Available online at www.sciencerepository.org

Science Repository

Research Article

Long-term dental adhesive toxicity on human gingival fibroblasts and epithelial cells

Costa A.R[1], Banzi E.C.F[1], Puppin-Rontani R.M[1], Tipton DA[2], Correr-Sobrinho L[1,3], Garcia-Godoy F[2] and Babu J.P[2*]

[1]Department of Restorative Dentistry, Dental Materials Division, Piracicaba Dental School, State University of Campinas-UNICAMP, Piracicaba, SP, Brazil

[2]Department of Bioscience Research Center, and Craniofacial Research center, College of Dentistry, University of Tennessee Health Science Center, Memphis, TN, USA

[3]Department of Restorative Dentistry, Dental Materials Division, Piracicaba Dental School, State University of Campinas-UNICAMP, Piracicaba, SP, Brazil

ARTICLEINFO

Article history:
Received: 17 April, 2019
Accepted: 30 April, 2019
Published: 12 June, 2019

Keywords:
Cytotoxicity
adhesive systems
human gingival epithelial cells
human gingival fibroblast cells
MTT assay
live/dead assay

ABSTRACT

Dentin adhesive system (DAS) components released can induce cytotoxic effects. We hypothesize that media obtained from long-term stored DAS are cytotoxic, depending on the DBS type/composition and modulation of cytotoxicity is time dependent. 0.2 mL DMEM media obtained from incubating DAS discs were filter-sterilized, added to the cultured cells (human gingival fibroblast and epithelial cells) for 24 hours and harvested up to 50 days. Cell toxicity was assessed using MTT cell viability assay and LIVE/DEAD backlight staining. PB was cytotoxic to both cell types only from 1h to 3 days. SE was non-toxic up to 1h, but it was up to 50th day, when the toxicity ceased. The SB was slightly more toxic than Scotch, but by day 45 no significant toxicity to the cells was observed. We concluded that all DAS tested were toxic for the cells analyzed, and cytotoxicity was composition/time dependent.

Introduction

Dental adhesive systems (DAS) were developed to enhance the bonding of resin-tooth interface, seal restoration, prevention of microleakage, secondary caries, and inflammatory pulpal response [1]. For the etch&rinse DAS, acid completely removes the smear layer, resulting in a significant permeability increase and releasing of uncured components and by-products, which may diffuse into dentinal tubules towards the pulp and cause potential damage to [2, 3]. On the other hand, self-etching agents are mainly suggested for deep cavity and permeable dentin, as they usually leave residual smear plug in the tubules, thus limiting the entrance of uncured resin monomers [2]. The incorporation of the antibacterial monomer 12-methacryloyloxydodecyl-pyridinium bromide (MDPB) containing Clearfil Protect SE to the self-etching agent would prevent bacterial infections at the bonding region. Since then, several studies have reported the MDPB effective bactericidal activity [4, 5]. There are many underlying reasons that could be attributed to the differences in cytotoxicity of comprehensive DAS, such as chemical composition and the type and quantity of leachable components, the time and types of light curing and the different methodologies used [3, 6-15].

Monomers like Bis-GMA, HEMA, UDMA, TEGDMA, initiators, organic solvents, water, and inorganic fillers are used as base substances in DAS composition (Table 1). Uncured and/or leachable monomers, and canphoroquinone (CQ), showed cytotoxic effects on experimental animals and on various types of human cells and oral tissues-derived [1, 10, 12, 13, 15-27]. Ratanasathien et al (1995) showed that cytotoxicity on fibroblasts depends on the monomer evolved (Bis-GMA> UDMA>TEGDMA> HEMA). However, the cytotoxicity effect was not only influenced by the individual component, but also by the synergistic or antagonistic interaction between them [7]. Although, some in vitro and in vivo studies have evaluated the cytotoxic effects of DAS on different cells, using different methodologies [20, 21, 23, 24, 27-30], but the results poorly contributed to a significant data correlation, since the same material tested in different assays yielded controversial results [31]. Currently, no study has shown the modulation of a DAS toxicity effects over an extended period of time. So, the hypotheses of this study

**Correspondence to: Jegdish P. Babu, Department of Bioscience Research Center, and Craniofacial Research center, College of Dentistry, University of Tennessee Health Science Center, Memphis, TN, 38163, USA; E-mail: jbabu@uthsc.edu*

http://dx.doi.org/10.31487/j.DOBCR.2019.03.01

are: 1. Photo-cured DBS are toxic to gingival fibroblast and epithelial cells, depending on the DBS type/composition in long-term evaluation; 2. Modulation of cytotoxicity is time dependent.

Materials & Methods

Preparation of adhesive system discs in conditioned media

Four DAS were used in this study (Table 1): self-etching [Clearfil SE Bond (Kuraray), Clearfil SE Protect (Kuraray), and Adper Scotch Bond SE (3M ESPE)], and an etch&rinse – [Adper Single Bond Plus (3M ESPE)]. DAS discs (6 mm diameter x 2 mm thickness) were prepared inserting 30µL of the material in a mold, and light-cured using a halogen-based light unit for 10sec (VIP light, BISCO). The discs were immersed in 1.0mL of sterile Dulbecco's Modified Eagle Medium (DMEM; Gibco; Grand Island NY), supplemented with 10% (v/v) newborn calf serum (Gibco) and 100g/mL gentamicin (Sigma-Aldrich; St. Louis, MO). They were incubated at 37°C for 1h, and 1-50 days. At each of tested interval, the media was removed and filter-sterilized using 0.22-µm size.

Cell cultures

I Epithelial Cells

Human gingival epithelial cells (S-G) were obtained from F.H. Kasten, East Tennessee State University, Quillen College of Medicine, Johnson City, TN. The cells were grown in DMEM; Gibco, supplemented with 10% (v/v) newborn calf serum (Gibco) and 100g/mL gentamicin (Sigma-Aldrich; St. Louis, MO) (termed complete growth medium) at 37ºC in a humidified atmosphere of 5% CO_2 in air. Cells were tested between passages 30-36th passages.

II Fibroblasts

Human gingival fibroblast cells line previously established in our laboratory at the University of Tennessee Health Science Center, Memphis, TN were used. The cells were routinely cultured as described for epithelial cells. Cells were tested between passages 10-15.

Treatment of cells with adhesive system discs

Human gingival epithelial or fibroblasts were trypsinized, washed with sterile PBS (Phosphatase Buffer Saline; Life technologies), resuspended to a concentration of $2x10^4$ cells/mL, and then seeded in 96-well culture plates (Costar/Corning) containing 0.5mL of complete growth medium. The cells were incubated for 24h at 37ºC in 5% CO_2, and 95% relative humidity. Then, the medium was removed from the wells; cells were washed once with 200µL sterile PBS, and then re suspended in 25µL of complete growth media along with 50µL of the DAS media (prepared as previously described). Cells were incubated for another 24h.

Cell viability assay

Effects of the toxicity of the testing DAS on cell viability were determined by MTT assay kit (Boehringer Mannhein Corp.,Indianapolis, IN, U.S.A). Briefly, the MTT (3-[4,5dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) was added to the cells at a final concentration of 0.5mg/mL and incubated for 4h at 37ºC. Purple formazan crystals produced from the MTT by metabolically active cells, were solubilized through overnight incubation with solubilization solution provided in the kit, at 37ºC. Aliquots of 150µL were removed of it and transferred to another 96-well plate, and then the absorbance was read at 570nm using an ELISA microtiter plate reader (BMG spectrostar spectrophotometer, BMG labs). Each experimental assay was repeated six times; all assays were performed in triplicate for each evaluated sample. Results were expressed as percentage (%) control (A_{540nm} in cells exposed to DMEM-agent only) by the following formula: Cell viability (%) =100x (OD_{mean}test group/OD_{mean}Control group).

Live/Dead Cells Viability Assay

Cytotoxicity of cultured epithelial cells following the exposure to DAS was measured using the LIVE/DEAD mammalian cell viability assay kit (Molecular Probes-Invitrogen, Carlsbad, CA). Fibroblasts and Epithelial cells ($2x10^4$ cells) were incubated with testing media or control media for 24h. Briefly, cells were rinsed once with and then stained with the fluorescent dye mixture prepared according to the manufacturer's kit instructions (a mixture of two dyes; SYTO 9 and Propidium iodide), for 15 minutes, and then washed with saline solution (PBS). The combination of two dyes distinguishes live cells from the dead ones, based on membrane integrity. The green fluorochrome (SYTO 9) can penetrate intact membranes, while the larger red fluorochrome (propidium iodide-P.I.) penetrates only compromised membranes of dead cells, resulting in red fluorescence by binding to the nuclear material (nucleic acid) of the cells. A standard curve was set up using known ratio of live (green) to dead (red) cells to facilitate the calculation of live/dead cells in the experiment. The florescence emissions were determined at 530±12.5nm and 645±20nm using the fluorescent reader (Spectra Max, Molecular Devices).

Statistical analysis

The statistical analyses of the data were performed by Kruskall-Wallis and Mann Whitney U with Bonferroni correction tests (α=0.05) tests, using SPSS 10.0 software (SPSS, Chicago, IL, USA). Statistical significance was determined at $p < 0.05$.

Results

Cytotoxic effect of DAS on human gingival fibroblasts and epithelial cells over time

Similar results were found for both cells type studied. For both cells types the four DAS evaluated in this study significantly differed (Figure 1), when analyzed by MTT viability assay. About 78% and from 76 to 92%, respectively, of the fibroblast and epithelial cells were viable after 60 minutes incubation in SE, SB and Scotch discs-conditioned media. However, the PB conditioned media collected from 1rst- to 3rd day showed significant high toxicity for both cells types (0% viable cells). The toxicity significantly diminished from 1week to 50, as the viability of cells increased from 80 to 83% during that period of time ($p<0.05$) on fibroblasts and about 96% for epithelial cells ($p<0.05$). Interestingly, for SE, although the highest cells viability was found on the 1h incubation, after 24h, no viability cells (fibroblasts and epithelial cells) were found, until day 30. About 65% of cells were viable again when they were

incubated with 40 to 50 days-old SE-conditioned media. Conditioned media obtained from SB and Scotch DAS exhibited similar degree of toxicity to fibroblasts (p>0.05). Media obtained from SB discs continued to exhibit toxicity to the fibroblasts and epithelial cells, for a period up to 50 days, as only 46-63% and 40-75%, respectively, of the cells were kept alive. The Scotch-conditioned media was somehow significantly more toxic to fibroblasts and epithelial cells than SB-conditioned media. The PB-conditioned media exhibited toxicity to the epithelial cells for one to three days; after that, MTT assay showed 82-96% of the cell viability for fibroblasts and epithelial cells. Overall, these results demonstrate a clear difference between the tested DAS toxicity to the cultured cells.

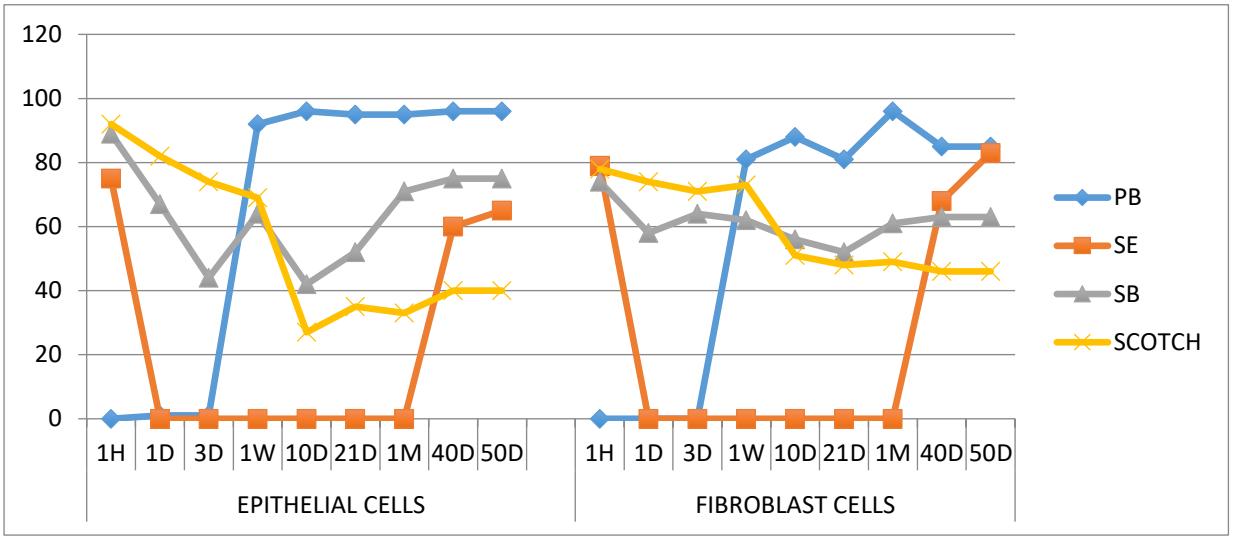

Figure 1: Cytotoxicity of adhesive systems on human epithelial and gingival fibroblast cells by MTT assay, over extended period of time.

Interestingly results can be observed when figure 1 was plotted in three main range of time (Figure 2). For both cell types, the rank order of toxicity was observed: In the first range period (1h-3days) PB>SE>SB>SB; for the second range (1week-21dys) SE>Scotch=SB>PB; and, in the last range period (1month-50days) Scotch=SE>SB>PB.

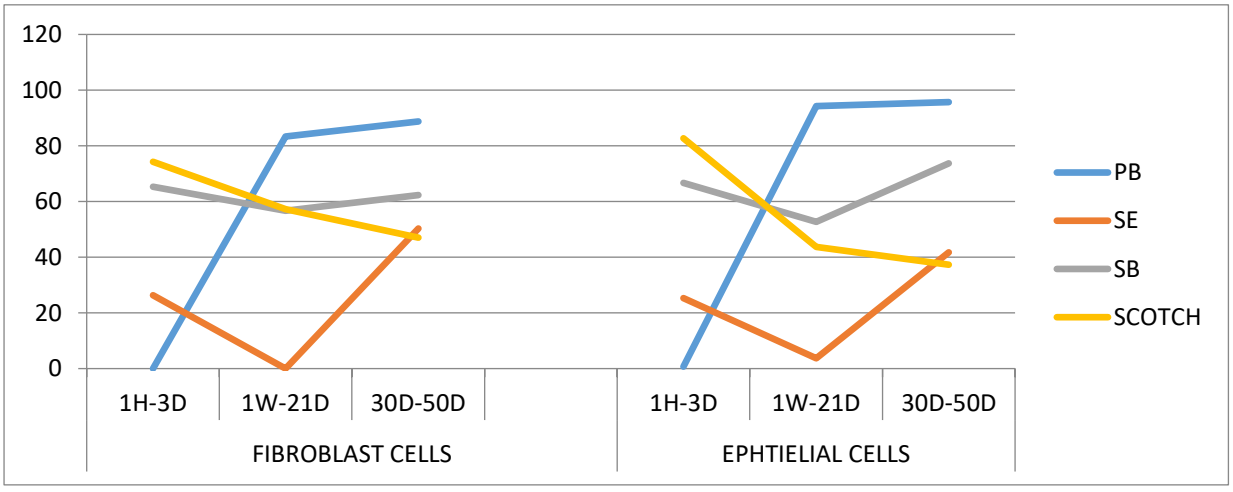

Figure 2: Cytotoxicity of adhesive systems on cultured fibroblast and epithelial cells at three ranges of time period (1h – 3 days; 1 week – 21 days; and, 1 month – 50 days), by MTT assay.

DAS effects on gingival fibroblasts and epithelial cells by Live/Dead assay

The results demonstrate that 75 to 79 % of the cells were still alive after incubation with 1-hour SE-conditioned media (Figure 3). Media collected from day 1 onwards exhibited toxicity up to day 30, with about 56% of viable fibroblasts, and only 10% of viable epithelial cells. Approximately, 71-78% of these cells were alive when incubated with 50 days-old SE-conditioned media. The PB-conditioned media from 1 hour and 1-day exposure were found to be very toxic to the cells as there were no live cells detected by the assay. Similar results were obtained from MTT assay. 78-80% of the cells were alive after treatment with 7-days-old PB cured media. The SB- and Scotch Bond-conditioned media did not totally kill the cells, but the percentage of live cells were slightly higher when incubated with 1 hour and 1-day-old cured media. However, cells treated with media obtained from 30-days and 50-days-old SB- and Scotch-cured discs showed significantly lower numbers of live cells when compared to the cells incubated with 1 hour to 7-days-old media. Overall, all these results were in agreement with the MTT cell viability methodology also used in this study.

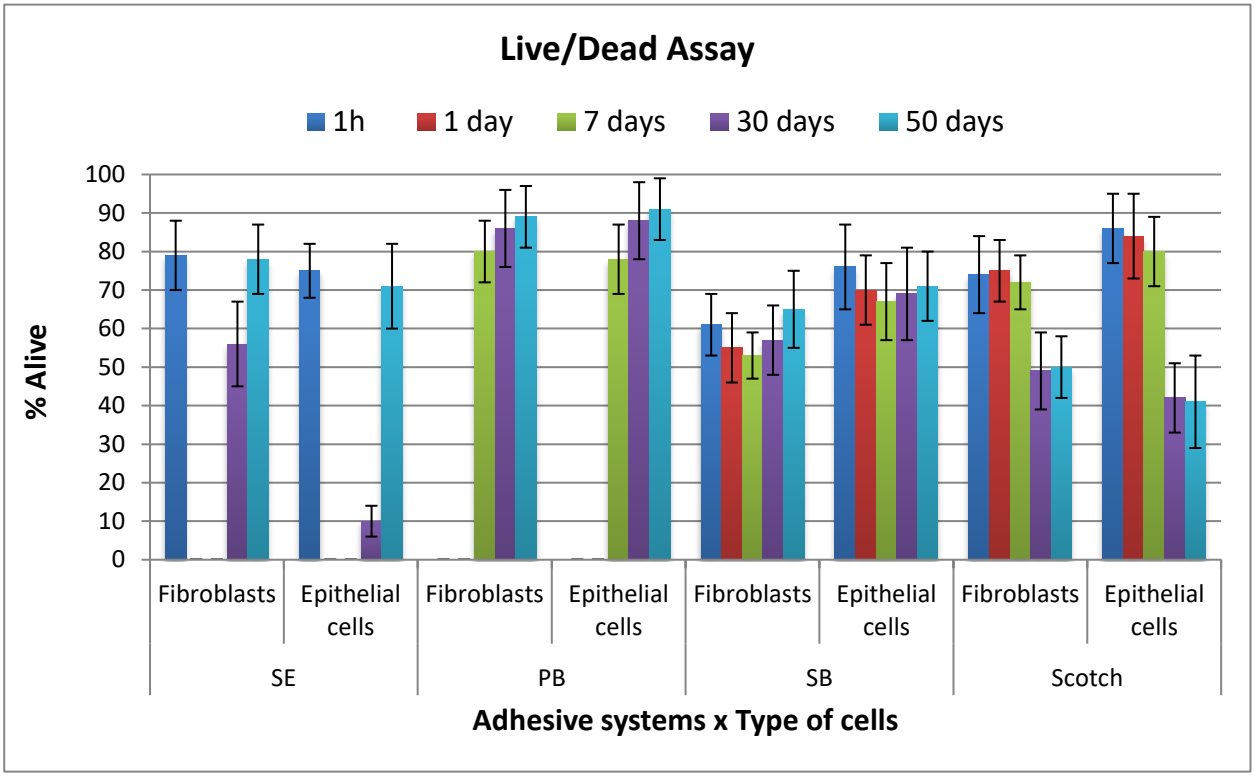

Figure 3: Effect of adhesive systems-conditioned media on both fibroblast and epithelial cells, by Live/Dead assay.

Discussion

Cell culturing methods are relevant and suitable for the evaluation of basic biological properties of dental materials. These methods are standardized and reproducible, making them quick and easy to be performed at relatively low cost [17, 32]. Furthermore, *in vitro* experiments have the advantage of easy-controlling experimental variables, which is often a problem for *in vivo* experiments [17]. Particularly, established cells were used in this experiment due to its easier maintenance and greater reproducibility in culture, compared to primary cells. Over the years, several *in vitro* and *in vivo* models have been used to test the cytotoxicity of adhesive bonding systems but results of such tests are not always suitable for comparison, even if offering some valuable information. Franz Alexander et al. (2009) demonstrated that data of cytotoxicity on culture cells are highly model-dependent, which indicates that the methodologies applied may be a major variable for these *in vitro* assays.

Adhesive systems are frequently used in deep cavities, which have less dentin thickness and higher permeability (more dentin tubules) or as pulp capping material. They can cause pulpal irritation, because of their components, such as monomers, solvents, acids and/or CQ, which may pass through dentinal pulp and reach the pulp tissue, as observed in previous *in vivo* studies [16,18–24]. The main goal of our study was to evaluate the toxicity of four polymerized adhesive systems by comparing conditioned media prepared from its discs at a extended time period, and also to determine the timing required for neutralization of the cytotoxic effects on cultured gingival fibroblasts and epithelial cells. Previous studies have used only short period of time to evaluate cytotoxicity of these systems, and not at extended periods as we demonstrated in this study. In addition, there are not reports in the literature showing the time required for an adhesive system to lose its toxic effects. This is a very important factor to be considered, since the toxic components should affect pulp cells as long as they are in contact with them.

Our data showed that PB was toxic to the human gingival cells up to seven days. From this point on, the cytotoxicity of PB decreased over time, showing, then 81% or more of viability for both cell types (Figure 1). The four adhesive systems evaluated differed significantly in their cytotoxic properties to the cultured established cells (Figure 2) at three ranges of time period considered in this study. Among the first range of time period (1h-3days), PB showed the highest cytotoxicity (lowest cell viability), but after that, for the other two range periods (1 week-21 days and 1month-50 days) PB appeared to be the lowest toxicity. Altogether, these findings could be associated with the chemical composition of this adhesive system, which, according to the (Table 1), contains different ingredients, including toxic monomers such as Bis-GMA and HEMA, MDPB, Fluoride, and Camphoroquinine (CQ) [4, 7, 10, 12, 13, 26, 27, 33].

It is known that the uncured and/or leachable resin monomers released from resin dental materials during the monomer-polymer conversion may be directly responsible, and a major reason, for cytotoxicity of the materials [6]. Moreover, the degree of this conversion of the resin composition and adhesive systems is not always complete. Thus suggesting, that resin monomers depending on the degree of polymerization will be as much cytotoxic as lowest polymerized resin-based materials are release, allowing the toxic substances to penetrate through the dentin and pulp. Importantly, leaching of monomers occurs not only during the setting period (degree of conversion or oligomer

polymerization), but also later when the material becomes degraded [33, 34].

In a recent study, El-Kholany et al. (2012) showed that the MDPB (12-methacryloyl-oxy-dodecyl pyridinium bromide) monomer might also modulated the toxicity of the adhesive system Clearfil Protect Bond, which showed the strongest cytotoxic effect on odontoblast cells during 24h, 48h and 72h incubation. On the other hand, as stated by Imazato et al. (1999), the cytotoxic potential of MDPB was considered to be low or similar to other monomers used for dental materials. Also, Demirci et al. (2008) attributed the cytotoxic effects of self-etching primers much more to the HEMA than to the MDPB. According to their findings, they could not conclude whether the MDPB monomer was potentiating the cytotoxicity, since this monomer had not increased the cytotoxic effects of Clearfil Protect Bond primer, when compared to the Clearfil SE Bond primer and other primers of the adhesive systems tested.

Thaweboon et al. (2003) stated that fluoride, if used at low concentration, might be a useful therapeutic agent for the treatment of pulpal disease, due to its capacity of stimulating proliferation and differentiation of dental pulp cells. At higher concentrations, however, it will have negative effects for this type of cells. Other studies have shown that CQ, the most commonly used photo-initiator in all adhesive systems, possess cytotoxic and mutagenic effects on experimental animals and on a variety of cells derived from human oral tissues [10, 12, 13]. Our adhesive systems were prepared according to the manufacturers' instructions (Table 1), similarly to how they are clinically applied in patients. Following, the discs (solid/cured masses) were incubated in the culture media for 1 hour, 24 hours, 72 hours, 1 week, 10 days, 3 weeks, 1 month, 40 days and 50 days, respectively, in order to assess their cytotoxicity. Yet, manufacturers do not provide the exact amounts of each chemical component. From the data presented here, it is difficult to conclude what is the main component of the tested adhesive systems responsible for the cytotoxic effects over extended period of time. Furthermore, Ratanasathien et al. (1995) previously stated that the cytotoxicity effect would be influenced by synergistic or antagonistic interactions of components rather than by only each one of the individuals.

Most of the *in vitro* studies assessed the cytotoxicity of adhesive systems on cells for a short period of time (from 1h to 72h) [8, 9, 11, 25-29, 31, 35-37]. The results reported are contradictory for the adhesive system PB, either at short or long-time exposure. Koulaouzidou et al (2007) suggested that PB caused the least toxic effects on fibroblast cell lines at 24h and 48h. However, in a recent work, El-Kholany et al. (2012) reported that PB had the highest cytotoxicity effects on odontoblast cells at 24h, 48h and 72h. As such, our study corroborates with El-Kholany's study lately published. On the other hand, Demirci et al. (2008) demonstrated that this adhesive induced cytotoxicity and genotoxicity in V79 cells after 24h, but if the dentin barrier was used, no toxic effects was observed for all dentin bonding agents tested. Furthermore, Sigush et al. (2009) evaluated the cytotoxicity of several adhesive systems-light curing units (LCUs) combinations used at longer exposure times (1st to 7th, 14th, 21st and 28th) for human gingival fibroblasts. Their study showed that the adhesive Clearfil Protect Bond was clearly dependent of LCUs. In addition, during the first few days there was a reduction of cellular viability and, after that, an increasing of it was observed for this adhesive, but then the adhesive system became cytotoxic again, and remained like that over time.

Our results are partially in agreement with Sigush et al. (2009), as PB was found to be high-toxicity in an initial phase, followed by an increase of cell viability (least toxic). However, unlikely their results, this adhesive remained toxic over extended period of time independent of LCUs. Although such diverse data are certainly difficult to understand, the different cytotoxic results for the tested adhesives maybe explained due to different methodologies employed; in our study, might have occurred the induction of interactions between the acidic monomers, co-monomers or others components, that, somehow, neutralized the cytotoxic effects after day 7 of exposure. It is worth mentioning that all these tests were repeated several times for an accuracy of the results. Because the literature reports the increasing of the cytotoxicity and degree conversion percentage (%DC) as the photo-activation time increases [8, 37], we sought to photo-activate the discs of adhesive systems following the manufacturers' instructions (i.e., for 10sec). Therefore, extending the curing of photo-activation time (20sec or more) should also be taken into account as a fundamental alternative for all the adhesive systems tested in this study. The combination between the insertion of the composite resin and the adhesive systems diminished a lot their cytotoxicity, probably due to overextended polymerization of the composite resins, as already reported by Kim et al. (2013).

Our results revealed the SE with almost no cytotoxic effects up to 1h for both type of gingival cells. However, the conditioned media collected from day 1 to day 30 was toxic to the cultured fibroblasts (none viable cells). Then, the toxicity diminished from day 40-50, as 68 to 83% of cells were still viable for fibroblast culture, and 60 to 65% cells were viable for epithelial cells. Overall, when SE was evaluated at three ranges of extended time period it remained cytotoxic and had the highest cytotoxic effects. Furthermore, no differences were observed between SE and Scotch at the latest range of time period (1month-50 days). In the other hand, SB system remained toxic the entire time, up to day 50. In addition, it is worth to mention that this total-etching adhesive system removes completely the smear layer, resulting in an increase of permeability, and releasing substances such as monomers, co-monomers and other additives that may diffuse into dentinal tubules towards the pulp and cause potential damage to the tooth [3]. The difference of cytotoxicity observed between PB and SE, may be attributed to the presence of photo-initiator camphoroquinine (CQ) inside the SE's primer only. The variability of HEMA and Bis-GMA amounts within each system could be another possible reason for that, since the manufacturer does not provide the exact amount of each component, nor as other components present within PB as previously reported, such as MDPB and sodium fluoride. However, the results of our study do not corroborate with those obtained by Ergun et al. (2007), that evaluated the cytotoxicity of SE and found the highest cell survival at 24h (81.84% viability) and 72h (69.8% viability).

Some of the adhesive systems used in our study, PB, SE and SB had both HEMA and Bis-GMA incorporated; Scotch, which is a one-step self-etching adhesive system, was basically composed by water and HEMA (Liquid A), and acidic monomers and co-monomers (Liquid B) (Table 1). Conditioned media obtained from the adhesive system Scotch exhibited 78% survival of fibroblast and 92% of epithelial cells at the beginning (up to 1h exposure). Then, the percentages start decreasing over time, with only 46% and 40% of viable fibroblast and epithelial

cells at day 50, respectively. Of the four-adhesive tested, Scotch showed the lowest cytotoxicity potential to the cells at the first range of time period (1h-3days), but after that it was found to have the highest toxicity effects (1month-50days) to the cells. Scotch system contains a lot of phosphate groups, which may activate or de-activate many enzymes within the cells [27]. Another explanation for the strongly increasing toxicity of Scotch would be the fact that this dentine bonding agent, which is one-step self-etch, behave as semi-permeable membranes due to its high hydrophilicity. In that case, the penetration of fluids and incomplete curing would be allowed, compromising the polymerization process of Scotch system, and consequent increase of their cytotoxicity [39]. Thus, we can suggest that such adhesives released uncured and/or leachable components to the culture medium over an extended period.

The different results found for the same adhesive system may be attributed mainly to the differences of methodologies used in this study, as well as to the chemical composition of each dental material, likely responsible for the cytotoxic effects of them. Thus, internationally standardized testing protocols are still needed in order to obtain good correlating results in the studies of human oral cells toxicity towards the adhesive bonding systems [40]. In that case, *in vitro* tests would have great potential to determine the toxicity of the dental materials in human oral cells and direct the care that must be taken in clinical procedures. Furthermore, for the use of these materials in deep cavities, or as pulp capping material, the application of a protect liner before applying the adhesive systems tested should yet be considered. Finally, further *in vivo* studies in animal models showing similar results are still needed, and recommended, to support our *in vitro* findings. That would also bring a better understanding of which components are present in these materials formulation that may be responsible for the major cytotoxic effects on oral cells.

In conclusion, all adhesive systems tested showed cytotoxicity on cultured gingival human fibroblasts and epithelial cells differing from each other in their degree of toxicity. Clearfil SE Protect (PB) was initially cytotoxic (up to day 3) but showed the lowest cytotoxicity degree along the time (up to day 50).

Acknowledgements

This investigation was supported in part by FAPESP, Sao Paulo State Agency for Research Funding, Sao Paulo, Brazil (#2010/07829-2).

References

1. Chen RS, Liu CC, Tseng WY, Jeng JH, Lin CP (2003) Cytotoxicity of three dentin bonding agents on human dental pulp cells. J Dent 31: 223-229. [Crossref]
2. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M et al. (2003) Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent 28: 215-235. [Crossref]
3. Ferracane JL, Condon JR (1990) Rate of elution of leachable components from composite. Dent Mater 6: 282-287. [Crossref]
4. Imazato S, Ebi N, Tarumi H, Russell RR, Kaneko T et al. (1999) Bactericidal activity and cytotoxicity of antibacterial monomer MDPB. Biomaterials 20: 899-903. [Crossref]
5. Banzi EC, Costa AR, Puppin-Rontani RM, Babu J, García-Godoy F (2014) Inhibitory effects of a cured antibacterial bonding system on viability and metabolic activity of oral bacteria. Dent Mater 30: e238-e244. [Crossref]
6. Ferracane JL (1994) Elution of leachable components from composites. J Oral Rehabil 21: 441-452. [Crossref]
7. Ratanasathien S, Wataha JC, Hanks CT, Dennison JB (1995) Cytotoxic interactive effects of dentin bonding components on mouse fibroblasts. J Dent Res 74: 1602-1606. [Crossref]
8. Kaga M, Noda M, Ferracane JL, Nakamura W, Oguchi H et al. (2001) The in vitro cytotoxicity of eluates from dentin bonding resins and their effect on tyrosine phosphorylation of L929 cells. Dent Mater 17: 333–339. [Crossref]
9. Lanza CR, de Souza Costa CA, Furlan M, Alécio A, Hebling J (2009) Transdentinal diffusion and cytotoxicity of self-etching adhesive systems. Cell Biol Toxicol 25: 533-543. [Crossref]
10. Volk J, Ziemann C, Leyhausen G, Geurtsen W (2009) Non-irradiated campherquinone induces DNA damage in human gingival fibroblasts. Dent Mater 25: 1556-1563. [Crossref]
11. Szczepanska J, Poplawski T, Synowiec E, Pawlowska E, Chojnacki CJ et al. (2012) 2-Hydroxylethyl methacrylate (HEMA), a tooth restoration component, exerts its genotoxic effects in human gingival fibroblasts trough methacrylic acid, an immediate product of its degradation. Mol Biol Rep 39: 1561-1574. [Crossref]
12. Volk J, Leyhausen G, Wessels M, Geurtsen W (2014) Reduced glutathione prevents camphorquinone-induced apoptosis in human oral keratinocytes. Dent Mater 30: 215-226. [Crossref]
13. Wessels M, Leyhausen G, Volk J, Geurtsen W (2014) Oxidative stress is responsible for genotoxicity of camphorquinone in primary human gingival fibroblasts. Clin Oral Investig 18: 1705-1710. [Crossref]
14. Ergün G, Eğilmez F, Uçtaşli MB, Yilmaz S (2007) Effect of light curing type on cytotoxicity of dentine-bonding agents. Int Endod J 40: 216-223. [Crossref]
15. Sigusch BW, Pflaum T, Völpel A, Schinkel M, Jandt KD (2009) The influence of various light curing units on the cytotoxicity of dental adhesives. Dent Mater 25: 1446-1452. [Crossref]
16. de Souza Costa CA, do Nascimento AB, Teixeira HM (2002) Response of human pulps following acid conditioning and application of a bonding agent in deep cavities. Dent Mater 18: 543-551. [Crossref]
17. Huang FM, Chang YC (2002) Cytotoxicity of dentine-bonding agents on human pulp cells in vitro. Int Endod J 35: 905-909. [Crossref]
18. Costa CA, Giro EM, do Nascimento AB, Teixeira HM, Hebling J (2003) Short-term evaluation of the pulpo-dentin complex response to a resin-modified glass-ionomer cement and a bonding agent applied in deep cavities. Dent Mater 19: 739-746. [Crossref]
19. Accorinte Mde L, Loguercio AD, Reis A, Muench A, de Araújo VC (2005) Adverse effects of human pulps after direct pulp capping with the different components from a total-etch, three-step adhesive system. Dent Mater 21: 599-607. [Crossref]
20. Koliniotou-Koumpia E, Tziafas D (2005) Pulpal responses following direct pulp capping of healthy dog teeth with dentine adhesive systems. J Dent 33: 639-647. [Crossref]
21. de Souza Costa CA1, Teixeira HM, Lopes do Nascimento AB, Hebling J (2007) Biocompatibility of Resin-Based Dental Materials Applied as Liners in Deep Cavities Prepared in Human Teeth. J Biomed Mater Res B Appl Biomater 81: 175-184. [Crossref]

22. Teixeira HM, Do Nascimento AB, Hebling J, De Souza Costa CA (2006) In vivo evaluation of the biocompatibility of three current bonding agents. J Oral Rehabil 33: 542-550. [Crossref]
23. Koliniotou-Koumpia E, Papadimitriou S, Tziafas D (2007) Pulpal responses after application of current adhesive systems to deep cavities. Clin Oral Investig 11: 313-320. [Crossref]
24. Nayyar S, Tewari S, Arora B (2007) Comparison of human pulp response to total-etch and self-etch bonding agents. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 104: e45-e52. [Crossref]
25. Koulaouzidou EA, Helvatjoglu-antoniades M, Palaghias G, Karanika-kouma A, Antoniades D (2008) Cytotoxicity evaluation of an antibacterial dentin adhesive system on established cell lines. J Biomed Res B Appl Biomater 84: 271-276. [Crossref]
26. Demirci M, Hiller K, Bosl C, Galler K, Schmalz G et al. (2008) The induction of oxidative stress, cytotoxicity, and genotoxicity by dental adhesives. Dent Mater 24: 362-371. [Crossref]
27. El-kholany NR, Abielhassan MH, Elembaby AE, Maria OM (2012) Apoptotic effect of different self-etch dental adhesives on odontoblasts in cell cultures. Arch Oral Biol 57: 775-783. [Crossref]
28. Vajrabhaya LO, Korsuwannawong S, Bosl C, Schmalz G (2009) The cytotoxicity of self-etching primer bonding agents in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 107: e86-e90. [Crossref]
29. Porto IC, Oliveira DC, Raele RA, Ribas KH, Montes MA et al. (2011) Cytotoxicity of current adhesive systems: in vitro testing on cell cultures of primary murine macrophages. Dent Mater 27: 221-228. [Crossref]
30. Kim MJ, Kim KN, Lee YK, Kim KM (2013) Cytotoxicity test of dentin bonding agents using millipore filters as dentin substitutes in a dentin barrier test. Clin Oral Investig 17: 1489-1496. [Crossref]
31. Tu MG, Liang WM, Wu TC, Chen SY (2009) Evaluation of cytotoxicity of resin bonding materials toward human oral epithelial cells using three assay systems. J Dent Sci 4: 178-186.
32. Souza NJ, Justo GZ, Oliveira CR, Haun M, Bincoletto C (2006) Cytotoxicity of materials used in perforation repair tested using the V79 fibroblast cell line and the granulocyte-macrophage progenitor cells. Int Endod J 39: 40-47. [Crossref]
33. Thaweboon S, Thaweboon B, Chunhabundit P, Suppukpatana P (2003) Effect of fluoride on human dental pulp cells in vitro. Southeast Asian J Trop Med Public Health 34: 915-918. [Crossref]
34. Goldberg M (2008) In vitro and in vivo studies on the toxicity of dental resin components: a review. Clin Oral Investig 12: 1-8. [Crossref]
35. Costa CA, Vaerten MA, Edwards CA, Hanks CT (1999) Cytotoxic effects of current dental adhesive systems on immortalized odontoblast cell line MDPC-23. Dent Mater 15: 434-441. [Crossref]
36. Elisabeth A Koulaouzidou, Maria Helvatjoglu-Antoniades, George Palaghias, Artemis Karanika-Kouma, Dimitrios Antoniades (2009) Cytotoxicity of dental adhesives in vitro. Eur J Dent 3: 3-9. [Crossref]
37. Bianchi L, Ribeiro AP, de Oliveira Carrilho MR, Pashley DH, de Souza Costa CA et al. (2013) Transdentinal cytotoxicity of experimental adhesive systems of different hydrophilicity applied to ethanol-saturated dentin. Dent Mater 29: 980-990. [Crossref]
38. Kim K, Son KM, Kwon JH, Lim BS, Yang HC (2013) The effects of restorative composite resins on the cytotoxicity of dentine bonding agents. Dent Mater J 32: 709-717. [Crossref]
39. Tay FR, Frankenberger R, Krejci I, Bouillaguet S, Pashley DH et al. (2004) Single-bottle adhesives behave as permeable membranes after polymerization. I. In vivo evidence. J Dent 32: 611-621. [Crossref]
40. Franz A, König F, Lucas T, Watts DC, Schedle A (2009) Cytotoxic effects of dental bonding substances as a function of degree of conversion. Dent Mater 25: 232-239. [Crossref]