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Cytotoxicity of Universal Dental Adhesives at Different Light-Curing Parameters

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Abstract

Introduction: Adhesive systems have been evolving and simplifying the number of steps to achieve adhesion. Adhesives come into contact withdifferent tissues in the oral cavity, leaving free monomers after the photocuring process, which can vary depending on the parameters of the lamp, affecting the corresponding cells. The purpose of this work is to evaluate cell viability after being in contact with universal adhesives that were light-cured with modified lamp parameters.

Materials and methods: Scotch Bond Universal (3M) and All Bond Universal(Bisco) were used in combination with two curing lights; BluephaseNmc and Valo. Twenty human teeth sectioned in half were used; adhesive process was carried out in two ways; one according to the manufacturer's instructions using each of the lamps; another, with the adhesive placement process according to the manufacturer but increasing the light-curing time; Subsequently, they were put in contact with mouse embryonic fibroblasts of the 3T3 cell line to determine the cytotoxicity of each group.

Results: The group with the least toxicity was obtained with an 800mW/cm2 lamp and 20 seconds of light exposure.

Conclusion: The power of photocuring lamps has an impact on cell viability; the higher the power, the greater the cell destruction.

Keywords: universal adhesives; adhesion; photocured; cytotoxicity; photocuring lamps

I. Introduction

Over the years dentistry has made great progress in all its areas, such is the case of adhesive systems, also known as bonding agents, which are solutions used to bond materials, made from resin, to the tooth structure, sealing and preventing leaks from the oral environment. (1) These adhesive systems, so called since they are made up of different steps and substances with a specific purpose, can also have different purposes depending

on the way they are used; they can be used not only as bonding agents but also for pulp capping, some authors claim that they can be used as direct pulp capping while others say that it is only for indirect pulp capping and for small to medium cavities. (2) Taking this into account, we can deduce that the adhesive systems come into contact with the different tissues of the tooth and therefore with its cells, having repercussions at this level due to the free monomers remaining after the light-curing process. This photocuring process is affected by the parameters of the lamp used, such as the power of the light, its direction and time, among others. Existing reports in the literature tell us that these free monomers cause changes in cell viability; Currently, the most widely used adhesive systems are universal adhesives, which are preferred by clinicians due to their simplification in the number of steps, as well as the fact that they can be used with different adhesive techniques. (3) The first attempt to bond an acrylic resin to the tooth is attributed to Hagger, who used Glycerolphosphate monomer (GPDM), which is still included in the formulation of some adhesives. Later, in 1952, Kramer and McLean studied GPDM and concluded that it promotes adhesion by penetrating the surface of the substrate and forming an intermediate layer, which, thirty years later, was named, by Nakabayashi, hybrid layer. (4) The mechanism of action of the adhesive systems is either to maintain or eliminate the layer of sludge or dentin, thus being self-etching or etching systems, respectively; followed by primer and bonding (5) Over time, adhesive systems have evolved, trying to simplify their use (6) and this has led to the classification of adhesive systems by generations. Being the 8th generation the one of interest in this study.

Regardless of the generation, the principle of adhesion is the diffusion of the monomer in the collagen fibers (7) and its subsequent polymerization by means of light emitted by lamps, the material that has not completely polymerized will have free monomers, which will end up being more prone to degradation. (8) Adhesive systems are commonly made up of bifunctional monomers and hydrophobic and hydrophilic monomers (6), having carboxylic acid or phosphoric acid derivatives and/or organic or mineral acid derivatives added, (1,6) solvents such as water, acetone or alcohol , amines, photoinitiators, filler particles. (9) For the development of universal adhesives, specific functional monomers have been investigated that are capable of reacting with different substrates, that are compatible with resins and resin cements and that have a hydrophilic and hydrophobic character, such as 10 mdp. (10) MDP forms hydrophobic nanolayers, this MDP chain begins as a hydrophilic chain that changes to hydrophobic when polymerized, this monomer has the ability to bind to different substrates such as dentin, titanium, metal alloys, ceramics. (11), is one of the most widely used monomers in universal adhesives that contains a dihydrogen phosphate group used to etch the tooth and a methacrylate group to interact with other monomers, promotes an inflammatory response and inhibits the differentiation of human pulpal odontoblasts, will interact with calcium produced by cells similar to odontoblasts. (12) The first reaction in adhesive systems is polymerization that requires the release of radicals and this ends when the radicals reacted with acrylic monomers in the adhesives, yet this conversion does not take place completely, leaving free radicals. These monomers can be ingested, inhaled, or spread through dental tissues. (6) Since the bonding materials are in direct contact with the tooth, it is very important that they are biocompatible (11) since they can change the biology of the epithelium and the dentin-pulp connective tissue. (6) Some of the components of these materials have been identified as biologically harmful, causing toxicity, allergies and even mutations. (8) Studies in the literature confirm the toxicity of free monomers and architectural and structural changes have been reported in epithelial cells due to the penetration of the primers, in addition to having a cytotoxic effect on gingival cells. (13). Human teeth have a neurosensory system given by the trigeminal nerve fibers. (14) Adhesives compromise cell viability, cell proliferation, enzyme activity, mitochondrial respiration, change in cell morphology, and mitochondrial respiration. (1) Universal adhesives have fewer hydrophobic monomers which makes them less cytotoxic. Elevated levels of reactive oxygen space formation are directly related to the control of cell death by antioxidant genes and proteins. (15) The degree of conversion by polymerization of the materials depends on different factors, such as the chemical structure of the monomers, the effectiveness of the photoinitiators, the distance between the light source and the material, the intensity of the light, the polymerization time, (8) light parameters, emission spectrum, energy density and light direction. (6)

There are studies where the effect of irradiation and the light source on the photopolymerization of adhesives was evaluated and the time in which the conversion of the adhesive was achieved varied between each brand, since a hermetic seal is not achieved with the adhesives, these and its free monomers come into direct contact with the dentinal tubules.

Tetrazolium salts consist of heterocyclic organic compounds, with the characteristic of producing formazan during reduction, this being insoluble and much more pigmented. They were prepared for the first time in 1894. Although from a histochemical point of view it could be considered that formazan is generated by tetrazolium salts, in reality, tetrazolium salts are produced in the formazan oxidation process. (16)

Among cell viability assays, the MTT assay is one of the most popular. This measures the metabolic activity of living cells. (17) The result is quite accurate, compared with other tests such as the Alamar Blue, which, like this one, small changes in the metabolic activity of the cell can generate large changes in the test and thus detect cellular stress due to the action of some toxic component. (18)

The hypothesis of this study is that the cytotoxicity of universal adhesives decreases at higher power and longer light exposure time of light-curing lamps.

II. MATERIALS AND METHODS

A total of 20 human teeth were used, collected from private dental offices and placed in physiological serum to keep them hydrated until the time of sample preparation; The teeth were sectioned distolingually with a diamond disc, obtaining a total of 40 samples. Once the dental organs were sectioned, they were placed in physiological serum to keep them hydrated. (Fig.1).

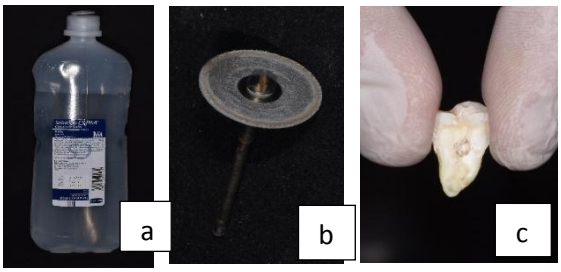

Fig. 1. a) Physiological serum; b) Diamond disc; c) Sectioned tooth.

Human teeth with extraction indicated for periodontal problems, extraction indicated for orthodontic reasons, and extraction of third molars were taken into account as inclusion criteria and as exclusion criteria, teeth with crowns destroyed by fracture or caries and teeth with infected dentin were taken into account. The adhesives selected for the present study were All Bond Universal from the Bisco trade house and Scotch bond Universal from the 3M trade house; Valo (VO) and Bluephase NMC (BP) lamps were used with 1000 mW/cm2 and 800 mW/cm2 as photocuring power, respectively. (Table 1) (Fig. 2) Groups of 5 samples were made to obtain 8 study groups, each group corresponded to an adhesive with a specific lamp and a certain exposure time to the light source (Fig. 3).

Table 1. Composition of adhesives

Adhesive	Manufacter	Composition	Reference
All-Bond Universal	Bisco, Inc. Schaumburg, IL 60193, USA	bis-GMA (20–50%), Ethanol (30–50%), 10-MDP (5–25%), HEMA (5–25%)	19, 20
Single Bond Universal	3M ESPE Dental Products, 3M Center, St. Paul, MN 55144-1000, USA	bis-GMA (15–25%), HEMA (15–25%), D3MA (5–15%), silane treated silica (5–15%), ethanol (10–15%), water (10–15%), 2-propenoic acid, 2-methyl-, reaction products with 1,10-decanediol and phosphorous oxide (P2O5) (1–10%), copolymer of acrylic and itaconic acid (1–5%), dimethylaminobenzoat(−4) (<2%)	20

Fig. 2. a) SBU and ABU adhesives; b) Valo lamp; c) Bluephase NMC lamp

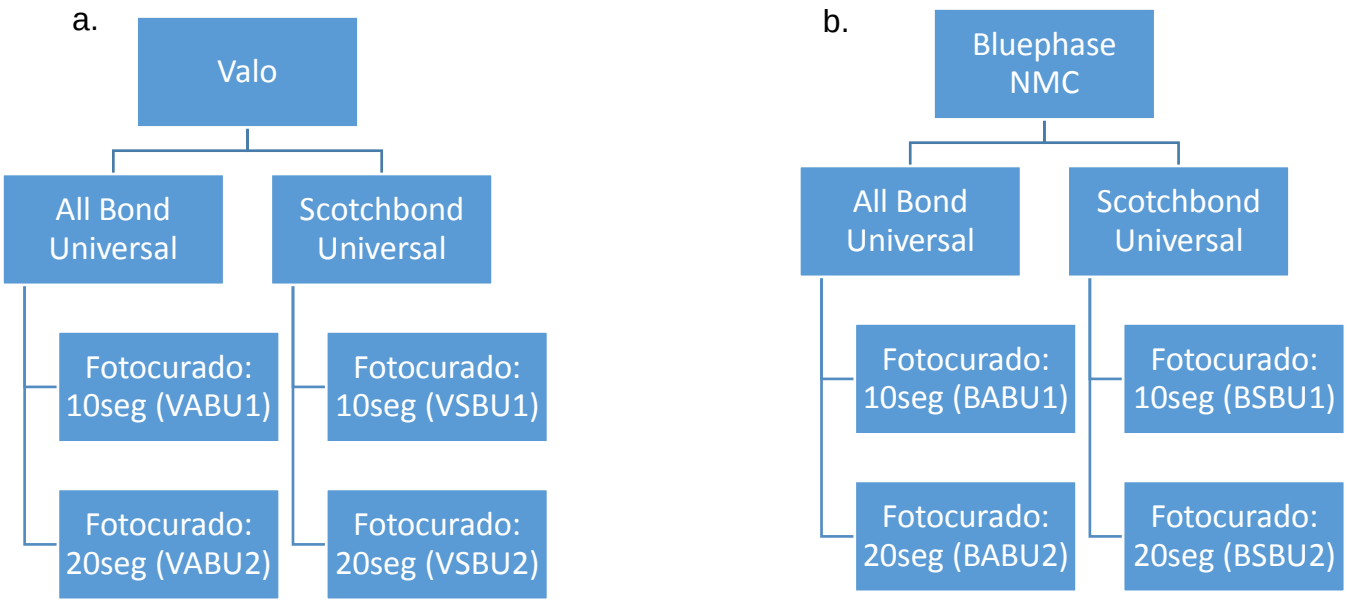

Fig. 3. Experimental groups. a) Valo lamp; b) Bluephase NMC Lamp

The adhesive was placed on the internal face of the sample, that is, in dentin with a self-etching technique and according to the manufacturer's instructions for half of the samples of each lamp and for the second half, the process of applying the adhesive on the dentin followed the same process indicated by the manufacturer, except at the time of light curing, the time was increased to 20 seconds. Following this process, for the cell viability test, the cytotoxic effect of the 8 groups of tooth with universal adhesive was evaluated in mouse fibroblast cells of the 3T3 cell line, using direct contact according to the ISO-10993-5 standard. : 2009. For the cytotoxicity test, a cell suspension of 40,000 cells per milliliter was prepared; samples of this were placed in a 96-well plate and were placed in direct contact with the cell suspension and 5 empty wells with growing cells were used as a control. In an inverted microscope, cell growth and the toxicity of the adhesive were observed, evaluating the quality of the monolayer of cells around the sample and using the MTT colorimetric test. Optical density was converted to percentage of controls for each cell culture. (21)

III. RESULTS

As a control, cells without treatment were used, which presented an elongated morphology, refractive to light and with an intact monolayer which covered the entire cell surface.

For the group corresponding to the treatment with All Bond Universal adhesive with a light curing time of 10 seconds (VABU1), a decrease in cells is observed, presenting spaces, which indicates cell death, compared to the control, in addition, a decrease in cells is observed. rounded shape in cells in response to treatment. (Fig.4a) For the group of the same adhesive and with 20 seconds of light curing (VABU2), cells can be observed at 90% confluence, decreased refringence and some of them with a more circular shape, which indicates morphological changes. (Fig.4b) For the group with which the Single Bond Universal adhesive was used and a light cure of 10 seconds (VSBU1) in the same way, more circular cells are observed, indicative of changes in morphology and spaces are observed as a result of cell death. (Fig.4c) Contrary to the group of the same adhesive and with 20 seconds of light curing (VSBU2) where fewer cell spaces are observed compared to the VSBU1 group, however, the absence of refringence is notable, which is indicative of a decrease in cell viability . (Fig.4d)

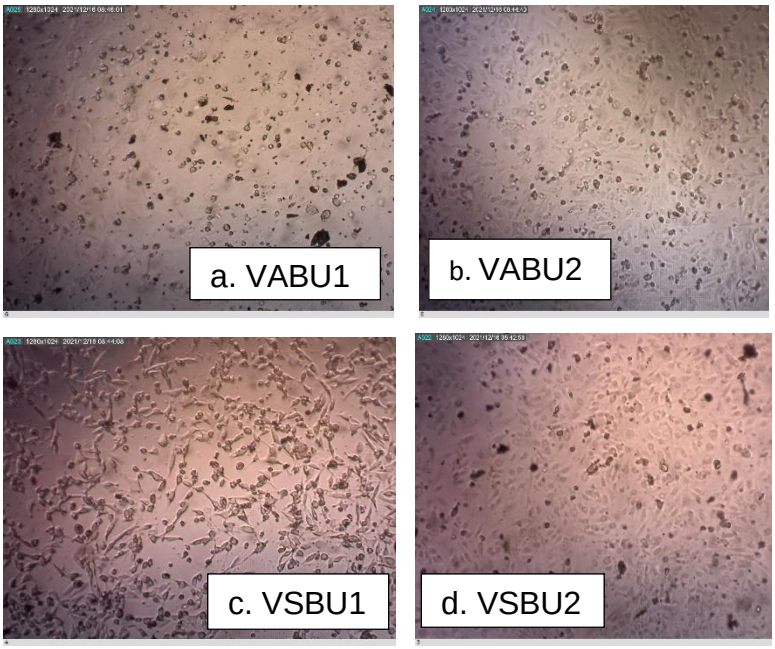

Fig 4. VALO lamp treatments. a. All Bond Universal with 10 second light cure; b. All Bond Universal with 20 second light cure; c. Single Bond Universal with 10 second light cure; d. Single Bond Universal with 20 seconds light cure.

For the group corresponding to the treatment with All Bond Universal and with 10 seconds of light curing (BABU1) we can observe that the cells are normal in morphology, are refractory and do not present spaces indicative of cell death. (Fig. 5a) In the group of the same adhesive and with 20 seconds of light curing (BABU2), although the sample seems to be somewhat detached, we can see how the monolayer of cells has a normal appearance. (Fig.5b) For the group corresponding to the treatment with Single Bond Universal and with 10 seconds of light curing (BSBU1), although a normal morphology is observed, we observe spaces consistent with cell death. (Fig.5c) In the sample of the same adhesive and with 20 seconds of light curing (BSBU2) we observed the detached monolayer but with a normal morphology as well as the confluence of the cells, indicative of greater cell viability compared to BSBU1. (Fig.5d)

Within the study groups, the group that obtained the lowest percentage of cell viability, with 7.15%, corresponds to the treatment with VALO lamp, All Bond Universal adhesive and a light-curing time of 20 seconds (VABU2); on the other hand, the study group with the highest cell viability, with 71.6%, corresponds to the adhesive, All Bond Universal, with a curing time of 20 seconds and with a Bluephase N MC (BABU2) lamp, as we can see in the graph of Figure 6.

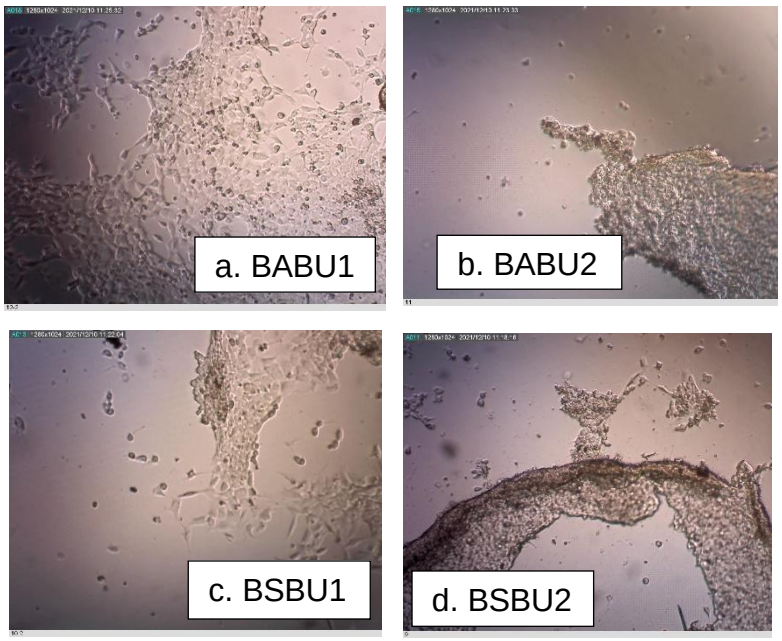

Fig 5. Treatments with Bluephase N MC lamp. a. All Bond Universal with 10 second light cure; b. All Bond Universal with 20 second light cure; c. Single Bond Universal with 10 second light cure; d. Single Bond Universal with 20 seconds light cure.

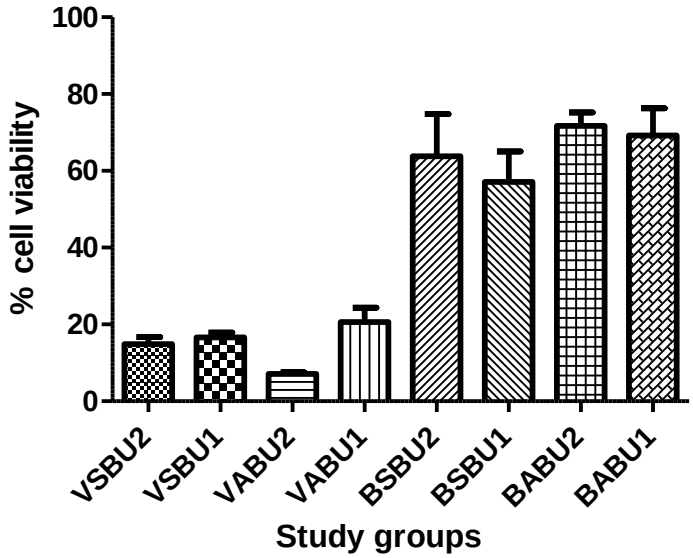

Fig. 6. Graph of cell viability percentage of each group.

For the interpretation of the data, an analysis of variance (ANOVA) was used, resulting in a value P= < 0.0001; This result indicates that a null hypothesis is rejected in which all treatments would have the same result and confirms that there is a statistically significant difference between several groups. (Table 2 and 3)

Table 2. One-way analysis of variance.

One- way analysis of variance	
P Value	< 0.0001
Measurements are sig. different (P < 0.05)	YES
Number of groups	8

Table 3. ANOVA

ANOVA	SS	df	MS
Treatment (between columns)	18372	7	2625
Residual (inside de columns)	3091	20	154.6
Total	21463	27	

SS (sum of squares); df (degrees of freedom); MS (square of means).

IV. DISCUSSION

The evolution of adhesives over time has given us the different generations that we have today, as there is the option of carrying out adhesion without the need to make an engraving and thus not expose the collagen fibers and that the adhesive penetrates more deeply would result in less cell toxicity than other generations of adhesives. (22) Therefore, this study did not compare the difference between performing or not recording and opting for a self-engraving technique.

In 2018 Leite et al. in their experimentation using SBU and evaluating cytotoxicity, using odontoblasts and analyzing cell viability using the Alamar Blue test, they determined that regardless of the way the adhesive was used, whether it was etching and rinsing or self-etching, the adhesive demonstrated have cell changes and reduction in cell viability by 88%; (23) however, in Leite's study, although the samples were manufactured according to the manufacturer's instructions, a 450 mW/cm2 lamp was used, that is, a lower power than the two lamps used in this study and as mentioned before , as the polymerization has a lower power in the lamp, this process may not be carried out properly, leaving a greater number of free monomers; and also taking into account that the power of the light used should be between 600 and 1000 mW/cm2, (24) the power of the light in the Leite study being below this.

In another study in the year 2021, Wawrzynkiewicz et al. determined in their cytotoxicity experimentation, with a colorimetric assay and using a 1000 mW/cm2 lamp, like one of the lamps in this study, that the SBU and ABU adhesives did not appear to be toxic (20). This is contrary to the results obtained in this experimentation and more specifically in the groups of the VALO lamp, which has a power equal to that of the Wawrzynkiewicz experiment, where the lowest percentage of cell viability was presented; however, in the Wawrzynkiewicz study a monocyte/macrophage peripheral blood cell line was used, ie a different line from the one used in the present study.

V. CONCLUSION

The hypothesis of the present study is rejected since, although the cytotoxicity decreased with longer photocuring time, this was only true for the groups worked with the 800 mW/cm2 Bluephase N MC lamp and not with the 1000 mW/cm2 Valo lamp. as expected and even having the lowest percentages of cell viability; which indicates that the most appropriate, regardless of the adhesive or the time of exposure to light, would be to work with a lamp of lower power than 1000 mW/cm2. This result opens a door for research where the cytotoxicity of the adhesive goes hand in hand with the light-curing power and not so much with the time of exposure to light or the composition of the adhesive.

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