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EVALUATION OF CYTOTOXIC PROPERTIES OF SOME DENTAL MATERIALS

Zbigniew Raszewski*

SpofaDental Kerr Company, Markova 238, 506-01 Jicin (Czech Republic)
*Present address: *Wyższa Szkoła Menedżerska Bialystok, Sobieskiego 3, 15-013 Bialystok (Poland)*
*e-mail: *Zbigniew.Raszewski@kavokerr.com*

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ABSTRACT

In this study we compared the cytotoxic properties of several dental materials on cell cultures. Six products chosen for the study were alginate impression material, zinc phosphate cement, tissue conditioner for temporary denture soft relining, acrylic temporary crown and bridge material, heat curing acrylic resin and acrylic teeth. The samples were tested for cytotoxic properties by MTT test on Vero CCL-81 (ATCC) cell line. The most cytotoxic potential was observed for zinc phosphate cement sample (cell survival, 5%) and highest biocompatibility for thermally polymerized acrylic resin (>70%). On exposure of dental materials to cytotoxic properties it did not completely cross out the product, being developed as a Medical Device. However, it requires further appropriate testing.

Key words: Acrylic resin, alginate impression material, cytotoxicity, zinc phosphate cement

INTRODUCTION

Some of the applications of medical materials are the products used in dentistry. They are useful in many applications: impression materials, cements for fixing of prosthetic fillings such as crowns or bridges, or materials for the production of removable dentures (Faggion, 2012). Some of them affect human body for a short period i.e. < 24 h while other materials remain in human body for a few days (acrylic dentures) to several years (cements, filling materials) (Tillberg *et al*., 2008; Haider *et al*., 2017}. The developed dental materials frequently used these days have almost remained unchanged for long. Of these, zinc oxide phosphate cement was invented over 150 years ago while others such as composite materials changed very quickly (Rueggeberg, 2002; Behr *et al*., 2003). A large part of the materials developed long ago have not thoroughly been investigated using modern tests. Their verification and usefulness have been proven over the years of clinical use (Faggion, 2012). The European Union has issued a special directive that requires manufacturers to test and re-analyze even those materials that are in use on sale since long (Faggion, 2012). Such retrospective studies are advantageous and explain a number of operational mechanisms of dental materials, thereby help in the design and implementation of new biocompatible products (Murray *et al*., 2007).

Some of the materials used in dental practice have rich database about their impact on cell cultures (composites, acrylics) [Schweikl *et al.*, 2006; Narvekar *et al.*, 2015] or alginates impression materials (Pithon *et al*., 2010). The class 1 materials (with a contact time of < 30 days) may adversely affect human body and consequently show negative effect on doctors, nurses or dental technicians, rather than the patients (Tillberg *et al*., 2008; Boraldi *et al*., 2009; Pithon *et al*., 2010; Priyaranjan *et al*., 2020). The present study was based on the hypothesis that the dental materials are biocompatible and aimed to compare the cytotoxic properties of some dental materials using cell cultures.

MATERIALS AND METHODS

Six different products of dental materials, chosen for the present cytotoxicity study, and their composition were as under:

Dental materials tested	Type of medical device	Main composition
Elastic Cromo (SpofaDental Jicin Czech Republic)	Class I contact time <5 min	Diatomic earth, sodium alginate, calcium sulphate dihydrate, potassium hexafluorotitane, trisodium phosphate, magnesium oxide
F.I.T.T (Kerr, Scafati, Italy)	Class I contact time <3-5 days	Poly(ethyl methacrylate), titanium dioxide, benzoyl peroxide, ethanol, phenyl benzoate
TAB2000 (Kerr Scafati Italy)	Class I contact time <30 days	Poly(methyl methacrylate), methyl methacrylate, dimethyl-p toluidine, benzoyl peroxide, titanium dioxide, pigments
Adhesor (SpofaDental, Jicin, Czech Republic)	Class IIa contact time a few years	Zinc oxide, boric acid, magnesium oxide, phosphoric acid
Superacryl Plus (SpofaDental, Jicin, Czech Republic)	Class IIa contact time 2-5 years	Poly(methyl methacrylate), methyl methacrylate, benzoyl peroxide, titanium dioxide, pigments
Mifam Super Lux (SpofaDental, Jicin, Czech Republic)	Class IIa contact time 2-5 years	Poly(methyl methacrylate), methyl methacrylate, benzoyl peroxide, titanium dioxide, pigments

Disc samples with 1 mm thickness and 5 mm diameter were prepared for the tests as per the manufacturer's recommendations for testing materials. Alginate impression materials powder was mixed with water and set at room temperature. The second group of products like acrylic dental resins were used after mixing the powder (PMMA) with liquid (MMA). Self-curing products were left at room temperature until full setting (FITT and TAB, 2000). Hot curing material Superacryl Plus was polymerized in boiling water during 60 min. Acrylic tooth material, Mifam Super Lux, was cut with micromotor (K9, Kavo, Biberach, Germany) to small discs. The samples were tested for cytotoxic properties by evaluating the cell survival test using MTT (methyl thiazolyl diphenyltetrazolium bromide, Sigma Aldrich, Praha Czech Republic) and Vero CCL-81 (ATCC, Warsaw, Poland) cell line. Cells were maintained in MEM medium containing 4% FBS in 37°C in humified atmosphere enriched by 5% CO_2. Before exposition, the cells were suspended on 96-well plate in density 1 x 10^4 cells 100 μL^{-1} in MEM per well. The cells were cultivated for 24 h to obtained 80% confluency; and then exposed to tested samples, positive (sodium lauryl sulphate solution) and negative controls (FBS) after 24 h. Cell medium was removed and 6 x 100 µL sample, positive control, negative control, blank samples were added to the individual wells. The test article extract was prepared in 1 x MEM cell growth medium (MEM supplemented with 10% fetal bovine serum extract) at the sample to extraction medium ratio of 6.0 cm^2 mL^{-1} and extracted at 37±1°C for 72±2 h. The sample was unchanged by extraction procedure and the extract was clear and free of particulates. After incubation with samples, the MTT test was applied using in final step 2-isopropanol (100 µg L^{-1} $well^{-1}$) with simultaneous shaking. The absorbance was detected at 570 nm. Based on the acceptance criteria for the procedure, it was judged that the viability of cells was more than 70% after application of the test sample during 24 h.

All the experiments were performed in triplicate for each parameter, which gives 18 repetitions for each parameter. Data were represented as mean ± standard error of the mean. Data were analysed by two-way ANOVA (in GraphPad Software Inc., San Diego, CA, USA), with confident level of 0.05 as a statistically significant.

RESULTS AND DISCUSSION

The results of cytotoxic properties of dental materials tested are presented in Fig. 1 and 2. The negative control (MEM) and blank sample (MEM with 4% FBS) both demonstrated no cytotoxic effect, thus cell oxidoreductive potential was undisturbed. The positive control (sodium lauryl sulphate) demonstrated significant cytotoxic impact even after the shortest time of incubation (2 h). The viability was less than 20% after 24 h. Additionally, the cytotoxic effect of positive control has to be proven in all concentrations. The most cytotoxicity occurred in Adhesor samples (c.a. 5%) ($p < 0.05$) and the highest biosafety was observed for Mifam, Superacryl and TAB 2000 (more than 70%) ($p < 0.05$). The posted hypothesis was not confirmed, because some of the materials used in dentistry adversely affected the growth of cell cultures. In dental technology different kinds of materials are used. Some of them evaluated in current study had no cytotoxic properties (hot cured acrylic resins (Superacryl Plus, Mifam Super Lux). However, some literature reveal that some acrylic materials strongly inhibit cell growth (Moharamzadeh *et al*., 2009; Goiato *et al.,* 2015). Such difference can be related with the method of polymerization of acrylic materials, and thus the content of residual monomer which remains unpolymerized inside the material and show huge impact on cell cultures (Schweikl *et al*., 2006). For this reason, the contact of patients with leaching methyl methacrylate may cause allergic reactions (Moharamzadeh *et al*., 2009; Mallikarjuna *et al.,* 2014). Some of the MMA can be washed out of the material by post-polymerization methods, some may be eluted with saliva or other consumed food, so after some time the unpolymerized monomer content is reduced (Rashid *et al.,* 2015). The other explanation for this difference is the time that was passed since the moment when material was polymerized and the cytotoxicity test performed (Mallikarjuna *et al*., 2014).

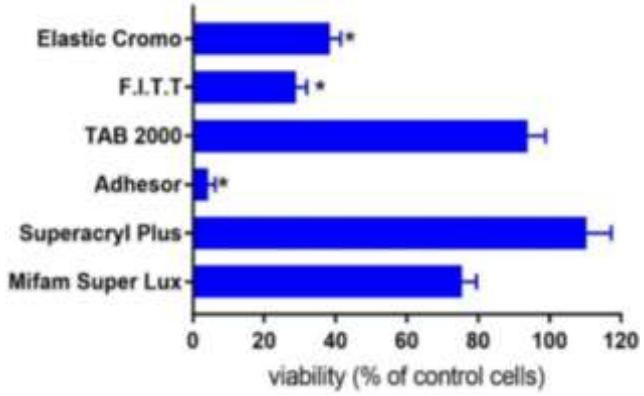

Fig. 1: Cytotoxic properties of dental materials are varied and related with the material composition [Control group 24 h exposition]

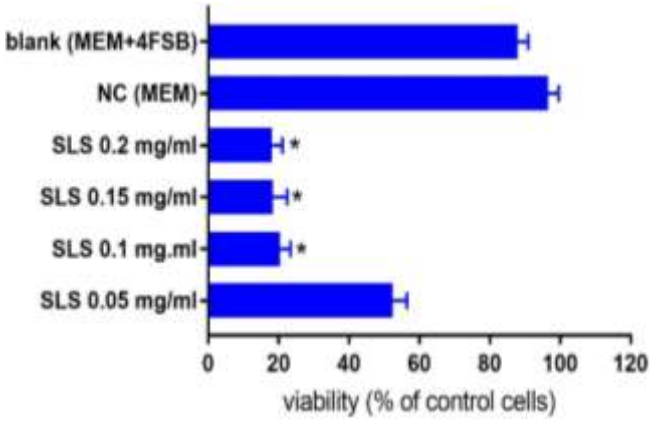

Fig. 2: Control cell viability Cytotoxic properies of dental materials 24 h contact

In case of impression materials such as alginates (Elastic Cromo) literature confirm their cytotoxicity; here the main factor may be the composition of materials (Pithon *et al.,* 2010; Pithon *et al.,* 2012). Time that elapse from the sample preparation to the exposition to the cell culture may also be important, as a large proportion of these materials react to form products with lower cytotoxicity. For example, sodium phosphate after reaction forms calcium phosphate, which is not irritant (Pithon *et al.,* 2010). The materials based on ZnO/phosphoric acid, which have successfully been used for over 150 years in dentistry as the first cement for filling. In the case of phosphate cements (in current study Adhesor), the literature describes them as cytotoxic materials (Mahasti *et al.,* 2011; Wassmann *et al.,* 2020; Diemer *et al.,* 2021). The low pH immediately after setting is the result of the interaction

of unpolymerized phosphoric acid. However, after a short period of 2-3 days, the pH rises to neutral. In this case, the place of application of the material as a cement or liner inside the teeth in too deep cavities is also very important. It is to avoid direct contact of such material with the pulp of tooth, without causing irritation (Behr *et al.,* 2009; Sahabi *et al.*, 2011; Koosha *et al.,* 2016; Marvin *et al.*, 2018).

The solvents (ethanol) contained in the material are often responsible for the cytotoxic properties as in the product F.I.T.T, where it is responsible for quickly gelling of the material on the denture. However, during clinical use, alcohol is washing out quickly within the first 24 h (Nishijima *et al.,* 2002; Abe *et al.*, 2003; Tay *et al.,* 2012; Lee *et al.,* 2018).

Conclusion:-The results obtained allow to make selection of appropriate raw materials, as the first screening test. However, if the cytotoxicity is achieved, this does not completely eliminate the product being developed as a medical device, but requires further appropriate testing, because materials in the oral cavity can have a completely different effect than on isolated cell cultures.

Conflict of interest: None. The research was carried out based on own funds.

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