Effects of composite restorations on nitric oxide and uric acid levels in saliva

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Abstract

Background and Aims: Dental materials that are used in dentistry should be harmless to oral tissues, and should, therefore, not contain any leachable toxic and diffusible substances capable of causing side effects. This study was intended to investigate the effects on salivary nitric oxide (NO) and uric acid (UA) levels after application of dental composite filling materials to healthy volunteers. **Materials and Methods:** A total of 52 individuals (32 female and 20 male) participated in the study. Filtek Z250 composite filling material (3M ESPE, St Paul, MN, USA) was applied to healthy volunteers. Saliva samples were collected before restoration (baseline) and 1 h, 1-day, 7 days, and 30 days after restoration. NO concentrations were measured using the Griess reaction method, and UA was measured using an enzymatic method. Data were analyzed using repeated measures ANOVA and the Bonferroni *post-hoc* test (α =5%). **Results:** NO values increased statistically significant after 7 days ($P < 0.05$). In addition, lower UA levels were determined compared to the baseline levels, but the difference was not statistically significant ($P > 0.05$). There was no correlation between NO and UA levels in saliva ($P > 0.05$). **Conclusion:** Composite resins activated the antioxidant system in saliva. However, further studies are now needed to confirm our findings and to permit a definitive conclusion.

Keywords: Composite resin, nitric oxide, saliva, uric acid

Introduction

Composite resins have improved significantly in structural terms in parallel with advances in adhesive technologies, and the use of these resins as an alternative to amalgam fillings has become widespread. Composite resins include the resin matrix, inorganic fillings, and bonding agent in general.[1] Dimethacrylates such as bisphenol-A diglycidyl methacrylate (Bis-GMA) and urethane dimethacrylate (UDMA) are the most widely used ingredients in the structure of the composite material. Triethyleneglycol dimethacrylate (TEGDMA) is added composite resins as a diluent and is present in certain bonding agents. Hydroxyethyl methacrylate (HEMA), found in bonding agents, also functions as a diluent, similarly to TEGDMA.[2]

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Resin-based materials can be polymerized chemically or by visible light. Not all monomers in composite materials are converted into polymers during the polymerization phase. Low levels of monomers are released into the environment immediately after polymerization. These unreacted monomers are known as residual monomers, and the release of these substances deteriorates the mechanical structure of the material and produces an adverse effect on biological properties.[3,4]

Secretions from several salivary glands produce saliva, a very complex biological fluid. Numerous compounds such as proteins, peptides, amino acids, hormones, electrolytes, and lipids are present in the structure of saliva.[5] Consisting of various molecules and enzymes, saliva acts as an antioxidant system. The most important of these molecules is the uric acid (UA), which constitutes approximately 70% of the total salivary antioxidant capacity. UA is, therefore, one of the most crucial antioxidant molecules in saliva. However, lipid soluble antioxidants only provide 10% of the total salivary antioxidant capacity.[6,7] UA is an end-product of purine metabolism and is produced in mammalian systems. UA exhibits free-radical-scavenging properties and is regarded as the most available antioxidant in human plasma.[8]

Nitric oxide (NO) is a crucial molecule for biological systems because of its physiological and pathophysiological mediator properties. There are two aspects to known biological functions of NO. The first, it acts as an endothelial-derived relaxer of vascular smooth muscle and inhibitor of platelet aggregation and adhesion. The second, it is a cytotoxic molecule influencing the ability of cells to kill bacteria, viruses, and protozoa, as well as tumor cells, by activating macrophages. The damaging effects of NO on cellular proteins, DNA and lipids are well known; and can lead to cell

death, tissue injury, and organ failure.[9,10] The antibacterial effects of NO are also well known. The molecule easily passes through cell membranes and can stimulate damage of micro-organisms through various mechanisms.[11] The production of highly toxic peroxynitrite and DNA damage are an example of the results of biological oxidation in mitochondria.[12] In order to produce peroxynitrite, and toxic effects on DNA and the enzymes involved in DNA repair, both NO and superoxide radicals must be present.[13]

This study was intended to investigate the effects of the composite filling material on salivary NO and UA levels after dental composite filling materials were applied to healthy volunteers. The null hypothesis of this study was that composite filling materials have no effect on human salivary NO and UA levels.

Materials and Methods

Ethical approval was received from the study, which was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from each of the participants prior to the study. The selection criteria for the study samples were: Age between 18 and 25, a negative previous medical history, not currently being in receipt of any medications, no previous fillings, nonsmoker status, no alcohol use, and no periodontal problems. A detailed examination of all teeth was carried out by a dental surgeon. The decayed, missing, and filled teeth (DMFT) index, which is based on the number of decayed, missing, and filled teeth, was used to classify the risk group. The study group consisted of participants with low levels of caries (DMFT score <1).

Eighty-four single-surface composite restorations were performed on 52 individuals (32 female and 20 male). The composite material used for dental restorations was Filtek Z250 (3M ESPE Dental Products, St Paul, MN, USA) color A2, lot N152614. The composition of the composite resin, as given by the manufacturer, was: TEGDMA 1–5%, Bis-GMA 1–5%, Bis-EMA 5–10%, and UDMA 5–10%. The adhesive Single Bond (3M ESPE Dental Products, St Paul, MN, USA) was used as a bonding agent. According to material safety data sheets (MSDS), single bond contains Bis-GMA 10–30%, HEMA 5–25%, and dimethacrylates 7–28%. The etchant was 38% phosphoric acid (Scotchbond Etchant gel; 3M ESPE). All dental treatments were performed at the same clinic by the same dentist. The restorative material was applied to cavities in line with the manufacturer's instructions. The composite filling material (Filtek™ Z250) was given an anatomical form inserting it into cavities in one piece not exceeding 2 mm in depth, followed by polymerization for 40 s with a light source (Elipar Freelight II, 3M-ESPE, St. Paul, MN, USA). The wavelength of the light source was between 430 and 480 nm, and the light intensity was approximately 1200 mW/cm^2. During the polymerization process, the tip of the light source was kept as close as possible to the restoration. The intensity of the light device was measured using a radiometer (Hilux Ultra Plus Curing Units, Benlioglu Dental, Ankara, Turkey). Finishing and polishing operations were completed using disks (Sof-Lex, 3M ESPE, St. Paul, MN, USA).

Unstimulated whole saliva samples were collected before restoration (baseline) and 1 h, 1-day, 7 days, and 30 days after restoration. For saliva collection, the patient was seated in a relaxed position with the head bent forward to allow saliva to accumulate in the anterior part of the oral cavity. The patient swallowed and saliva was then collected for 15 min in Eppendorf tubes. The participants had been instructed not to eat or to drink (water was allowed) for 2 h before saliva sample collection. Each specimen was centrifuged at 3000 rpm for 10 min, and the supernatant was used as the saliva sample for analysis of its components. All samples were stored at −80°C until analysis.

A commercial kit supplied by Roche Diagnostics GmbH, Sandhofer Strasse 116, D68305 Mannheim, USA was used to determine UA concentration in the saliva samples. NO concentrations were measured using the Griess reaction method.[14] Briefly, to 50 μL of saliva sample were added 100 μL of 14 mM sulfanilamide in 2 N HCl, 100 μL of 4 mM N-(1-naphthyl)-ethylenediamine (NED) in water, and 750 μL of 0.2 M KCl-HCL (pH 1.5). The samples were incubated at 37°C for 10 min and were then centrifuged at 5000 rpm for 10 min. Absorbance was measured at 540 nm, and sodium nitrite was used as a standard.

Statistical analysis

Statistical analysis was performed SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) software. Repeated measures analysis of variance was used in the assessment of oxidative status over time, and in the case of differences the Bonferroni *post-hoc* test was applied to determine the source. The independent-samples *t*-test was used to determine, if there was a statistically significant difference in enzyme levels between the sexes. Pearson's correlation analysis was used to examine the association between variables (α =5%).

Results

The independent-samples *t*-test revealed higher NO levels in saliva in females (12.94 ± 5.31 μM) than males (12.29 ± 4.03 μM), ($t = -0.470$, $P = 0.641$). However, higher UA levels were determined in males (3.59 ± 1.95 mg/dL) than in females (2.89 ± 1.72 mg/dL). The differences were not statistically significant ($t = 1.360$, $P = 0.180$).

Table 1 shows the means and standard deviations of NO and UA levels in saliva samples for different time periods. No statistically significant differences were determined among NO values measured at baseline, 1 h and 1-day after restoration ($P > 0.05$). A statistically significant difference was determined between NO values measured 7 days after

Table 1: Mean±SD values of NO and UA quantified in saliva samples baseline and after treatment (1 h-30 days) (*n*=52)

	NO (μM)	UA (mg/dL)
Baseline	12.69±4.82[a]	3.16±1.82[ab]
1 h after treatment	11.34±5.62[a]	3.32±1.75[b]
1-day after treatment	12.64±9.51[a]	2.94±1.68[a]
7 days after treatment	22.81±4.49[b]	2.95±1.66[ab]
30 days after treatment	19.65±7.47[b]	2.61±1.67[a]
P	0.001	0.001

Within each column, means with the same superscript letters are not statistically different from each other ($P<0.05$). NO: Nitric oxide; UA: Uric acid SD: Standard deviation

restoration ($P < 0.05$). However, no statistically significant difference was observed between NO values measured 7 days and 30 days after restoration [$P > 0.05$, Table 1 and Figure 1]. Analysis performed for UA revealed no statistically significant difference between the groups ($P > 0.05$). A small increase was observed in UA values measured 1 h after restoration [$P < 0.05$, Table 1, Figure 2]. There was a negative correlation between NO and UA levels in saliva but not a significant one. Pearson correlation was −0.059, and significant (2-tailed) was 0.347.

Discussion

This study evaluated the effects on salivary NO and UA levels within scheduled time intervals following the application of a composite filling material. Our review of the literature revealed no previous studies examining the effects of composite filling material on salivary NO and UA levels.

Even though the composite structure is stable, it can be degraded due to inadequate polymerization and the effect of oral fluids.[3] As a result, residual monomers, filling particles, and other components can be released into the oral environment.[15] These products pass into the saliva, come into contact with the mucosa and can even enter the dental pulp through dentin tubules.[16] Saliva was examined in this study because it is the first medium to come into contact with the composite restoration.

Previous studies have investigated the effects of residual monomers released from restorative materials on cariogenic microorganisms. These microorganisms cause secondary caries by entering between the restorative material and the cavity wall as a result of polymerization shrinkage.[17] Residual monomers serve as a good substrate for these microorganisms. The effect of composite fillings on plaque accumulation and on increasing gingival plaque flora has been reported to exacerbate gingival inflammation and to trigger the release of inflammatory cytokines by the host against that inflammation.[18,19]

Decreased oral cavity oxygen tension facilitates bacterial survival. Antimicrobial oxides of nitrogen produced by the oral immune system may also stimulate this pathogenic process. An increased intake of nitrate is known to reduce the ability to convert nitrate into nitrite, which is a protective pathway against cariogenic bacteria. Bacteriostatic and bactericidal effects against acidogenic and cariogenic, Streptococcus mutans have been observed due to acid production in the presence of nitrite.[20]

NO metabolites are responsible for indicating oxidative and nitrosative stress in the human body.[21,22] NO is produced via the action of nitric oxide synthase (NOS). Small amounts of NO are thought to be physiological and protective, whereas large amounts of NO, produced by inducible NOS are proinflammatory and injurious.[23] Moreover, the NO interface with oxygen radicals such as superoxide, leads to the formation of new compounds (like peroxynitrite) with a greater capacity to damage cells.[24] *In vitro* studies have shown that levels of intracellular reactive oxygen species (H_2O_2, superoxide anion, and hydroxyl radical) increase when exposed to TEGDMA, HEMA, or other substances released from composite resins.[25,26] In our study, the increasing NO level at scheduled time intervals may be associated with residual monomers released into saliva after restoration. Larsen and Munksgaard[27] asserted that esterase and hydrolase enzymes in saliva and organic acids in food can attach to materials with a resin–based surface and may lead to softening and dissolution. The activity of these enzymes can vary among individuals. Finer and Santerre[28] determined that pseudocholinesterase and cholesterol esterase enzymes can exist at excessive levels in human saliva such as to deteriorate the structure of the composite material.

Despite the differences between the methods used, resin-based materials have been known to be cytotoxic for more than a decade.[29] Cell culture studies have revealed that the monomers released from resin-based materials produce reactive oxygen species and affect the redox balance in the cell.[30] *In vitro* studies have shown that levels of intracellular reactive oxygen species increase when exposed to TEGDMA, HEMA, or other substances released from composite resins.[25,26] Previous studies have reported that the monomers released from composite filling materials increase bacterial growth and cause glutathione depletion, which is a key factor leading to pulp or gingival cell apoptosis at the molecular level and induces the production of reactive oxygen species while causing various allergic reactions.[29]

UA has the ability to bond with NO to form 6-aminouracil, clinically important. This mechanism may help explain the inhibition of endothelial function by UA under conditions of oxidative stress in which intracellular glutathione is depleted. It may also help explain the role of UA as antioxidant in

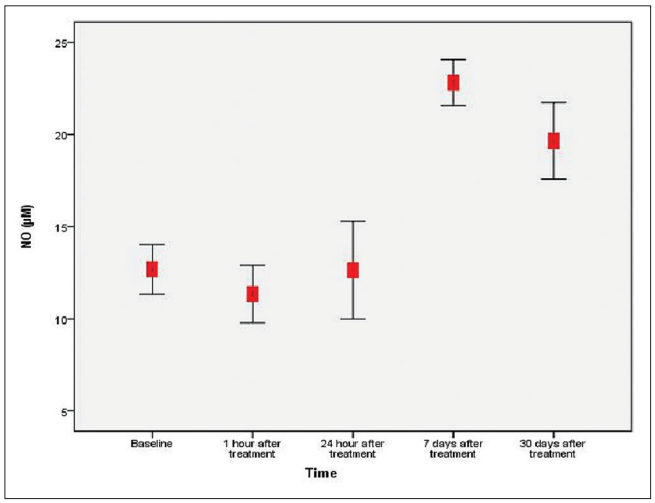

Figure 1: The changes of nitric oxide levels for different time periods

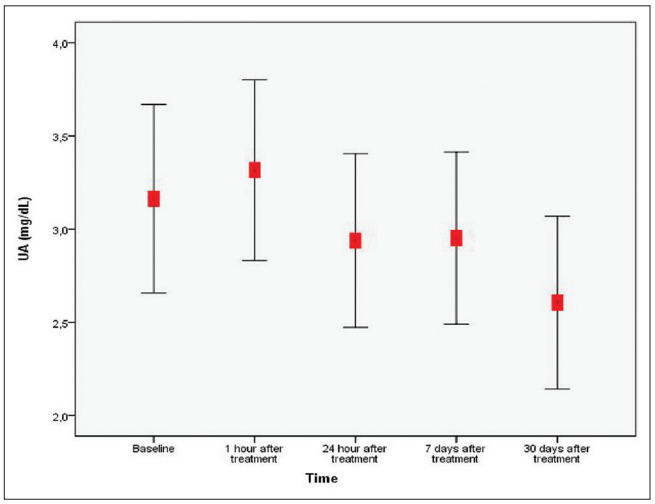

Figure 2: The changes of uric acid levels for different time periods

inhibiting the ability of exogenous peroxynitrite to uncouple endothelial NO synthase. However, high UA concentrations are associated with reduced intracellular NO levels.[8]

In our study, NO levels increased after 1-week of treatment and UA levels increased after 1 h of treatment. Other studies in the literature have also described NO level increases in terms of oxidative stress conditions. Our study suggests that the increased NO level may be caused by bioactive materials released into saliva from the composite filling. UA levels may have decreased in order to reduce the accumulated oxidant products (peroxynitrite, etc.) Even 1-month after treatment NO levels were still significantly higher than baseline. This situation is due to continual monomer release into saliva. Monomers released into saliva subsequent to an application of composite filling material were measured at specific time intervals (1 h to 30 days) in a previous study.[31] The amount of monomers released into saliva exhibited a statistically significant increase within 7 days, and the maximum amount of release was generally observed at 7 days.

In our study, UA levels were lower than at baseline 1-month after treatment, which may be associated with the increased NO levels. In addition, there was a negative albeit insignificant correlation between NO and UA levels in saliva ($r = -0.059$, $P = 0.347$). We suggest that decreased levels of UA, the most abundant antioxidant in saliva, may be caused by increased reaction with NO due to increase NO levels.

Conclusion

The hypothesis set out in the introduction that composite filling materials have no effect on human salivary NO and UA levels, was disproved. Within the limitations of this *in vivo* study, composite fillings affect the oxidative redox balance. However, more studies are needed to confirm our findings and to reach a definitive conclusion.

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