ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2020; 25(2): 1328-1333
doi: 10.25083/rbl/25.2/1328.1333

Received for publication, April, 1, 2018
Accepted, September, 20, 2018

Original paper

In vitro evaluation of cytotoxicity of modern dental adhesive systems

IRINA-MARIA GHEORGHIU[1], ALEXANDRU ANDREI ILIESCU[2], SANZIANA SCĂRLĂTESCU[1], ANDREI ILIESCU[1], PAULA PERLEA[1]

[1]Faculty of Dental Medicine, University of Medicine and Pharmacy "Carol Davila" Bucharest, Romania

[2]University of Medicine and Pharmacy of Craiova, Romania

Abstract Dental adhesive systems come in contact with dental and gingival tissues, mostly with odontoblasts and often with gingival fibroblasts. Therefore, their biocompatibility is mandatory. The aim of this in vitro study is to evaluate the cytotoxic effect of four dental adhesive product: Adper™ Single Bond Plus Adhesive (3M ESPE), Gluma Comfort Bond™(Kulzer), OptiBond™ All-In-One (Kerr) and Prime & Bond NT™ (Dentsplay) in human gingival fibroblast culture. The test was accomplished at 24-hour interval, over a four-day span. The cytotoxic effect was determined using quantitative and qualitative parameters: number of dead and detached cells from the cellular layer and microscopic cellular alterations caused by components released from dental adhesives in contact with cell culture. Statistic analysis of fibroblasts mortality rate induced by investigated adhesives highlights the lowest cytotoxicity for OptiBond™ All-In-One(Kerr) and the highest for Adper™ Single Bond Plus Adhesive (3M ESPE). The microscopic evaluation revealed different levels of induced cell stress, morphological alterations and lethal effects on fibroblasts.

Keywords Dental adhesive, cytotoxicity, human fibroblasts.

To cite this article: GHEORGHIU I-M, ANDREI ILIESCU A, SCĂRLĂTESCU S, ILIESCU A, PERLEA P. *In vitro* evaluation of cytotoxicity of modern dental adhesive systems. *Rom Biotechnol Lett*. 2020; 25(2): 1328-1333. DOI: 10.25083/rbl/25.2/1328.1333

*Corresponding author: IRINA-MARIA GHEORGHIU, Faculty of Dental Medicine, University of Medicine and Pharmacy "Carol Davila" Bucharest, Romania
E-mail: igheorghiu@hotmail.com

Introduction

Presently the "*biological danger*" focuses everyone's attention. This issue is definitely of a paramount importance in the dental field since various substances and materials that are used as therapeutic agents remain in contact with the oral tissues for a long period of time. Despite its definition meaning the absence of any harmful, negative interaction with the host tissues of a foreign substance, biocompatibility is a concept of extreme complexity. Accordingly, the need to ensure a high biological tolerability of the dental materials meets an ethical and deontological requirement (I. SUCIU et al [1]).

Current operative dentistry is governed by the idea of dental adhesion. As such, all modern dental materials allow minimally invasive preparation of carious and non-carious lesions due to their capacity to chemically adhere at the dental hard tissues rather than by mechanical retention (C. VARLAN et al [2]). Adhesion takes place through a hybrid layer, created at the dental-restorative material interface, which facilitates a good marginal sealing of the cavity and a successful protection against de microbial infiltration (A. de ALMEIDA NEVES & al [3]).

In order to achieve the dental adhesion of composite resins, it is necessary to use specific adhesion systems, commonly called "*dental adhesives*". Since the late 1970s, when the first generation of dental adhesives appeared, the techniques, methods and materials developed spectacularly in many directions, aiming to simultaneously provide increasing adherent capacity, simplified work and high biological tolerance.

Adhesive dental systems are placed in intimate contact with the tooth hard tissues and gingiva, primarily with the odontoblast cells, through their extensions located into the dentinal tubules. Any damage to the odontoblasts has as consequence the cessation of their function to producing dentin (O. CORTES & al [4]). Regarding the carious lesions situated in the cervical area of the tooth, in close proximity to the gum, the restorative and adhesive dental materials come into contact with the gingival fibroblasts. Therefore, it is extremely important that the dentinal adhesives to exhibit a reduced cytotoxic potential on the pulp-dentine and periodontal complexes (M. BOURBIA & al [5]; M. MOGA & al [6]).

Adhesion to enamel and dentin relies on different mechanisms which share the necessity of using dental adhesives. However, during the first stage of adhesion is mandatory to achieve by conditioning an acidic demineralization ("*etching*") in order to generate a high energy surface of the tooth hard tissues. The adhesive systems consist of an acid, mostly the phosphoric acid in a concentration of 30-40%, or an acidic monomer.

The second step of adhesion ("*priming*") requires the use of primer systems that are applied to moisten the previously demineralized dentin so that to assure the penetration and subsequent inter-penetration into the dentin's collagen structure of the adhesive bonding during the following and last stage of adhesion (third stage). Considering the chemical structure, the numerous adhesive systems are acrylic resin monomers (acrylates, methacrylates) in combination with water, organic solvents (ethanol, acetone), starters (amines, camphorquinones), inhibitors (butylhydroxytoluene, monomethyl ether hydroquinone), stabilizers (butylhydroxytoluene), inorganic filler particles, glutaraldehyde, and MDPB monomer.

Current techniques for dental adhesion are either "*total etch*" or "*self etch*". The latter are less biologically aggressive, as the phosphoric acid used during the demineralization step is replaced by acid monomers which allow the etching process without having to be removed by irrigation and subsequent drying.

Dental adhesives have now reached the seventh generation, their specific properties being dramatically improved, at the same time as the increase in biocompatibility (J.M. da SILVA & al [7]).

The biological effect on toth and periodontal tissues are obviously induced by the substances included in the composition of these dental products. These are identified by *in vitro* and *in vivo* studies that quantify cytotoxicity, genotoxicity or mutagenic capacity in cell or tissue cultures (I. SUCIU & al [8]; D.C. NUTA & al [9]).

The effect of different dental substances or materials on cellular metabolism, cellular changes (enzyme activity determination), growth (proliferation rate), viability (identification and cell survival count or cell death rate) as well as changes in cell morphology as a direct expression of the toxicity of the investigated product are concluded and analized through *in vitro* cell culture tests (D. MAROVIC & al [10]; E.A. KOULAOUZIDOU & al [11]). Presently for assessing *in vitro* the cytotoxicity of dental adhesives numerous models of cellular lines are running as screening procedures.

Materials and Methods

In this *in vitro* study, the cytotoxicity of dental adhesives in their un-polymerized phase was evaluated on a cell culture of human gingival fibroblasts in a dynamic manner, at 24-hour intervals on a 4-day period. The investigated parameters were: cellular mortality rate and cell morphological alterations.

There were tested 4 undiluted dental adhesives in liquid form, as follows:

1. **Adper™ Single Bond Plus Adhesive (3M ESPE)** is a 5th generation adhesive used in total etch technique. Its composition is: BisGMA, HEMA, dimethacrylates, ethanol, water, a novel photoinitiator system and a methacrylate functional copolymer of polyacrylic and polyitaconic acids.

2. **Gluma Comfort Bond™ (Kulzer)** is a 5th generation single component dental adhesive ethanol based used in total etch technique. The composition is: methacrylate, 4-META, ethanol, photoinitiators, glutaraldehyde.

3. **OptiBond™ All-In-One(Kerr)** is a 7th generation adhesive system, which is used in self etch technique, in one single step. The composition is: monomers (glycerol phosphate dimethacrylate – self-etching adhesive monomer and co-monomers including mono- and di-functional methacrylate monomers); solvents (water, acetone and

ethanol), photo-initiator (camphorquinone based), fillers (three nano-sized fillers), fluoride-releasing fillers (sodium hexafluorosilicate and yterbium fluoride).

4. **Prime & Bond NT™ (Dentsplay)** is a 5th generation acetone based adhesive which combines primer and adhesive in a single bottle and it is used in total etch technique. Its composition is: di- and trimethacrylate resins, PENTA (dipentaerythritol penta acrylate monophosphate), nanofillers(amorphous silicon dioxide), photo-initiators, stabilizers, cetylamine hydrofluoride, acetone.

The gingival samples

Subsequent to the accepted informal content, gingival biopsies were harvested under surgical conditions in the upper molars area from 4 young healthy patients: 2 men and 2 women. The gingival samples were preserved less than 1 hour in vials with sterilized phosphate-buffered saline. After that the samples were introduced in 5 vials (2 ml testing tubes) with growing medium Eagle for fibroblasts (10% calf fetal serum, 50 IU/ml penicillin and 50 micro-grams/ml streptomycin) and stored in thermostat at 37°C in a milieu of 95% air and 5% carbon dioxide saturated in water vapors. The achieved fibroblasts density was 7.39 x 10000 cells/cm^2. Later on the Eagle medium was sucked in and the fibroblasts were preserved in thermostat for 96 hours at 37°C in a non-serum milieu with the tested substances (0.05 ml substance/2 ml cell culture). Milieu's pH in all 5 vials (1 for control + 4 for tested adhesives) ranged between 7.20-7.30.

The death of fibroblasts was established by counting in the haemocytometer after a previous detachment of cellular layer and cells staining with trypan blue. The cytotoxicity of dental adhesives was assessed by comparing the number of dead cells to the initial amount of fibroblasts and by microscopic examination of the fibroblasts layer as well.

Results

The test results of fibroblast cell death rates are shown in table 1 and the graphic representation is in Figure 1.

Table 1. Cell mortality (%) induced by the investigated dental adhesives in human fibroblasts cell culture

DENTAL ADHESIVES	24 hours	48 hours	72 hours	96 hours
Adper™ Single Bond Plus Adhesive (3M ESPE)	12.34	19.66	23.76	28.39
Gluma Comfort Bond™ (Kulzer)	8.37	16.33	18.05	25.96
OptiBond™ All-In-One (Kerr)	4.31	6.23	8.37	11.42
Prime&Bond NT™ (Dentsply)	8.41	9.15	13.11	16.14
Control	3.62	4.12	5.39	6.86

The mortality rate provoked by all 4 tested dental adhesives was significantly higher than that of control group ($p<0.05$). Though all dental adhesives tested in this study demonstrated a certain cytotoxic effect, the statistic analysis (Fischer and *t* – Student tests) of the mortality of the fibroblasts cell culture showed a minimal cytotoxicity for OptiBond™ All-In-One (Kerr) and a maximal one for Adper™ Single Bond Plus Adhesive (3M ESPE).

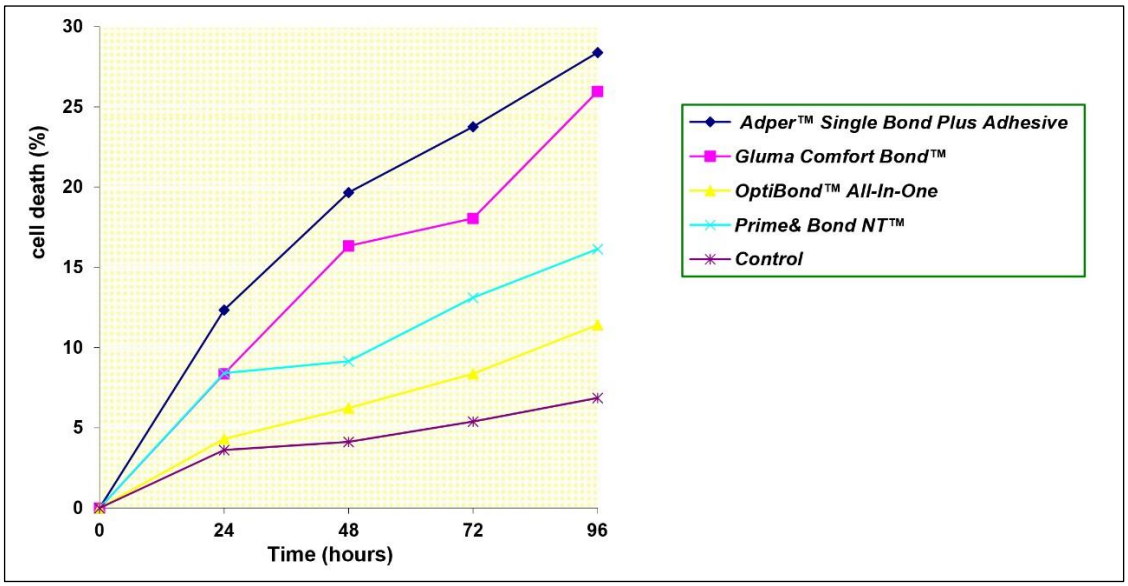

Figure 1. The graphic representation of the evolution of the death cells for the gingival culture of human fibroblasts, in the presence of tested dental adhesives products.

Associated with quantitative determinations, the microscopic examination reveals specific aspect of biological toxic effect of dental adhesive on fibroblasts culture. First of all, a limited number of dead and detached cell are observed (Figure 2). As the experiment progresses and the cells are exposed to adhesives to a longer period of time, appear major degenerative morphological alterations: cellular membrane disintegration (Figure 3), and the nuclei and nucleoli feature is modified (Figure 4). A large number of dead cells are present finally and a clear-cut delimitation is visible between dead and surviving fibroblasts (Figure 5).

The aspects are differentiated according to the toxicity level of the tested substances on the cells from the cellular monolayer.

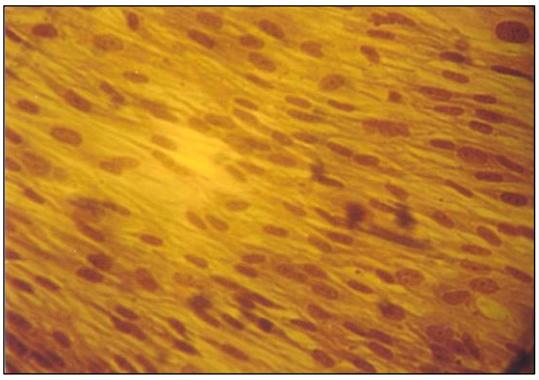

Figure 2. Gingival fibroblasts culture at 24 hour mark, **OptiBond™ All-In-One (Kerr**) lot, (MGGx20 stain). Dead and detached cells in suspension.

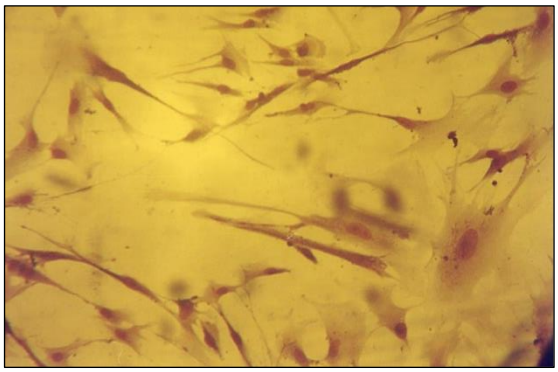

Figure 3. Gingival fibroblasts culture at 96 hour mark, **OptiBond™ All-In-One (Kerr)** lot, (MGGx40 stain). Visible degenerative morphological alterations of cellular membranes and significant number of dead and detached fibroblasts.

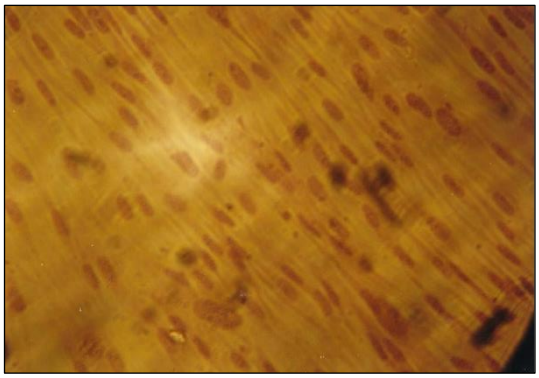

Figure 4. Gingival fibroblasts culture at 24 hour mark, **Adper™ Single Bond Plus Adhesive (3M ESPE)** (MGGx20 stain). Major cell alterations of cytoplasma and nucleoli. Cell membrane damage.

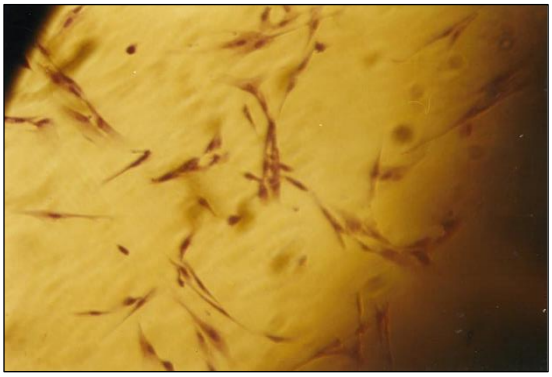

Figure 5. Gingival fibroblasts culture at 96 hour mark, **Adper™ Single Bond Plus Adhesive (3M ESPE)** (MGGx40 stain). A large number of dead cells, expression of this product toxicity.

Discussions

First of all the experimental observation of the primary culture of fibroblasts with respect to the cell mortality rate reveals very low mortality rates in the control group compared to all the tested dental adhesives.

During the first 24 hours of study the mortality had a relatively high increase for all tested products, but more noticeable for Adper™ Single Bond Plus Adhesive (3M ESPE) and Gluma Comfort Bond™ (Kulzer), while the lowest value was recorded by OptiBond™ All-In-One (Kerr).

In the next day (24-48 hours), the mortality rate is moderately increasing, with a higher level for some substances compared to others. This evolution observed in OptiBond™ All-In-One (Kerr) was almost linear, with a moderate effect to inducing the cellular mortality.

During the last day of study (72-96 hours) was recorded a significant increase in cell mortality induced by Adper™ Single Bond Plus Adhesive (3M ESPE). Although at 72-hour was estimate for Comfort Bond™ (Kulzer) an intermediate level, below the level of the Adper™ Single Bond Plus Adhesive (3M ESPE), at the end of the study (96-hour) the cell death rate was 25.96%, which means a very high level of toxicity. For the period of last 72-96 hours follow-up in case of OptiBond™ All-In-One (Kerr) and Prime & Bond NT™ (Dentsply), unlike the aforementioned dental adhesives, the percentage of detached cells increases very little, almost linearly.

Therefore Adper™ Single Bond Plus Adhesive (3M ESPE) showed the highest cytotoxicity effect since the cell mortality rate increased progressively during the 96 hours experiment (4 times more dead cells compared with control). In follows in descending order Comfort Bond™ (Kulzer) while Prime & Bond NT™ (Dentsply) and OptiBond™ All-In-One (Kerr) induced much lower cell death rates, close to half the maximum value.

Adper™ Single Bond Plus Adhesive (3M ESPE) is a well-known dental adhesive system. Regarding the adhesion to enamel and dentin it is almost considered as "*gold standard*". This dental product provides high adhesion strength but one of its major components, Bis GMA, is highly cytotoxic, as we also found in our study. HEMA, which is also a component of Adper™ Single Bond Plus Adhesive it has lower cytotoxicity and, due to its low molecular weight, facilitates the dentinal penetration of the adhesive system. Hence the global cytotoxic effect of this adhesive system is amplified through the apoptosis mechanism.

Gluma Comfort Bond™ (Kulzer) contains glutaraldehyde, which proved to be highly cytotoxic substance. These results of the present study are consistent with literature.

Prime & Bond NT™ (Dentsply) is a mild-etch dental adhesive with pH 2.5-3, ensuring a less aggressive cellular and tissue effect. It contains dipentaerythritol pentacrylate phosphate (PENTA) as an alternative phosphate ester monomer for bonding methacrylate-based resins which has been shown to be less cytotoxic than conventional acrylic resin monomers.

In our study, the OptiBond™ All-In-One (Kerr) has been identified as the least cytotoxic product among all investigated adhesive systems. It belongs to the 7th generation of dental adhesives and is used in *self etching* techniques. OptiBond™ All-In-One (Kerr) contains glycerol phosphate dimethacrylate, methacrylate monomers and other components that could be responsible for its biocompatibility (L. VAJRABHAYA et al [12]).

Though hard to be quantified unlike cytotoxicity level, it has to be highlighted that the visible morphological changes of fibroblasts cells are strongly associated with the biological incompatibility of dental adhesives. Occuring after the first 24 hours of intimate contact between fibroblasts and dental adhesives the deleterious cell effects are gradual and progressively expressed.

Therefore the quantitative measurement of the death cells can be accepted as a valuable test to easily quantifying the cell sensibility threshold for a certain dental material. This test may be significant when the mortality rate in a monolayer achieves minimum 10% of the cells found in the certain tissue region.

The cell changes in the OptiBond™ All-In-One (Kerr) group are discreet, whereas for Adper™ Single Bond Plus Adhesive Adhesive (3M ESPE) we have observed in high proportion evident signs of cell deaths after various stages of degeneration paved the way. The microscopic images obtained are relevant for the cytotoxic effect of the tested dental adhesives which initially was revealed by detached cells from the fibroblast monolayer. Subsequently, degenerative changes affecting the cell membrane and nucleus have been identified as well. These changes lead, as they evolve over time, to the appearance of a large number of dead cells. What is observed is that the cytotoxicity scale expressed by cell death rates is consistent with the microscopic observation of cellular appearance.

All these cell degenerative effects are mainly generated by residual monomers of the dental adhesives. Numerous *in vitro* studies showed that such highly cytotoxic substances are Bisphenol A-glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA), hydroxyethyl methacrylate (HEMA), and triethylene glycol dimethacrylate (TEGMA). However, previous studies revealed that HEMA and TEGMA seem to be less aggressive in cell culture (V. JANKE & al [13]; I.C. PORTO & al [14]; L. STANISLAWSKI & al [15]). It was also imagined even a toxicity scale of monomers from dental adhesives, as follows: Bis-GMA > UDMA > TEGDMA > HEMA (S. RATANASATHIEN & al [16]).

It is noteworthy that, depending on the degree of dentin prmeability, in dental practice a lot of factors contribute to the amplification or attenuation of the cytotoxic effect of dental adhesives. These are: the *etching* versus *self etching* technique, its accuracy, the local status regarding the depth of the cavity and the way of excavating the altered tissues (I.M.GHEORGHIU & al [17]).

Therefore, a direct clinical extrapolation of *in vitro* results wold not be possible, as the biocompatibility studies on cell cultures can not quantify the contribution of other extrinsic factors such as the interposition of a dentin layer having the buffering capacity or protective response mechanisms from some organs and systems, mainly localized blood-borne elements that are involved in immune defensive mechanisms (A. NARVEKAR & al [18]).

Conclusions

The results of this study which investigates cytotoxicity of four dental adhesive systems showed that Adper™ Single Bond Plus Adhesive (3M ESPE) presented the highest level of cytotoxicity, while OptiBond™ All-In-One (Kerr) is the product which determined the lowest rate of cell death. The microscopic evaluation revealed different levels of induced cell stress, morphological alterations and finally lethal effects on fibroblasts.

Aknowledgements

For this research article, the authors have equal contributions.

References

1. SUCIU I, SORITAU O, GHEORGHE I, LAZĂR V, BODNAR DC, DELEAN AG, SCĂRLĂTESCU SA, STANCIU R, BURLIBAȘA M, DIMITRIU B. Biocompatibility testing on cell culture of some root canal sealers used in endodontics. *Rom. Biotechnol. Lett.*, 21(3): 11543-11549 (2016).
2. VÂRLAN C, DIMITRIU B, STANCIU D, SUCIU I, CHIRILĂ L. Situația actuală a adeziunii la structurile dure dentare. SSER – Societatea de Stomatologie Estetică din Romania, București, pp. 8-10, 16-22 (2011).
3. DE ALMEIDA NEVES A, COUTINHO E, CARDOSO MV, LAMBRECHTS P, VAN MEERBEEK V. Current concepts and techniques for caries excavation and adhesion to residual dentin. *J. Adhes. Dent.*, 13:1 (2011).
4. CORTES O, ALCAINA A, BERNABE A. Biocompatibility Evaluation of Four Dentin Adhesives Used as Indirect Pulp Capping Materials. *Acta. Stomatol. Croat.*,51(2): 113-121 (2017).
5. BOURBIA M, FINER Y. Biochemical Stability and Interactions of Dental Resin Composites and Adhesives with Host and Bacteria in the Oral Cavity: A Review. *J. Can. Dent. Assoc.*, 84:i1 (2018).
6. MOGA M, BOȘCA AB, SORIȚĂU O, BĂCIUȚ M, LUCACIU O, VIRAG P, ILEA A, DÎRZU N, CÂMPIAN RS. Nicotine Cytotoxicity on the Mesenchymal Stem Cells Derived from Human Periodontium. *Rom. Biotechnol. Lett.*, 21(4): 11763-11772 (2016).
7. DA SILVA JM, RODRIGUES JR, CAMARGO CH, JR. FERNANDES VV, HILLER KA, SCHWEIKL H et al. Effectiveness and biological compatibility of different generations of dentin adhesives. *Clin. Oral Investig.*, 18(2):607-13 (2014).
8. SUCIU I, VÂRLAN C, DIMITRIU B, SORIȚĂU O, AMZA O, MOLDOVEANU GF, BURLIBAȘA M, GHEORGHE I, LAZAR V, GHEORGHIU I. Biocompatibility Study of Several Esthetic Dental Restorative Materials. *Rom. Biotechnol. Lett.*, 21(2):11438-11442 (2016).
9. NUTA DC, MĂRUȚESCU L, MISSIR AV, MORUSCIAG L, CHIRITA C, CURUTIU C, BADICEANU CD, PAPACOCEA MT, LIMBAN C. *In vitro* evaluation of the antimicrobial activity of N-phenylcarbamothioyl benzamides against planktonic and adherent microbial cells. *Rom. Biotechnol. Lett.*, 22(6): 13163-13168 (2017).
10. MAROVIC D, TADIN A, MLADINIC M, JURIC-KACUNIC D, GALIC N. *In vitro* detection of DNA damage in human leukocytes induced by combined effect of resin composites and adhesive systems. *Am. J. Dent*., 27:35-41 (2014).
11. KOULAOUZIDOU EA, HELVATJOGLU-ANTONIADES M, PALAGHIAS G, KARANIKA-KOUMA A, ANTONIADES D. Cytotoxicity of Dental Adhesives *In Vitro*. *Eur. J. Dent.*, 3(1): 3-9 (2009).
12. VAJRABHAYA L, PASUK A, HARNIRATTISAI C. Cytotoxicity evaluation of single component dentin bonding agents. *Oper. Dent.*, 28:440-444 (2003).
13. JANKE V, NEUHOFF N, SCHLEGELBERGER B, LEYHAUSEN G, GEURTSEN W. *TEGDMA* causes apoptosis in primary human gingival fibroblasts. *J. Dent. Res.*, 82(10):814-8 (2003).
14. PORTO IC, OLIVEIRA D, RUELE R, RIBAS K, MONTES M, CASTRO C. Cytotoxicity of current adhesive systems: *In Vitro* on cell cultures of primary murine macrophages. *Dent. Mater.*, 27(3):221-8 (2011).
15. STANISLAWSKI L, LEFEUVRE M, BOURD K, SOHEILI-MAJD E, PERIANIN A. *TEGDMA*-induced toxicity in human fibroblasts is associated with early and drastic glutathione depletion with subsequent production of oxygen reactive species. *J. Biomed. Mater. Res. A.*, 66:476-482 (2003).
16. RATANASATHIEN S, WATAHA JC, HANKS CT, DENNISON J.B. Cytotoxic interactive effects of dentin bonding components on mouse fibroblasts. *J. Dent. Res.*, 74:1602-1606 (1995).
17. GHEORGHIU I.M, MARASCU V, STAICU D, ZMARANDACHE D, PERLEA P. Comparative ultrastructural analysis of dentin surfaces mechanically prepared using carbon steel conventional burs and polymer burs. *Rom. Biotechnol. Lett.*, 22(4): 12775-12784 (2017).
18. NARVEKAR A, RAMAMOORTHI M. Cytotoxic effects of restorative biomaterials on dental pulp – a systematized review. *W. J. Pharm. Pharmaceut. Sci*., 4:239-266 (2015).