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In vitro cytotoxicity of dental adhesives: A systematic review

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ABSTRACT

Objectives. The increased demand for esthetics and minimally invasive tooth restorations resulted in a rapid development of adhesive dentistry. However, much controversy remains about the safe use and cytotoxic effects of different groups of dental adhesives. The present study performed a systematic review to identify the answer to the following question: are self-etch adhesives more cytotoxic than those employing the etch-and-rinse system?

Methods. This systematic review was performed in accordance with the PRISMA statement; a quality assessment for in vitro studies was conducted using the ToxRTool. Specific search strategies were developed and performed in the electronic databases MEDLINE via PubMed, Cochrane Library, Scopus, Web of Science, and LILACS/BBO. After removal of duplicated studies and application of the exclusion criteria, ten eligible articles were selected and submitted to a qualitative descriptive analysis comparing both groups of dental adhesives. Most in vitro test systems employed pulp cells or gingival fibroblasts.

Results. The methodologies presented great variability regarding the exposure to the test materials. Only four studies assessed the role of the degree of conversion of the materials in their toxicity, with conflicting results.

Significance. While the lack of methodological standardization among the studies still hinders the establishment of a relationship between type of dental adhesive and toxicity, studies employing dentin barrier systems indicate greater cytotoxicity for etch-and-rinse adhesives.

Contents

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Introduction

The increased demand for esthetics and minimally invasive tooth restorations resulted in a rapid development of adhesive dentistry. Such developments gave rise to different types of dental adhesives (DAs), which can be classified based on their bonding mechanism and clinical application approach into etch-and-rinse and self-etch adhesives [1]. DAs can also be categorized according to the mechanism of adhesion/clinical steps into single-step or two-step systems. The conventional two-step system employs conditioning with 37% phosphoric acid and subsequent application of the adhesive, which is provided in two bottles (primer + adhesive). The two-step self-etching system is composed of one bottle with acid monomers (acid primer), and another bottle with balanced concentrations of hydrophilic and hydrophobic monomers. Single-stage adhesives have the acidic primer with the adhesive resin of the two-step self-etching systems in one bottle, known as "all in one" [2].

As materials in close contact with biological tissues (i.e., teeth and oral soft tissues), either directly or indirectly, biocompatibility is one of the most critical requirements for dental adhesives [3]. It has been demonstrated that components of adhesives and restorative resins such as HEMA (2-hydroxyethyl methacrylate) and TEGDMA (triethylene glycol dimethacrylate), respectively, can diffuse through the dentinal tubules and reach the pulp tissue in concentrations considered toxic to the pulp cells [4]. In fact, the incomplete polymerization of DAs leaves a certain percentage of residual monomers [5]. The degree of conversion (DC) is the extent to which carbon double bonds (C=C) of resin monomers are converted into carbon single bonds (C—C) during the polymerization reaction of dental adhesives. In light-cured polymerization, this process is influenced by various parameters such as light source, viscosity and thickness of the adhesive layer, temperature, air inhibition, presence of solvents, concentrations, types and mixtures of photoinitiators, co-initiators, stabilizers and inhibitors, as well as types and proportions of monomers and fillers [6].

The release of resin monomers was proposed to be one of the possible causes of the adverse effects of dentin adhesives [7]. Wegehaupt et al. [6] found a higher degree of conversion in the conventional adhesives when compared to the self-etch ones, and associated this to the release of monomers and cytotoxicity of adhesives. The cytotoxicity of DAs may cause not only inflammation or necrosis of the oral mucosa but also allergic reactions [8] and would be of increased relevance when in contact with vital dentin, or in cases of accidents with gingival tissue [9]. Therefore, the in vitro evaluation of dental adhesives cytotoxicity becomes indispensable for safety assessments.

However, whether some groups of DAs are more prone to induce cytotoxicity than others is controversial, depending on the correlation between the etching system and the release of monomers [10]. Such debate might be increased by the fact that studies employ different methodologies, with some variables that may influence the outcome of cytotoxicity assays [11–14]. Therefore, aiming to shed some light on this theme, the present work performed a systematic review to identify the following focused question: are self-etching adhesives more cytotoxic than the etch-and-rinse systems?

Materials and methods

Protocol

This descriptive qualitative systematic review was performed based on the PRISMA statement [15] and the handbook from the Office of Health Assessment and Translation (OHAT – NIH) for in vitro toxicological studies.

Information sources and search strategy

The search process was performed under the guidance of a librarian. Specific search strategies were developed for each database and performed up to January 9th 2018, in different electronic databases; MEDLINE via PubMed, Cochrane Library, Scopus, Web of Science, and Latin American and Caribbean Health Sciences Literature database (LILACS)/Brazilian Library in Dentistry (BBO) (Table 1). No restrictions were placed on the publication date, while there was a limit in languages (English, Portuguese, French, and Spanish). Adaptations were made to adopt the same terms on the different search engines of the two other databases in combination with database-specific filters, where available.

This systematic review included studies according to the PICOS format [16], as follows: Population (P): pulp and gingival cells; Intervention (I): etch-and-rinse adhesives; Comparison (C): self-etch adhesives; Outcome (O): presence of increased cytotoxic effects; Setting (S): in vitro assays. The grey literature

Table 1 – Electronic database and search strategy. (09/01/2018).

PubMed		
#1 adhesives[MeSH Terms] OR adhesive*[Title/Abstract] OR adhesive system*[Title/Abstract] OR dental adhesive*[Title/Abstract] OR adhesive material*[Title/Abstract] OR etch-and-rinse adhesive*[Title/Abstract] OR total-etch adhesive*[Title/Abstract] OR dental bonding[MeSH Terms] OR bonding agent*[Title/Abstract] OR dental bonding agent*[Title/Abstract] OR dental bonding[Title/Abstract] OR light-curing of dental adhesives[MeSH Terms] OR light-curing of dental adhesives[Title/Abstract] OR Dentin-Bonding Agents[Mesh]	#2 (self-etch adhesive*[Title/Abstract] OR self-etching adhesive*[Title/Abstract] OR all-in-one adhesive*[Title/Abstract] OR one-bottle adhesive*[Title/Abstract]	#3 toxicity tests[MeSH Terms] OR cytotoxicity[Title/Abstract] OR cytocompatibility[Title/Abstract] OR toxicity test*[Title/Abstract] OR mutagenicity tests[MeSH Terms] OR genotoxicity test*[Title/Abstract] OR genotoxic effect*[Title/Abstract] OR genotoxicity[Title/Abstract] OR biocompatible materials[MeSH Terms] OR biomaterial*[Title/Abstract] OR cell culture techniques[MeSH Terms] OR cell culture*[Title/Abstract] OR cell survival[MeSH Terms] OR cell viabilit*[Title/Abstract]
#1 AND #2 AND #3		
Scopus		
#1 (TITLE-ABS-KEY ((adhesives OR “adhesive system” OR “dental adhesives” OR “adhesive material” OR “etch and rinse adhesive” OR “total etch adhesive” OR “bonding agent” OR “dental bonding agent” OR “dental bonding” OR “light curing dental adhesive”	#2 (TITLE-ABS-KEY ((“self etch adhesive” OR “self etching adhesive” OR “all in one adhesive” OR “one bottle adhesive”)))	#3 (TITLE-ABS-KEY ((cytotoxicity OR cytocompatibility OR “toxicity test” OR “genotoxicity test” OR “genotoxic effect” OR genotoxicity OR “biomaterial” OR “cell culture” OR “cell viabilit”)))
#1 AND #2 AND #3		
Web of Science		
#1 adhesives OR “adhesive system” OR “dental adhesives” OR “adhesive material” OR “etch and rinse adhesive” OR “total etch adhesive” OR “bonding agent” OR “dental bonding agent” OR “dental bonding” OR “light curing dental adhesive”	#2 “self etch adhesive” OR “self etching adhesive” OR “all in one adhesive” OR “one bottle adhesive”	#3 cytotoxicity OR cytocompatibility OR “toxicity test” OR “genotoxicity test” OR “genotoxic effect” OR genotoxicity OR “biomaterial” OR “cell culture” OR “cell viabilit”
#1 AND #2 AND #3		
LILACS and BBO		
#1 adhesives OR “adhesive system” OR “dental adhesives” OR “adhesive material” OR “etch and rinse adhesive” OR “total etch adhesive” OR “bonding agent” OR “dental bonding” OR “light curing dental adhesive”	#2 “self etch adhesive” OR “self etching adhesive” OR “all in one adhesive” OR “one bottle adhesive”	#3 cytotoxicity OR cytocompatibility OR “toxicity test” OR “genotoxicity test” OR “genotoxic effect” OR genotoxicity OR “biomaterial” OR “cell culture” OR “cell viabilit”
#1 AND #2 AND #3		
Cochrane Library		
#1 MeSH descriptor: [Adhesives] explode all trees #2 (adhesive* or adhesive system* or dental adhesive* or adhesive material* or etch-and-rinse adhesive* or total-etch adhesive*) #3 #1 or #2 #4 MeSH descriptor: [Dental Bonding] explode all trees #5 (bonding agent* or dental bonding agent* or dental bonding) #6 #4 or #5 #7 MeSH descriptor: [Light-Curing of Dental Adhesives] explode all trees #8 light-curing dental adhesives #9 #7 or #8 #10 #3 or #6 or #9	#11 (self-etch adhesive* or self-etching adhesive* or all-in-one adhesive* or one-bottle adhesive*) #12 MeSH descriptor: [Toxicity Tests] explode all trees #13 (cytotoxicity or cytocompatibility or toxicity test*) #14 #12 or #13 #15 MeSH descriptor: [Mutagenicity Tests] explode all trees #16 (genotoxicity test* or genotoxic effect* or genotoxicit) #17 #15 or #16 #18 MeSH descriptor: [Biocompatible Materials] explode all trees #19 biomaterial*	#20 #18 or #19 #21 MeSH descriptor: [Cell Culture Techniques] explode all trees #22 cell culture* #23 #21 or #22 #24 MeSH descriptor: [Cell Survival] explode all trees #25 cell viabilit*

(produced on all levels of government, academics, business and industry, in print and electronic formats, but not controlled by commercial publishers) was explored using the System for Information on Grey Literature in Europe (SIGLE) database. A hand search was performed in the reference lists of critical primary studies for additional relevant publications.

Study selection and data collection process

The studies were selected according to the eligibility criteria based on the PICOS strategy. Two aspects were considered for inclusion: (i) cytotoxicity tests and (ii) comparison between conventional and self-etch adhesives. The exclusion criteria were: clinical studies, case reports, review articles, retrospective studies, editorials, opinions, surveys, guidelines, conferences, commentary articles, and in vivo animal studies. Papers that evaluated adhesives without comparing the different generations were excluded.

All titles and abstracts of articles initially retrieved in the search were analyzed and selected by the eligibility criteria, and duplicates were eliminated. The titles and abstracts were read independently by two reviewers (M.Z.S and I.P.C.), and the articles that were compatible with the inclusion criteria were selected so that the full texts were examined to confirm their eligibility. Any disagreement on the eligibility of studies was solved through discussion and consensus, and in case of disagreement, a third reviewer (G.G.A.) decided whether the article should be included.

For data extraction, items of scientific and technical information were tabulated and analyzed with Microsoft Office Excel 2013, and two reviewers conducted the analyses independently. The data extracted included the study year of publication and author(s), adhesives used, types of cell lines, cell culture methodologies, parameter evaluated for cell viability, use of controls, and study outcomes.

Quality assessment

The ToxRTool (Toxicological data Reliability Assessment Tool) was developed within the context of an ECVAM funded project to provide comprehensive criteria and guidance for evaluations of the inherent quality of toxicological data [17]. In this review, the tool was used to assess the inherent quality or reliability at the methodological level. It consists of an 18-point rating checklist, which considers the description of methodological aspects of each study, such as identification of test substance and test system, study design, and documentation of results. Articles with less than 11 points are considered unreliable, studies with 11–14 points are reliable with possible restrictions, and studies with 15–18 points are considered reliable without restrictions.

Results

Selection of articles

The electronic screening returned 140 entries and, after removal of duplicated studies, a total of 18 works was evaluated for eligibility. After agreement among reviewers, the exclusion criteria were applied, and eight non-eligible articles were excluded, for the reasons indicated on the flow diagram (PRISMA format [15]) of Fig. 1.

Quality assessment according to the ToxRTool

The ten eligible articles were submitted to an assessment of the inherent quality of toxicological data, based on the ToxRTool, employing an 18-point rating for in vitro studies. As shown in Table 2, all studies were considered reliable without restrictions (scoring over 15 points) and thus, no study was removed from the review due to high risk of bias.

Main characteristics of the selected articles

Table 3 shows the ten selected studies and their main characteristics. Most of the selected studies used murine cell lines (7 out of 10 studies), while only four studies [6,10,18,19] used human pulp fibroblasts. One study compared results for both rat pulp cells and human fibroblasts [19].

All studies had a mean exposure time of 24 h, but two studies also assessed other experimental periods (48 and 72 h) [10,20]. Regarding the method of exposure, five studies [10,18,19,21,22] employed the production of extracts by the immersion of the specimens into the cell culture media, while five other studies [6,13,20,23,24] employed discs as dentin barriers, including dentin discs of bovine origin [6] (Fig. 2). The use of standardized methods, such as those preconized by the International Organization for Standardization (ISO), were cited in only four studies [6,10,13,20]. Most of the studies used the MTT cytotoxicity assay [10,13,18,21–24], while one study used the modified sulforhodamine B (SRB) assay [19], one used the lactate dehydrogenase assay (LDH) [6], and another assessed cytotoxicity through the fluorescent V-FITC/PI live-dead staining assay [20]. Regarding the use of controls, a single study simulated the extraction process by using a recognized biocompatible medical material (medical grade silicone) as a negative control [13], while all other studies used the unexposed medium as the control for cell survival. Very different substances were used as positive controls for cytotoxicity, such as ethanol [10], glass powder and polyacrylic acid compounds [13], hydrogen peroxide [22,23], primer [20] or even an etch-and-rinse adhesive [24].

According to Table 3, the studies reported different outcomes regarding the relationship between etching system and cytotoxicity, ranging from no significant difference between etch-and-rinse and self-etch adhesives [6,18,22], to higher cytotoxicity for either the conventional etch-and-rinse adhesives [13,19,20,24] or for the tested self-etch adhesives [10,21,23].

Discussion

The clinical relevance of the use of dentin adhesives has led to several modifications in their composition and presentation, culminating in different generations of DAs. Consequently, the need to evaluate the biocompatibility of each novel material has led to cell culture studies as a primary approach to safety before pre-clinical and clinical evaluations. The resulting lit-

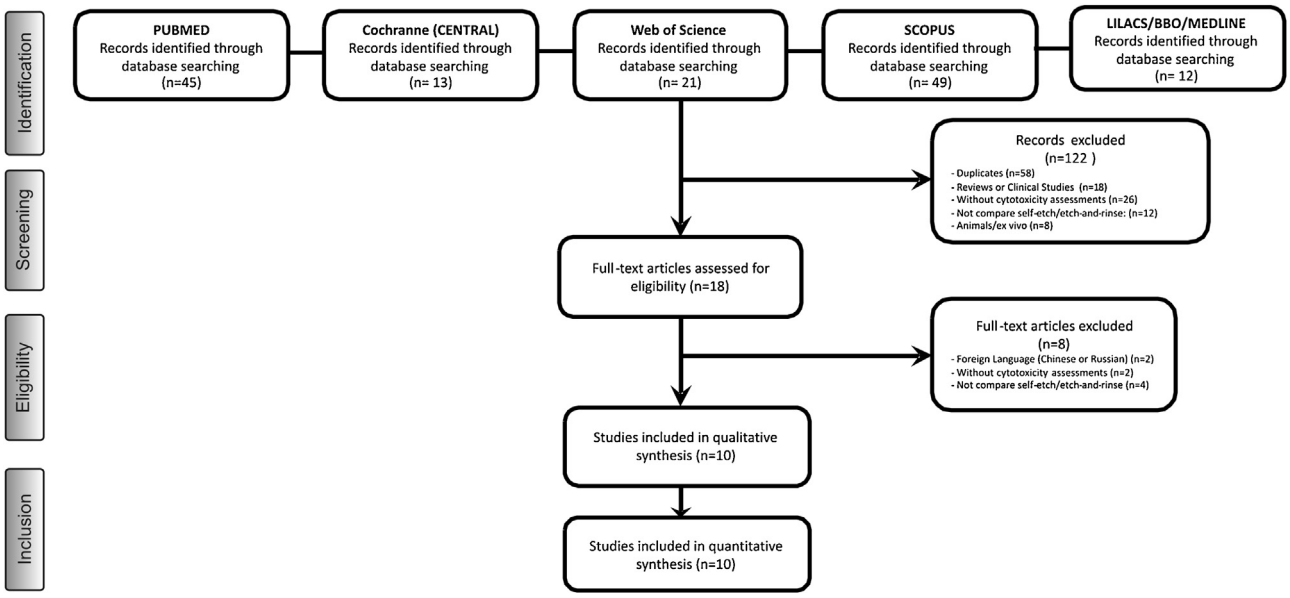

Fig. 1 – Flow diagram of the screening and selection process, according to the PRISMA Statement (15).

Table 2 – Quality assessment of the selected articles according to the ToxRTool in vitro criteria.

Author/year of publication	Group I: test substance identification (4)	Group II: test system characterization (3)	Group III: study design description (6)	Group IV: study results documentation (3)	Group V: plausibility of study design and data (2)	Total	Reliability categorisation
Almaroof et al., 2017 [10]	4	3	6	3	2	18	Reliable without restrictions
Jiang et al., 2017 [13]	4	3	6	3	2	18	Reliable without restrictions
Wegehaupt et al., 2016 [6]	3	3	6	3	2	17	Reliable without restrictions
Lee et al., 2016 [21]	4	3	5	3	2	17	Reliable without restrictions
Bianchi et al., 2016a [23]	4	3	6	3	2	18	Reliable without restrictions
Bianchi et al., 2016b [22]	4	3	5	3	2	17	Reliable without restrictions
Tuncer et al., 2012 [20]	4	3	6	3	2	18	Reliable without restrictions
Cavalcanti et al., 2010 [18]	3	3	5	3	2	16	Reliable without restrictions
Koulaouzidou et al., 2009 [19]	3	3	5	3	1	15	Reliable without restrictions
Lanza et al., 2009 [24]	3	3	6	3	2	17	Reliable without restrictions

erature has the potential to provide an insight on the impact of such modifications in the biocompatibility of dental adhesives. In this context, the present literature search identified studies assessing and comparing the cytotoxicity of members of two main groups of adhesives: etch-and-rinse and self-etch DAs.

By employing distinct methodologies of exposure and cell viability evaluation, rather different outcomes were observed in the comparisons of etch-and-rinse and self-etch DA's cytotoxicity. Cytotoxicity was often evaluated using classical methods, such as the reduction of tetrazolium salts (MTT and XTT). This preferred use of MTT probably reflects the fact that this method is widely described in the literature and recommended by international standards such as the ISO 10993 series [25]. Nevertheless, toxicologists have established new paradigms of product safety analysis involving the assessment of other parameters that contribute to the understanding of the mechanisms involved in the toxicity pathways, more than providing evidence for cell death [26]. Although these paradigms and related methodologies (such as proteomics, genomics, and pathway analyses) do not yet permeate the research on dental materials, several parameters were often used in many of the selected studies to support the observation of toxicity by single endpoints. These

Table 3 – Main characteristics of the selected articles.

Author/year of publication	Total-etch adhesives tested	Self-etch adhesives tested	Cell type	Method of exposure	Cytotoxicity tests performed	Study controls	Other parameters assessed	Outcome
Almaroof et al., 2017 [10]	Scotchbond-multipurpose (SBMP) + modified SBMP	Clearfil universal bond (CUB) + modified CUB	Primary human gingival fibroblasts	Resin disc samples in 3 mL of fibroblast medium 24 and 48 h exposure	MTT assay (ISO 10993-5:2009)	Negative: Cell + media Posivitve:10% ethanol	Degree of conversion (DC), curing temperature, thermal properties, contact angle, water sorption and solubility, push-out, antibacterial assay.	All adhesives showed acceptable biocompatibility. The CUB group exhibited lower survival (80%).
Jiang et al., 2017 [13]	GLUMA bond 5	GLUMA self etch + single bond universal	L929 cell cultures Three-dimensional (3D) culture	Human dentin disks in a dentin barrier test. 24 h exposure	MTT assay (ISO 7405: 2008)	Negative: medical-grade silicone Positive: powder: glass powder, polyacrylic acid, diphenyliodo-nium chloride Liquid: cam-phorquinone, ethyl-4-dimethylaminobenzoate, HEMA, water		Etch-and-rinse adhesive produced significant cytotoxicity in deep cavities.
Wegehaupt et al., 2016 [6]	OptiBond TM FL (Kerr Corporation, Orange, United States)	OptiBond All-In-One (Kerr Corporation, Orange, United States),	Dental pulp cells and gingival fibroblast primary cultures	Dentine discs from bovine incisors Dentine discs were covered with 3 ml cell culture medium 24 h exposure	Lactate dehydrogenase (LDH) (ISO 10993-5:2009)	Control: untreated group	DC	No significantly increased cytotoxicity compared with the control group OptiBond™ FL showed a higher degree of conversion than OptiBond® All-In-One
Lee et al., 2016 [21]	Adper Single bond 2 (SB).	Adper easy bond (EB), Xeno V (XV), iBond (IB), AdheSE One (AO), Clearfil SE primer (CS)	Mouse odontoblast cell line (MDPC-23)	The adhesives were diluted with the cell culture medium to 0.5% 24 h exposure	MTT assay	Control: cell culture	-Ph determination, Flow Cytometric Apoptosis Assay, SEM.	All the one-step self-etching adhesives tested showed increases in apoptotic activity.
Bianchi et al., 2013a [23]	3-Step etch-and-rinse adhesive systems (R1), simplified etch-and-rinse adhesive (R2)	Simplified self-etch adhesive R3 and R4	Mouse odontoblast cell line (MDPC-23)	Dentin discs in vitro pulp chambers 24 h exposure	MTT assay	Negative: no adhesive Positive: 29%hydrogen peroxide	- SEM, Cytometric assay, DC	R3 presented significantly higher Succinic dehydrogenase (SDH) production than R1, which was not significantly different from control.

Bianchi et al., 2013b [22]	Three-step etch-and-rinse adhesive or two-step self-etch systems. (R1 and R2), simplified etch-and-rinse adhesive (R3)	Simplified self-etch adhesive (R4 and R5)	Mouse odontoblast cell line (MDPC-23)	Sterile filter paper discs were impregnated of each adhesive. All discs were placed in culture medium 24 h exposure	MTT assay	Negative: no adhesive Positive: 29%hydrogen peroxide	- Total protein (TP) production, alkaline phosphatase (ALP) Activity, Cytometric assay, *scanning electron microscope* (SEM), DC	R5 was the most cytotoxic adhesive. R2 and R3 were not toxic.
Tuncer et al., 2012 [20]	Scotchbond Multi-Purpose and XP Bond	Xeno V, Clearfil Protect Bond, and AdheSE	3T3 mouse fibroblast cell line	The adhesives were exposed to cells, and were analyzed in a dentin barrier test 24 and 72 h exposure	Live-dead fluorescence staining assay.	Positive control: the primers Negative control: cell culture inserts without test materials	- SEM, apoptosis, cell proliferation.	Etch-and-rinse dentin adhesives were more cytotoxic than self-etch adhesives.
Cavalcanti et al., 2010 [18]	Single bond (SB)	Clearfill Protect Bond primer (CP) or Clearfill Protect Bond resin (CB)	Human pulp fibroblasts (FP5 cell line)	media containing the substances leached or dissolved from the test materials (of 0.2 g of materials per mL of culture medium) 24 h exposure	MTT assay	- Control: fresh cell culture medium	- Graph shows only the total number of cells	Single Bond and Clearfill Protect Bond primer release substances that decrease cell viability of human dental pulp cells in culture.
Koulaouzidou et al., 2009 [19]	Admira bond (VOCO), Gluma Comfort Bond	Clearfil Liner Bond 2 V, ED Primer II (Panavia F2.O), NanoBond	rat pulp cells (RPC-C2A) and human lung fibroblasts (MRC5)	Test materials were prepared and were placed in culture medium (100 μL of each adhesive was applied in 10 ml sterile Vials) 24 h exposure	modified sulforhodamine B (SRB) assay	- Negative Control: 100 μL of DMEM		In both cell lines, the most toxic effects were observed with etch-and-rinse adhesives

– Table 3 (Continued)

Author/year of publication	Total-etch adhesives tested	Self-etch adhesives tested	Cell type	Method of exposure	Cytotoxicity tests performed	Study controls	Other parameters assessed	Outcome
Lanza et al., 2009 [24]	Single bond (SB)	Clearfil SE Bond (CSE), Clearfil Protect Bond (CPB), Adper Prompt (PR), and Xeno III (XE)	Mouse odontoblast cell line (MDPC-23)	dentin disks were produced from human molars 24 h exposure	MTT assay	- Positive Control: Single Bond (SB) - Negative Control: phosphate buffer solution (PBS).	- Morphological analysis by electron microscopy scanning SEM, mass spectrometry.	One step total-etch was the most aggressive

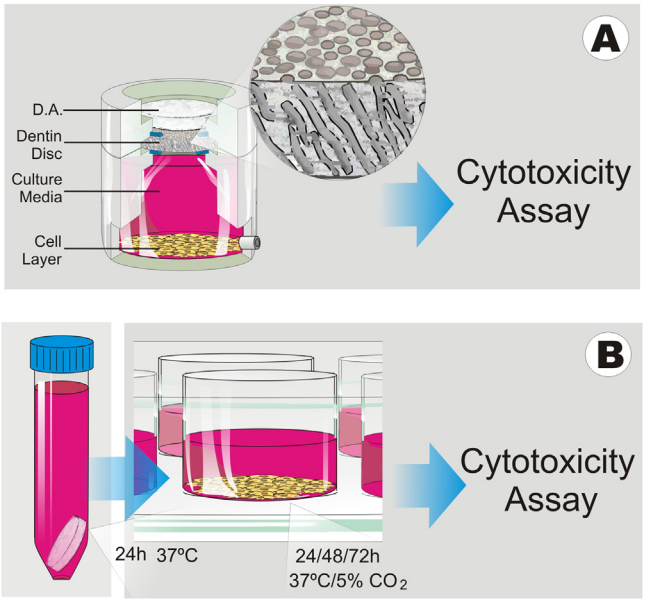

Fig. 2 – Methodological approaches for exposure of cells to the dental adhesives (DAs) during in vitro cytotoxicity testing. (A) Dentin barrier systems employ human or animal (porcine or bovine) dentin disks of different thickness separating cells from the test material. Therefore, released toxicants such as monomers from incomplete conversion should diffuse and transverse the dentinal tubules in order to affect cells. Such systems may employ both mono-laminar and tri-dimensional cell culture, and the flow of culture media may be both inward and outward, simulating pulp chamber fluid pressure. (B) The most common method of exposure is the indirect approach, where the specimen (test material) is immersed on extraction media (usually cell culture medium), which is conditioned by incubation at 37 °C for different times (often 24–72 h) with or without mixing. Cells sub-cultured in multi-well plates are subsequently exposed to such conditioned media, when possible released toxicants may act on cell viability.

include the observation of cell morphology through scanning electron microscopy [20–24], the investigation of necrosis or apoptosis pathways [20,21], or the evaluation of cell cycle effects through flow cytometry [21–23]. Nevertheless, while these assays indeed help to identify the main outcomes of the selected studies, their limited use indicates a clear need to invest in more advanced methods for safety evaluation of dental adhesives.

One important observation of this literature search is the low adherence to methodological standardization among studies. Of the ten articles selected, only four [6,10,13,20] declared the use of standardized methods such as those preconized by the International Standards Organization. The relevance of methodology standardization to evaluate the quality of biotechnological products and processes aimed at bioengineering and medical/dental biomaterials has been increasingly recognized in the scientific community [27]. Considering the importance of risk assessment of materials in

close contact with human tissues, the adherence to international standards [28] may fulfil other roles than just providing the basis for traceability in the regulatory approval of products. Standardization may also contribute to the harmonization of results, allowing easier comparisons of studies, and helping to reduce confusion and conflicting results in science [29].

The cell types used in the selected studies, odontoblasts or fibroblasts (mainly from gingiva or pulp), reflected the main tissues exposed to DAs. The assessment of cytotoxicity in both cell types is very important, as these tissues (gingiva and dental pulp) might respond in different ways when challenged with dental materials, and their ways of exposure are clinically different, due to the presence or not of hard dental tissues [6]. It is worth mentioning that among the selected papers, eight used immortalized cell lines [10,13,19–24], while only two [6,18] used primary cells. There is a wide variety of culture techniques for the study of pulp behavior. Non-immortalized human pulp cells, which have not undergone any genetic alteration, might be the most indicated study model since it maintains the characteristics of the original tissue [14]. Only three studies [6,10,18] of this review used human cells, and one [10] found a significant difference between the adhesives tested, with higher cytotoxicity reported for self-etch adhesives.

Another change of paradigm proposed for the cytotoxicity testing in the 21st century is the gradual substitution of the two-dimensional (2D) monolaminar cell culture by micro-tissue three-dimensional (3D) models [30]. Three-D models better mimic the tissue microenvironment since cellular communication occurs through factors concentrated in the micro-tissue interstitial fluid (while in 2D models these factors are usually diluted in culture media), or through the highly increased cell-cell or extracellular matrix-cell interactions [11]. Among the studies included in this review, only one [13] used a 3D culture model, with a polystyrene scaffold with four fiber layers, where cells were cultured inside a simulated pulp chamber. The fact that this is one of the most recent papers in the review may be an indication that these paradigms are slowly approaching dentistry research.

Regardless of the test system (cell model) employed, one important factor related to the toxicity of DAs and other dental materials is their leachability of toxic components. Considering composite materials, the incomplete conversion of the polymer matrix may favor chemical instability and release of toxic agents into the biological media [6]. In this sense, the relationship between the cytotoxicity of the tested DAs and their degree of conversion (DC) has been measured in four selected studies [6,10,22,23] of the present review. Unfortunately, no consensus was found regarding their main outcome and the results from DC. While Bianchi et al. [23] and Wegehaupt et al. [6] confirmed the expected pattern of higher toxicity for adhesives with lower DC, the studies of Almaroof et al. [10] and Bianchi et al. [22] failed to confirm such relationship. Furthermore, no relationship was identified between DC and differences between self-etch and etch-and-rinse materials in the selected studies.

While the data of the selected articles altogether did not reinforce a direct relationship between degree of conversion and cytotoxicity of the different groups of DAs, some patterns may be observed when considering the permeability of toxic agents and the in vitro method of exposure (Table 4). In the works that used the dentin barrier method [13,20,24], the etch-and-rinse adhesives were identified as the most cytotoxic. Methods using dentin as a barrier (Fig. 2A) are intended as an improvement of safety assessments of dental materials by helping to understand how the characteristics of the dentin substrate can affect the molecular diffusion of toxic agents to the pulp tissue [24]. Therefore, such methodological difference may explain the different results reported by studies that employed the direct exposure to extracts of the adhesives in the culture medium (Fig. 2B) [10,21,23], which found greater cytotoxicity for the self-etch adhesives. Regarding the use of dentin barrier, the difference in toxic behavior between the adhesive systems may be attributed to the fact that etch-and-rinse systems involve the use of phosphoric acid, which completely removes the smear layer and smear plugs while enlarging the lumen of the dentinal tubules by the dissolution of the peritubular dentin. Therefore, they may favor the transdentinal diffusion of various substances, including toxic compounds [31]. It is important to observe that, while the principle of the dentin-barrier test may represent a better simulation of the clinical application of dental restorative materials, as compared to the traditional in vitro exposure methods, this approach also requires careful consideration. The standardization of human dentin permeability is necessary for the dentin barrier test to produce consistent results [24], since dentin has a complex structure and can yield results with significant heterogeneity that may affect the cytotoxic levels of the tested material [13]. In fact, dentin permeability strongly varies from individual to individual, as well as from different portions of the same tooth, mostly due to differences in the structure of dentinal tubules. Therefore, permeability and cytotoxicity tests should not limit the dentin area to small portions that do not represent the average cavity size or dentin permeability observed in clinical settings [13].

This review aimed to search the scientific literature and identify patterns that could contribute to evidence-based dentistry regarding the choice for the safest dental adhesive system. Among the study limitations, the search strategy including only articles comparing both groups in the same test, which was necessary to enable the evaluation of experimental data, led to a quite limited number of works considering the available literature on DAs. The overall variety of methodological parameters also impaired the performance of more profound comparisons, such as a meta-analysis, or even the establishment of strong correlations by qualitative evaluation of the results. Also, it is possibly a source of confusion and conflicting results, such as those regarding the biocompatibility of the different presentations of the Clearfill, largely studied as the first self-etch adhesive in the market, which was identified as both more cytotoxic [18,21] and more biocompatible [19,20,24] than the tested etch-and-rinse materials. The difficulty in observing the relationship between DAs cytotoxicity and the degree of conversion might also be a result of this methodological variability and the fact that only 40% of the studies investigated this important parameter. Nevertheless, this review identified evidence that the mode of exposure of the material to the cells influences the outcome, and the dentin barrier with all its advantages seems to be promising to promote reliable studies. However, it is also very clear that

Table 4 – Relationship between the method of exposure and main outcome of the selected studies.

	Main Outcome		
Method of exposure	Self-etch DAs as more toxic	Etch-and-rinse DAs as more toxic	Similar toxicity
Studies employing dentin barrier tests	–	Jiang et al. [13] Tuncer et al. [20] Lanza et al. [24]	–
Studies employing direct exposure to extracts	Almaroof et al. [10] Lee et al. [21] Bianchi et al. [22]	Koulaouzidou et al. [19]	Wegehaupt et al. [6] Bianchi et al. [23] Cavalcanti et al. [18]

the standardization of exposure protocols and the assessment of both the leakage of toxicants and advanced toxicity parameters are needed to better understand the safety of both dental adhesives and dental materials in general.

Conclusions

While the lack of methodological standardization among studies comparing self-etch and etch-and-rinse DAs hinders the establishment of a relationship between the type of dental adhesive and toxicity, studies employing dentin barriers indicate greater cytotoxicity for etch-and-rinse adhesives.

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