Interactions Between Dental Composite Resins and Saliva
A comparative biochemical in vitro study

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This in vitro study analyses the biochemical interaction between saliva and three types of dental composite resins (a direct resin, an indirect resin and a dual-cure resin used for cementation of indirect dental restorations). The resin samples were obtained following a specific protocol and in line with the producers’ recommendations; the resin samples were incubated with saliva samples collected from 19 healthy volunteers. The obtained results showed that the tested composite resins did not produce significant changes in oxidative stress parameters that were analysed (albumin, uric acid, GGT / gamma glutamyl transferase, OXSR-1 / oxidative stress responsive kinase 1) and do not influence the inflammatory salivary status reflected by the levels of IL-6 – an inflammatory marker.

Keywords: dental composite resins, saliva, oxidative stress, biocompatibility

The composite resins represent a dental material with significant importance in today’s dentistry, offering excellent results and high biocompatibility with the oral environment. The specialised literature is rich in topics related to composite resins for dental use, addressing, especially, topics such as: the mechanical behaviour of composite resins; biocompatibility; correlations between the physico-chemical properties of composite resins and their biocompatibility; the elements released by the composite resins immediately post-polymerisation and at different time intervals; conversion rate; specific techniques to ensure the photopolymerisation depth; the adhesion shrinkage of composite resins; improvement of composite resins with certain substances that confer antisepctic abilities; the cytotoxic effect of dental composite resins on pulp cells or on gingival fibroblasts etc.

On the other hand, saliva is a valuable source of relevant information regarding the clinical status, as it contains salivary biomarkers specific to certain conditions (periodontal, peri-implant, malignant or oral mucosal disorders - for example oral lichen planus), and qualitative changes in the composition of these biomarkers could have a diagnostic value by identifying patients with susceptibility to the disease, by identifying the areas with active disease or by predicting sites that will have an active disease in the future and / or that shall serve as reference points for monitoring the effectiveness of the therapy [1-3]. These aspects are all the more interesting as medicine is increasingly focused on the idea of personalised treatment and monitoring of patients [4].

However, in the literature there are relatively few in vivo or in vitro studies that test the antioxidant activity of composite resins for dental use. In this scientific research, we have analysed the biochemical interaction between saliva and three different dental composite resins: a resin used for obtaining direct dental restorations, a resin used for obtaining indirect dental restorations, as well as a composite resin used for cementation of indirect dental restorations. In the study, certain salivary parameters are evaluated – albumin, uric acid, GGT / gamma glutamyl transferase, OXSR-1 / oxidative stress responsive kinase 1 - for the purpose of analysing the antioxidant activity of these composite resins, as well as the levels of IL-6.

Experimental part
Sample preparation
The dental composite resins tested in this study were as under: composite resin for direct dental restorations - GC G-ænial Posterior (GC Corporation/Tokyo/Japan); composite resin for indirect dental restorations - Gradia Lab Indirect Restoration System (GC Corporation/Tokyo/Japan); composite resin for cementation of indirect dental restorations - G-Cem Link Force (dual cure resin cement) (GC Corporation/Tokyo/Japan) –<br>GC G-ænial Posterior (GC Corporation/Tokyo/Japan) is a radio-opaque photopolymerisable composite resin, with outstanding aesthetic results, high resistance to cracking and bending and a low elasticity coefficient. The material is made up, in synthesis, of organic matrix (UDMA / Urethane dimethacrylate / dimethacrylate co-monomer),
filler (silica, strontium and fluoro lantanoid, fluoro-aluminosilicate, silica vapours), pigment and photoinitiators [5,6]. GC Gradia Plus HB / Heavy Body (GC Corporation / Tokyo / Japan) is a nano-hybrid composite, photopolymerisable, with ultra-fine fillers with very high density and homogeneous distribution, mixed in the resin matrix, which gives it outstanding mechanical properties. Its resistance to abrasion and fracture is considered to be superior to other indirect composite resins [7, 8]. G-CEM Link Force (GC Corporation / Tokyo / Japan) is a universal cement, ideal for use when additional retention is required, including for CAD/CAM, ceramic or hybrid prosthetic restorations. It is useful for fixing more opaque or dense dental restorations, as it has an efficient self-polymerisation mode. The dental resin composite samples, obtained according to a predetermined protocol, had the form of a cylindrical disc, 30mm in diameter and 2mm thick, being made with some conformers of metallic alloy (Cr-Co), obtained by CAD/CAM technology by milling (subtractive method) (fig. 1). In obtaining the composite resin samples, the exact recommendations of the producers were respected. We obtained two samples from each type of composite resin. The codings established for the composite resin samples were as follows: DR = directly composite resin; IR = indirectly composite resin; CR = composite resin for cementation.

Saliva collection

The selection of patients participating in this study was performed at the Prosthetics Clinic of the Faculty of Dental Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, by two specialist dentists, following a pre-established protocol. Samples of 1.0 - 2.0 mL of unstimulated saliva were obtained from 19 healthy volunteers, in the morning, between 9 and 10 AM. The saliva samples were collected in special sterile test containers. All subjects were asked not to eat, brush their teeth or use mouth rinse for at least 2 h prior to sample collection. All participants in this scientific research voluntarily accepted to be included in the study and signed an informed consent.

Saliva analysis

After collection, saliva samples were immediately incubated for 12 hours, at 37°C, with samples of dental materials (one dental material sample / 500µL of saliva). After the incubation period, the saliva samples were centrifuged for 10 min at 3000 rpm to remove bacterial and cellular debris. After collection, the saliva control samples were immediately centrifuged for 10 min at 3000 rpm to remove bacterial and cellular debris. All the determinations were performed using the supernatant. Salivary uric acid, GGT, albumin, OXSR-1 assayed in the incubated and control samples, immediately after sample centrifugation. The concentrations of all salivary parameters were expressed relative to the salivary concentration of albumin in order to avoid the salivary flow influence. Salivary albumin, uric acid and GGT were measured using analysing kits from Biosystems (Barcelona, Spain) on a biochemistry automatic analyser (oxidative stress responsive kinase 1) and of IL-6.

The experimental results show that the tested composite resins do not alter the antioxidant capacity of the incubated saliva, meaning that they do not influence the oxidative stress in the oral environment or the inflammatory status as reflected by the levels of IL-6.

The biocompatibility of composite resins has been demonstrated in vivo, on experimental animals (mice), the results of the experiments showing that they do not induce cytological changes in the microscopic and haematological tests [9].

Another study [10] investigated the potential for proliferation and differentiation of pluripotent mesenchymal cells by interacting with some resin-based restorative materials, using a typical pluripotent precursor cell line, C2C12 (RIKEN Cell Bank, Tsukuba Science City, Japan).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Uric acid mg/mg alb.</th>
<th>GGT U/mg alb.</th>
<th>OXSR-1 mg/mg alb.</th>
<th>IL 6 pg/mL</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR</td>
<td>1.83 ± 0.22</td>
<td>3.2± 1.2</td>
<td>0.53± 0.04</td>
<td>&lt;5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>IR</td>
<td>1.89±0.29</td>
<td>3.4±1.4</td>
<td>0.6±0.04</td>
<td>&lt;5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>CR</td>
<td>1.84 ±0.25</td>
<td>5.3±0.9</td>
<td>0.58±0.06</td>
<td>&lt;5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Control</td>
<td>1.81±0.26</td>
<td>3.4±1.1</td>
<td>0.52±0.07</td>
<td>&lt;5</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Table 1

EXPERIMENTAL DATA EXPRESSED AS MEANS - SD
The composite resins based on bis-GMA (bisphenol-A glycidyl methacrylate) / triethylene glycol dimethacrylate (TEGDMA) and resins based on 4-methacryloxyethyl trimellitic anhydride (4-META) / methyl methacrylate (MMA) have better biocompatibility for the C2C12 cells than the glass-ionomer cement modified with resin containing HEMA (hydroxyethyl methacrylate), suggesting a potential advantage of using both types of resins.

Walters et al. (2016) studied composite resins containing urethane dimethacrylate (UDMA) or bisphenol A glycidyl methacrylate (Bis-GMA) with poly (propylene glycol) dimethacrylate (PPGDMMA) or triethylene glycol dimethacrylate (TEGDMA). It has been found that UDMA (urethane dimethacrylate) has a significantly better degree of conversion, biaxial bending resistance and depth polymerization rate, as compared to Bis-GMA, without an increased adhesion shrinkage. It has been suggested that PPGDMA exhibits enhanced cytocompatibility compared to TEGDMA [11].

Other authors [12] confirmed that all monomers have a dose-dependent cytotoxic effect, but the hierarchy of cytotoxicity following the assays was GMA > TEGMA > HEMA. This toxicity induced by the resin monomers was significantly reduced by co-treatment with NAC (N-acetylcysteine), an antioxidant. The authors also confirm a dose-dependent genotoxicity of the resin monomers, which induced the formation of multinucleated cells in fibroblast cells. Similar to the effects on cytotoxicity, NAC (N-acetylcysteine) reduced the number of micronuclei in the cells, as compared to the resin monomers. The preventive effects of NAC were also observed against the apoptosis induced by the monomer in the pulp cells; furthermore, it is noted that glutathione depletion and oxidative stress are responsible for the mutagenicity and apoptosis induced by GMA, TEGMA and HEMA.

The same fact is supported by another study [13], which showed that monomers reduce the level of naturally occurring glutathione (GSH), which protects the cell structure from destruction caused by reactive oxygen.
species (ROS). Intracellular depletion of glutathione may contribute significantly to cytotoxicity, as increased levels of ROS may activate pathways leading to apoptosis.

The compositions of composite resins have been permanently improved over time with various substances that increase their quality; for example, because composite resins used to restore dental hard tissue accumulate biofilm, quaternary ammonium poly (ethylene imine) / QAP-PEI nanoparticles have been developed for an additional antibacterial activity of composite restorative resins [14].

The results we obtained in this study are favourable, being, generally, in agreement with those obtained by other studies found in the specialised literature; the problem related to the interaction between composite resins and saliva, to the biocompatibility of composite resins, is associated with pros and cons, which may or may not be found in the results of the present study.

Thus, in the specialty literature, there are scientific papers that highlight the toxic effects of composites on dental or gingival pulp cells. Restoration materials based on composite resins release monomer immediately after the adhesion but also at a distance in time; in addition, residual monomers stimulate the development of karyogenic bacteria at the interface between the restorative material and the cavity walls [15,16], which favours the appearance of secondary caries and the marginal degradation of restorations. Sisman et al. [17] confirm in a study in the cytotoxicity of five composite resins (of the bulk fill composite resins type) that the viability of pulp stem cells in WST-1 (Water-soluble tetrazolium 1 / WST-1 assay) analysis was reduced during the incubation period. On the other hand, Goncalves et al., in 2018, [18] conclude that it is reasonable to conclude that BPA is present in WST-1 ( Water-soluble tetrazolium 1 / WST-1 assay ) of the additives that can induce cytotoxicity [20]. The presence of dental composites may be associated with the release of monomer immediately after or an hour after their placement. Celik N et al. [24], studied, in another train of thoughts, that the cytotoxic and oxidative effects manifested in the gingival fibroblasts by four dental restoration materials: a microhybrid composite resin, a compomer, a glass ionomer and a silver amalgam. They studied the total antioxidant capacity (TAC) and the total oxidant status (TOS); the highest level of TAC was present for the glass ionomer - after 7 days, which prevented an increase of TOS; finally, it was appreciated that all the tested materials showed a cytotoxic and pro-oxidative potential.

The cytotoxic effect of the composite resins used in direct restorations on neuronal cells has been shown to be greater than that shown on the pulp cells or on the gingival fibroblasts: in this study, it is specified that all the analysed resins showed a certain cytotoxic effect ( some cytotoxic effects ) and the production of IL-6 increased [25].

Another important aspect is that regarding the subgingival placement of the odontal restorations; the composite resins may interfere with gingival healing, with the restoration of gingival insertion, affecting the periodontal status. In a relatively recent study [26], the MTT test (a colorimetric test that highlights the metabolic activity of cells) was used to analyse the cell adhesion of gingival fibroblasts and osteoblasts on samples of composite resins with the surface modified or not by sand blasting (50 𝜇m or 110 𝜇m Al₂O₃), as well as chromatography (high-performance liquid chromatography / HPLC) to determine the release of residual monomer after 1 hour, 1 day and - respectively - 7 days. Compared with the control group, the cell adhesion of fibroblasts and osteoblasts was significantly lower when testing the selected composite resins, without major differences between the different types of resins; the amount of residual monomer was reduced, but sufficient to indirectly affect the cell adhesion.

Dental materials’ pro or antioxidant activity in saliva has been also analysed in the context of biochemical interactions of polymeric resins used for occlusal splints and saliva [27]: 3D printed and milled polymeric resins do not influence the redox status markers (uric acid, TAC, GGT, OXSR-1) or inflammatory markers (IL-2, IL-6), but the self-cured acrylic resin produced an important decrease in the salivary TAC and uric acid.

Regarding the composite resins used for fixing the indirect restorations, it is appreciated, after some studies, that the dual-cure resins have a significantly lower toxicity than the self-adhesive resins; in addition, the eventual cytotoxicity of the cementum is reduced over time, due to the buffering effect attributed to saliva [16, 28].

There are permanent, interdisciplinary concerns in the current scientific research, regarding the development and study of dental materials, and their biocompatibility, their interaction with the oral environment are topics of interest [29-31]. The scientific research carried out in this paper highlights, joining other confirmations in the literature, that the composite resins studied do not induce significant statistical changes (p > 0.05) of the levels of the studied parameters: (uric acid, GGT / gamma glutamyl transferase, OXSR-1/ oxidative stress responsive kinase 1) and of the levels of IL-6.

In the study performed by Kloukos et al. [22], it is considered that it is reasonable to conclude that BPA is released after placing some sealants in the oral cavity and that the largest quantities are detected in the saliva immediately after or an hour after their placement.

Relatively recently, in 2017, Berge et al., [23] argue that the presence of dental composites may be associated with a slightly higher concentration of unconjugated BPA in the saliva. However, further studies using sensitive analytical methods are needed before firm conclusions can be drawn. The influence of other factors such as food consumption and time of the day for collecting saliva samples should be also considered. It is concluded that, in fact, the relative contribution of existing polymer-based dental fillings to total BPA exposure appears to be low.

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saliva and do not modify the inflammatory status. These results have clinical-practical applicability, joining those of the specialized literature that confirm, increasingly, the biocompatibility of modern dental composite resins.

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References

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