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ORIGINAL ARTICLE

Effectiveness and biological compatibility of different generations of dentin adhesives

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Abstract
Objectives Besides possessing good mechanical properties, dental materials should present a good biological behavior and should not injure the involved tissues. Bond strength and biocompatibility are both highly significant properties of dentin adhesives. For that matter, these properties of four generations of adhesive systems (Multi-Purpose/Single Bond/SE Plus/Easy Bond) were evaluated.
Materials and methods Eighty bovine teeth had their dentin exposed (500- and 200-μm thickness). Adhesive was applied on the dentin layer of each specimen. Following that, the microshearing test was performed for all samples. A dentin barrier test was used for the cytotoxicity evaluation. Cell cultures (SV3NeoB) were collected from testing materials by means of 200- or 500-μm-thick dentin slices and placed in a cell culture perfusion chamber. Cell viability was measured 24 h post-exposition by means of a photometrical test (MTT test).
Results The best bonding performance was shown by the single-step adhesive Easy Bond (21 MPa, 200 μm; 27 MPa, 500 μm) followed by Single Bond (15.6 MPa, 200 μm; 23.4 MPa, 500 μm), SE Plus (18.2 MPa, 200 μm; 20 MPa, 500 μm), and Multi-Purpose (15.2 MPa, 200 μm; 17.9 MPa, 500 μm). Regarding the cytotoxicity, Multi-Purpose slightly reduced the cell viability to 92 % (200 μm)/93 % (500 μm). Single Bond was reasonably cytotoxic, reducing cell viability to 71 % (200 μm)/64 % (500 μm). The self-etching adhesive Scotchbond SE decreased cell viability to 85 % (200 μm)/71 % (500 μm). Conversely, Easy Bond did not reduce cell viability in this test, regardless of the dentin thickness.
Conclusions Results showed that the one-step system had the best bond strength performance and was the least toxic to pulp cells. In multiple-step systems, a correct bonding technique must be done, and a pulp capping strategy is necessary for achieving good performance in both properties.
Clinical relevance The study showed a promising system (one-step self-etching), referring to it as a good alternative for specific cases, mainly due to its technical simplicity and good biological responses.

Keywords Dentin adhesives · Bond strength · Cytotoxicity

Introduction

Concepts of modern dentistry have been based on the theories of health promotion, prevention, and aesthetics. For that matter, the advent of new materials and techniques has been related to the current development of dentistry and based on scientific evidences, which associate functionality with the significance of such concepts.

Considering that, the development of "adhesive dentistry" came with the idea of improvement of dentin adhesives. A greater performance on the quality of adhesion was followed by a technical difficulty during clinical application, representing a need for multiple steps, hindering the work of professionals, and also showing variance in the adhesion

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results [1]. Currently, there is a trend for developing simpler systems, which involve fewer operative steps, reducing the possibility of failure, such as excessive etching or dryness of the dentin, and thus avoiding problems on adhesion [2].

Dentin bonding agents have been classified into generations. Perdigão et al. [3] have proposed a simplified rating, taking into account only the current available products. The adhesives have then been classified into four types—the total-etching multibottle and one-bottle systems and the self-etching multibottle and one-bottle systems. Good effectiveness on the adhesion results has been described for the total-etching systems in the clinical literature. However, these authors have stated that these systems show a complex application technique [1, 2, 4]. Recently, all-in-one self-etching adhesives were introduced in the market in order to fulfill the expectations of clinicians, who have been looking for less technique-sensitive formulations and a simplified application procedure [5], though Sasakawa et al. [2] draw attention to the fact that this simplification might lead to a less efficient adhesive property.

An important aspect with regard to the adhesion capacity of adhesive systems is the substrate where it is applied. The inorganic composition of dentin, the mesh of collagen, and humidity and its relation with the pulp tissue make it the real "Achilles heel" of adhesive dentistry [3, 6]. This can be observed in the data found in the literature, with bond strength values ranging from 5 to 48 MPa [2, 7].

Many studies have attempted to assess the significance of using dental adhesives not only as bonding agents but also as protectors of the dentin pulp complex. Schmalz et al. [8] firmly indicate them as liners only for shallow and medium cavities, while others [9, 10] suggest that they can also be used in deep cavities and even for direct pulp capping, without causing damage to structures or adhesion failures.

In order to achieve clinical success, it is necessary for dental adhesives to provide good physical properties. However, due to the fact that dental adhesives are in close and extended contact with vital dentin, biocompatibility becomes a property of supreme importance. Thus, dentin adhesives should have their biological behavior studied before their clinical use [11]. For this purpose, animal experiments and cell culture tests have been available. Animal experiments for cytotoxicity tests of dental materials are time-consuming, expensive, and a theme for extensive public discussions. Cell culture methods, on the other hand, are better standardized and reproducible. They are faster and easy to perform at relatively low costs [12].

There are no investigations which associate the bond resistance and biological behavior of different generations of dentin adhesives in deep cavities. Hence, it has been taken into account that differences in the results are due not only to the composition of the adhesives but also to the variations in the application technique and in the dentin structure. In view of those facts, the aim of this study was to evaluate four different adhesive systems concerning the microshearing bond strength to bovine dentin in the simulation of deep cavities. The "in vitro" cytotoxicity of these adhesive systems was also evaluated on a molecular level by means of a dentin barrier test device and MTT assay in bovine dental pulp fibroblasts. The hypothesis states that the bonding system with a greater effectiveness in the bond strength test will present a higher degree of toxicity, causing injury to pulp cells.

Material and methods

Four different adhesive systems were evaluated regarding the microshearing bond strength to bovine dentin and cytotoxicity by means of a dentin barrier test. The materials and their compositions are listed in Table 1.

Microshearing bond test

Eighty bovine incisors were used in the experiment. The roots were sectioned in accordance to the tooth axis by using a low-speed device, and the pulp chamber was accessed in order to remove the pulp tissue. Following that, the enamel surface was worn in a chopper for a plaster aiming for dentin exposition.

The samples were divided into two groups of 40 teeth each (group 1, teeth worn until a 500-μm dentin thickness remains; group 2, teeth worn until a 200-μm dentin thickness remains). The remaining dentin thickness was measured by means of calipers. Afterwards, the specimens were placed in a silicon array and included in acrylic resin (Jet Classic) so that the buccal surface of the teeth remained exposed.

The specimens were stored in distilled water for 24 h at 37 °C, and the dentin surface was further polished by means of a circular polisher (Eros), following a decreasing order of grit grinding disks (600, 800, 1,200) in order to provide a smooth and uniform surface.

The samples were once more stored for 24 h, and 40 teeth from each group were divided into four subgroups, with 10 teeth each, according to the adhesive system used:

Groups 1MP and 2MP: Scotchbond Multi-Purpose (3 M ESPE) - (n=10)
Groups 1SB and 2SB: Single Bond 2 (3 M ESPE) - (n=10)
Groups 1SE and 2SE: Adper SE Plus (3 M ESPE) - (n=10)
Groups 1 EB and 2 EB: Adper Easy Bond (3 M ESPE) - (n=10)

The bonding procedures were carried out for each group according to the manufacturer's instructions. The samples were placed in a metal device, containing a Teflon split with

Table 1 Materials used in the study

Material	Description	Composition	Manufacturer
Adper Scotchbond Multi-Purpose	Total-etching multibottle system	Primer: HEMA, polialcenoic acid Adhesive: Bis-GMA and HEMA	3 M Co., Seefeld, Germany
Adper Scotchbond Single Bond	Total-etching one-bottle system	Bis-GMA and HEMA,dimetacrilates, ethanol, polialcenoic acid	3 M Co., Seefeld, Germany
Adper Scotchbond SE Plus	Self-etch multibottle system	Acidic primer: HEMA, ink, surfactant Adhesive: UDMA, TEGDMA, HEMA	3 M Co., Seefeld, Germany
Adper Easy Bond	Self-etch one-bottle system	Bis-GMA, HEMA, dimetacrilate, ethanol, polialcenoic acid	3 M Co., Seefeld, Germany
CaGPG 14	Positive control (DBT)	Polyacrilic acid, HEMA, ethyl 4-dimethyl-aminobenzoat, camphoroquinone	University of Regensburg, Germany
President regular	Negative control (DBT)	Polymer preparation and compounds	Coltène AG, Altstätten, Switzerland

an inner hole of 2-mm diameter and 2-mm height. Tygon tubes (2 mm ×1 mm height and 0.8-mm internal diameter) R-3603 (Norton Performance Plastic Co., São Paulo, Brazil) were used to delimitate the adhesion area and to prepare the samples for the microshearing test. The composite resin Filtek Z250 (3 M ESPE, 3 M Co., St. Paul, MN, USA) was applied in single increment and light-cured for 40 s by means of a calcium hydroxide applicator (SS White Duflex, São Paulo, Brazil), and small cylinders were obtained for the mechanical test. The specimens were removed from the matrix, and an additional 40-s curing was performed. All specimens were immersed in distilled water and stored in a bacteriological incubator at 37 °C for 24 h and then submitted to the microshearing test. This test was carried out in a universal testing machine (DL-2000, EMIC, São José dos Pinhais, Paraná, Brazil) with a load cell of 50 kg. A metal base was used in order to place the testing samples correctly, and a 0.2-mm-diameter orthodontic wire was fixed on top of the mobile end of the machine. The wire was looped flush around the composite resin cylinder and positioned in the adhesive interface. The machine was set to operate at a crosshead speed of 0.5 mm s^{-1} [13] (Fig. 1).

Statistical analysis was performed by means of the Mann–Whitney U test (α=0.05) (SPSS, version 18.0; SPSS, Chicago, IL, USA).

Dentin barrier test

Transfected bovine pulp cells SV3NeoB were maintained in a growth medium (MEMα, Gibco BRL, Karlsruhe, Germany), supplemented with 20 % fetal bovine serum, and three-dimensional cultures of these cells were cultivated as previously described [14].

After the incubation period of 14 days, the three-dimensional cultures were introduced into a dentin barrier testing system.

Dentin slices (500±20 μm and 200±20 μm) were cut from extracted bovine incisors. The smear layer on the pulpal side was removed by etching it with 50 % citric acid for 30 s, and the slices were then sterilized. A commercially available cell culture perfusion chamber (Minucells and Minutissue GmbH, Bad Abbach, Germany) was separated into two compartments by the dentin disk. The three-dimensional cell culture tissues were placed in direct contact with the pulpal side of the dentin disk and held in place by the stainless steel holder. The chambers were perfused with 0.3 mL/h assay medium for 24 h, simulating a healthy pulp. Following that, the perfusion was switched off, and the adhesive applications were done in the upper compartment in direct contact with the cavity side of the dentin disks. All steps were carried out according to the manufacturer's instructions, and the adhesive systems were polymerized for 20 s. Subsequently, the pulpal part of the perfusion chamber containing the cell cultures was perfused now with a 2 mL/h medium throughout an incubation period of 24 h, simulating the occurrence of an inflammation. After an exposure period of 24 h, the survival cell in the exposed three-dimensional cultures was determined by the MTT assay as described [12]. The median optical density values obtained from tissues exposed to a polyvinylsiloxane impression material (President regular, Coltène AG, Altstätten, Switzerland) were used as a negative control reference (100 % survival cell). The cytotoxicity of the samples was referred as a percentage of the negative control tissues. Each experiment was performed with five replicates and carried out at least for two times. Statistical analysis was performed by means of the Mann–Whitney U test (α=0.05) (SPSS, version 18.0; SPSS, Chicago, IL, USA).

Results

Microshearing bond test

The median values for the investigated adhesives are shown in Fig. 2. The results are similar when evaluated for the

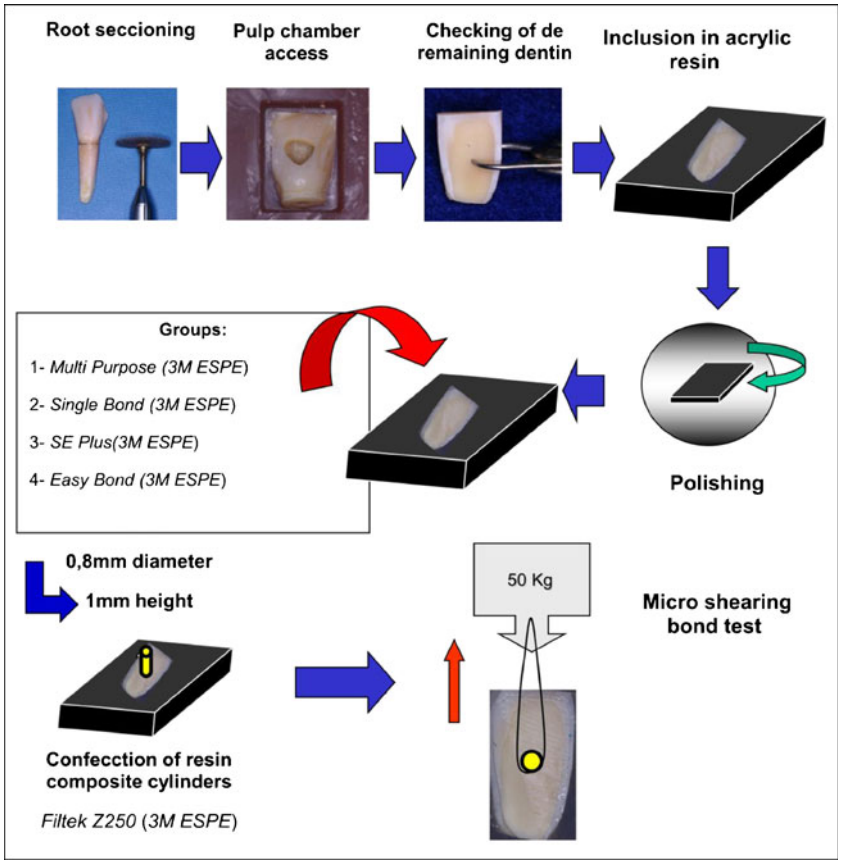

Fig. 1 Schema of the microshearing bond test

same thickness of dentin. It seems that the self-etching "one-step" system (Easy Bond) was more efficient with regard to the bonding strength (21 MPa, 200 μm; and 27 MPa, 500 μm) for both dentin thicknesses, followed by SE Plus (18.2 and 20), Single Bond (15.6 and 23.4), and finally the three-step system Multi-Purpose (15.2 and 17.9). For all systems, there was a greater resistance to bonding for the 500-μm remaining dentin compared to the other tested thickness (200 μm).

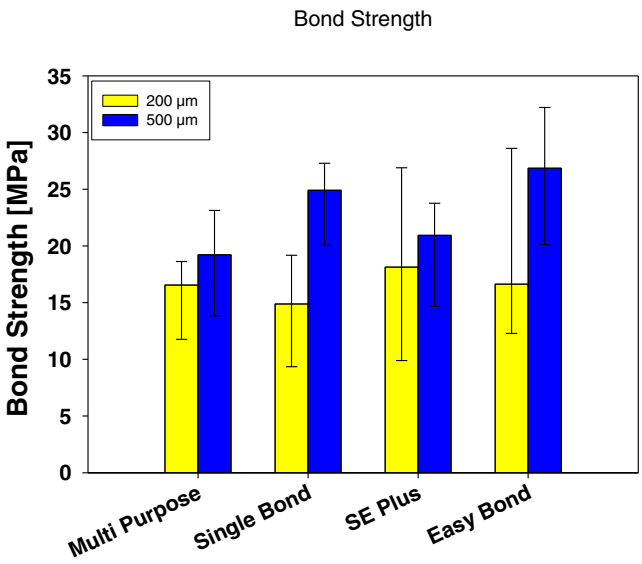

Fig. 2 Median and 25–75 % quantile values from 10 independent specimens for both (200 and 500 μm) dentin thicknesses

When analyzing the median values for the bonding resistance of the various systems at the same thickness, statistical difference was observed only between the values for the adhesives Easy Bond and Multi-Purpose in the 500-μm dentin thickness (p=0.035). No statistical difference was observed for both dentin thicknesses, except for the Single Bond system (p=0.023).

Dentin barrier test

Data collected on the dentin barrier test associated to the dentin adhesives are shown in Fig. 3. A vinyl polysiloxane (President) and a light curing material, developed at the University of Regensburg (CaGPG 14), were used as negative and positive controls, respectively. As expected, CaGPG 14 was the most toxic material for both dentin thicknesses, leading to about 38 % reduction in cell survival in the 500-μm dentin slices and to 6 % reduction in the 200-μm dentin slices, when comparing to the cell cultures exposed to President.

Twenty-four hours post-exposition, Adper Scotchbond Multi-Purpose reduced the cell survival rate to 92.5 % in the dentin thickness of 200 μm and to 93.7 % in the 500-μm

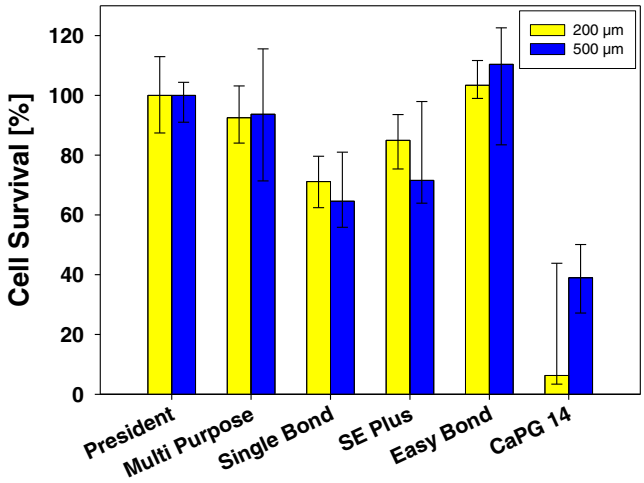

Fig. 3 Cell survival of three-dimensional cultures in a dentin barrier test device when exposed to dentin adhesives. Percentage of the negative control cultures: President 100 %. Median and 25–75 % quantile values calculated from independent experimental cultures: for President, n=20, and for each adhesive system, n=10 (for both dentin thicknesses of 200 and 500 µm)

slices, showing the low cytotoxicity of the material. There was a greater reduction in the cell survival rate caused by the Adper Scotchbond Single Bond, 71.1 % in the dentin thickness of 200 µm and 64.6 % when used in the 500-µm thickness, which could be considered as a moderate toxic material.

Regarding the self-etching adhesives, the Scotchbond SE Adper decreased the cell survival rate to 85 % and 71 % in the dentin thickness of 200 and 500 µm, respectively, thus showing a moderate toxicity. On the other hand, Adper Easy Bond did not reduce cell survival, regardless of the dentin thickness used, characterizing it as a non-toxic material for the current experimental conditions.

Median values for the cell survival rates obtained from the positive control group showed statistical difference compared to all the other groups. The negative control group (President) showed statistical difference only when compared to the adhesives Adper Scotchbond Single Bond and Adper Scotchbond SE (moderate toxicity), but no significant differences were observed when compared to the low- and non-toxic materials (Table 2).

Discussion

The dental adhesives tested in the present study are all from the same manufacturer, and the main purpose of this strategy was to analyze not only more than the composition, but also the influence of the application technique on the studied properties.

For the bond strength and the dentin barrier test, bovine teeth were used. This choice was based on the literature; several authors performed comparisons between human and bovine teeth and qualify those to be used in tensile tests [15, 16]. Regarding the dentin barrier test, Galler et al. [17] reported that due to the similarities in the composition, density, and diffusion of human and bovine dentin, they can be used for the test.

The total-etching systems showed lower bond strength values when compared to the self-etching systems, which contradicts the findings of other studies [1, 4, 18, 19]; nevertheless, it is known that these systems have the disadvantage of technical complexity, mainly related to the time of dentin conditioning and humidity [1, 20, 21]. Sano et al [20] states that the degree of demineralization is often greater than the capacity of penetration of adhesive resins, resulting in a void and a non-hybridized demineralized zone, which may result into microleakage, losses on bonding strength, and post-operative sensitivity. This point and the technical complexity of the application may justify the fact that total-etching systems showed lower values compared to the self-etching systems. In our study, the highest values were obtained by the one-step self-etching system, which is in agreement with several authors [22, 23]. These systems use hydrophilic and acidic monomers, which are able to simultaneously demineralize and penetrate the enamel and dentin. In addition to the simplification of the bonding procedure and the potential decrease in technique sensitivity, the simultaneous procedure of demineralization of tooth structure, diffusion, and embedding of the monomer around dentinal collagen fibers should provide optimum infiltration. It should then form a resin-saturated interdiffusion zone with no empty spaces, and this would result in higher values of bonding, even though the area is not as thick as in the total-etching systems [24, 25].

When adhesive systems are placed onto deep cavities, their direct toxic effects will most likely be on the pulp cells beneath the dentin. According to Schmalz et al. [26] the in vitro dentin barrier test system for cytotoxicity tests might mimic clinical situation, which is better than direct cell–material contact in vitro methods, and it has the potential to, at least partially, replace animal experimentation.

There is a consensus that dental adhesives can be cytotoxic to pulp cells when applied in deep cavities [8, 11, 27]. Several studies have shown the toxicity of dental adhesives, but the etiology of pulpal responses has not been completely comprehended, as well as the effect of adhesives on the cell cycle [28, 29]. Some authors attribute this toxicity to the monomers, reporting the small methacrylates HEMA and TEGDMA as moderately toxic [29–31]. The complete polymerization of composite and adhesive systems is hardly achieved, so different components of adhesive materials can be released when in a moist environment [30]. When applied in deep cavities, these residual monomers may reach the pulp by diffusion, and in etched dentin, this penetration tends to be higher. Some concentrations can be toxic to the pulp cells, causing inflammation and tissue disorganization.

Table 2 Statistical analysis of cell survival rate in the dentin barrier test

	President	Adper Scotchbond Multi-Purpose	Adper Scotchbond Single Bond	Adper Scotchbond SE	Adper Easy Bond	CaGPG 14	200-μm dentin thickness
President	ns	ns	0	0.014	ns	0	
Adper Scotchbond Multi-Purpose	ns	ns	0.003	ns	0.035	0	
Adper Scotchbond Single Bond	0	0.011	ns	0.043	0	0	
Adper Scotchbond SE	0.005	0.043	ns	ns	0.001	0	
Adper Easy Bond	ns	ns	0.001	0.009	ns	0	
CaGPG 14	0	0	0.001	0	0	ns	
500-μm dentin thickness							200 μm×500 μm

ns not significant

The severity of the pulpal reaction may be influenced by several factors, such as material composition, procedure, and clinical application [32]. The total-etching system Scotchbond Single Bond was found as the most toxic in this study, with a moderate toxicity, which is consistent with several studies that used conventional monolayer cell cultures [27, 33, 34]. Scotchbond Multi-Purpose showed a low toxicity, which is also in agreement with other authors [35]. The verified toxicity of these systems can be mostly attributed to dentin permeability.

The self-etching system, Easy Bond, showed no toxicity, which is also consistent with other studies in the literature [8, 17, 36]. Other authors observed the toxicity of these systems mainly due to the direct contact with cells in culture [25]. In a dentin barrier test, however, the dentin may protect cells in the underlying three-dimensional cultures from damage [37]. Besides the protective effect of dentin, one-bottle systems do not promote a very deep dentin demineralization [17]. It is likely that there was no sufficient penetration of the material, which might explain the non-toxicity of these materials noticed in the present investigation.

According to our results, all materials studied showed a compatible performance for clinical use in both aspects. Nonetheless, some care must be taken when using these products. Regarding bond strength, it is important to note, especially for total-etching systems, that it is necessary to perform the technique correctly in order to achieve the maximum bonding capacity, taking care with the conditioning time, dentin moisture, and correct application of the agents and following the instructions for the time required for evaporation of solvents and penetration of the adhesive. Regarding the biological action, the investigation showed that most of the tested adhesive presents a certain degree of toxicity, inducing apoptosis or interfering with the cell cycle, which consequently, also interferes in dentin regeneration. It was also shown that although the remaining dentin promotes a pulp capping in deep cavities, this protection is not sufficient to prevent penetration of residual monomers. Therefore, another strategy should be chosen for such cases in order to protect the dentin–pulp complex and allow the regeneration of the tissues involved.

The one-step self-etching system showed the best bond strength values and no toxicity to cells. The results demonstrated a promising system, which represents a good alternative for specific cases, particularly because of its technical simplicity and good biological tissue responses. Yet, it is important to emphasize that the effectiveness of a material is related to several properties, which require laboratory investigations to evaluate other aspects and longitudinal studies.

This study has some limitations, which are inherent to all in vitro researches. In spite of being something sacred in the literature, the limitation of using bovine teeth should be cited. In the specific case of the dentin barrier test, dental bovine pulp cells were used because of the need of working with a 3D cell culture, which is possible only with this kind of cells. Furthermore, when an aggression on pulp cells occurs, an inflammation happens, which is difficult to simulate, since an in vitro study does not reproduce the defense reactions of the tissues, which is another limitation of our study [17]. The results show a tendency, and a careful analysis is necessary in order to convey the findings to clinical practice.

Based on the results, we reject the hypothesis since the system with the best bond strength performance was the least toxic to pulp cells.

Conflicts of interest The authors do not have conflict(s) of interest to declare.

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