Syntac® combined with the VOCO Polofil Lux, compared to the extracts of the other LCU combinations. During the plateau phase, the extracts of Clearfil™ Protect Bond in combinations with the Swiss Master Light® and the VOCO Polofil Lux showed a significantly smaller reduction in cell viability compared to the samples polymerized with the LED light curing unit. No significant difference was found between the two halogen-based LCUs. The results were similar for the extracts of the Syntac® adhesive system. For Syntac®, the influence of the LCUs became evident especially by the fact that the toxicity of the extracts after polymerization with the VPL was significantly lower throughout the observation period than after polymerization with the other LCUs. Altogether, Adper™ Prompt™ L-Pop™, Prime & Bond® and iBond™ show a distinctly lower influence of the light curing unit. At some times within the observation period, though, their toxicity levels were also found to be lower with the halogen-based polymerization units. A significantly lower cytotoxicity compared to the samples polymerized with the LED light curing unit was observed especially during the rise phase, whereas in the plateau phase the differences between the light curing units were insignificant. The extracts of the groups with the halogen-based units, though, tended to also have a weaker cytotoxic action on the HGFs. Other studies investigating the influence of different light curing units on the quality of polymerization also showed that the material properties and the cytotoxicity of the dental adhesive was influenced by the polymerization device employed [10,20]. As shown in the present study, the halogen-based light curing units have some advantages in this respect over the LED units; another current study report comes to a similar result [20]. On the other hand LED LCUs have other benefits such as a lower temperature increase in the pulp or a longer lifetime at constant irradiation when compared with halogen LCUs [41].

The present results show that the adhesives iBond™, Prime & Bond NT® and Adper™ Prompt™ L-Pop™, which are distinguished by their relatively low toxicity, also are least influenced by the light curing units used. An influence of the light curing unit on cytotoxicity was established specially for the adhesives Clearfil™ Protect Bond and Syntac®. After polymerization with the halogen-based LCUs Swiss Master Light® and VOCO Polofil Lux, altogether better cell viability results were observed than after the use of the LED unit.

In practice, such adhesives should be used that not only establish a good adhesive bond with the hard tooth tissue but also feature a high biocompatibility. Biocompatibility can be optimized with a good matching of dental adhesive and light curing unit, especially for the adhesive systems Clearfil™ Protect Bond and Syntac®. This is a way to reduce possible toxic influences, especially on the pulp–dentin system and the oral mucosa.

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