

Bisphenol A Blood and Saliva Levels Prior To and After Dental Sealant Placement In Adults

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Introduction

Occlusal sealants in permanent molars demonstrate caries-preventive effects, lasting 15 to 20 years.^{1,2} Dental sealants differ from restorative composite fillings. Unfilled pit and fissure dental sealants contain only the dimethacrylate resin component of composite dental materials made of an organic monomer, bisphenol A-diglycidyl methacrylate (bis-GMA). This is the most commonly used resin matrix which is formed by reacting glycidyl methacrylate with bisphenol A (BPA).³ Additional monomers, including acrylates and methacrylates, may be added to bis-GMA to dilute the resin and make the sealant material more flowable.⁴ One of the most common monomers added to bis-GMA is BPA, which is a hormonally active, synthetic chemical and part of a broad group of chemicals known as endocrine disrupting compounds.⁴ More specifically, BPA is a xenoestrogen, which mimics the relative bioactivity of estrogen.⁵

Among all xenoestrogens, BPA has received increased attention due to its pervasive presence in the environment and ubiquitous human exposure. BPA is used in the manufacture of polycarbonate plastics and epoxy resins and leaches from food and beverage containers, baby bottles, children's toys and dental sealants.⁶⁻⁸ BPA leaches from some formulations of dental sealants, if not completely polymerized, may be released into the oral cavity as a result of enzy-

Abstract

Purpose: This study examined the effects of a widely used (Delton® Pit & Fissure Sealant – Light Cure Opaque, DENTSPLY Professional, York, PA) pit and fissure sealant material on bisphenol A (BPA) levels in blood and saliva, among both low and high-dose groups over time.

Methods: A convenience sample of 30 adults from the Old Dominion University population were randomly and evenly divided into 2 independent variable groups: a low-dose group (1 occlusal sealant application) and high-dose group (4 occlusal sealant applications). A 2 group, time series design was used to examine the presence and concentration of BPA in serum and saliva after sealant placement. Differences comparing low-dose and high-dose groups were examined 1 hour prior (baseline), 1 hour post, 3 hours post and 24 hours after sealant placement, as measured by a direct-competitive BPA Enzyme Linked ImmunoSorbent Assay (ELISA). Hypothesized outcomes were evaluated by applying a parametric, 2 way ANOVA for repeated measures technique to data on the 30 participants ranging in age from 18 to 40 years, and were of mixed gender and ethnicity.

Results: BPA was detected in the saliva of all participants prior to sealant placement and ranged from 0.07 to 6.00 ng/ml at baseline. Salivary BPA concentration levels peaked over a 3 hour period following sealant placement and returned to baseline levels within 24 hours. BPA was significantly elevated at all post-sealant placement time periods for both the low-dose (1 occlusal sealant application) and high-dose (4 occlusal sealant applications) groups with peak levels of 3.98 ng/ml and 9.08 ng/ml, respectively. The blood serum did not contain BPA at any point in this investigation.

Conclusions: Exposure to BPA from sources other than dental resins contributes to salivary baseline concentration levels and indicates environmental exposure and use of products containing BPA. Use of specific molecular formulations of dental sealant material determines the release of BPA, therefore, dental sealant materials should be reviewed independently when questioning the release of BPA from dental sealants. In addition, dosage amounts of the dental sealant material used in this study do not influence the serum concentration levels of BPA. Further research is needed to examine the cumulative estrogenic effects of BPA from dental sealants.

Keywords: sealants, dental, bisphenol A, estrogenic

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matic activity within saliva, and may be systemically absorbed by the patient.⁹⁻¹¹

Perinatal low-dose exposure to BPA results in functional and morphological alterations of the rodent

genital tract and mammary glands, which may predispose the tissue to earlier onset of disease, increased infertility and mammary and prostate cancer, as demonstrated *in vitro*.¹² Fluctuations in hormonal exposure, especially estrogen during fetal development, is also thought to be a factor in prostate, breast and uterine cancers.^{13–16} Although research shows that BPA leaches from the dental sealant into the saliva, the idea that it may be absorbed systemically into the blood or may have cumulative effects in the body should be a concern to all oral health care professionals because of the known xenoestrogenic effects of BPA.

It is crucial for dental professionals and the public to know if pit and fissure dental sealants that contain BPA pose a hidden risk to BPA exposure. This study measured BPA in the serum and saliva of adults after placement of dental sealants and the rate and time BPA might be released from a light cured dental sealant.

Dental Sealants

Dental caries is a preventable disease, but still remains the most common chronic disease of childhood in the United States, occurring 5 to 8 times more frequently than asthma, the second most common chronic childhood disease.¹⁷ According to Healthy People 2010, focus area 21, the increased use of dental sealants and fluoridated toothpastes, community water fluoridation and stable dietary practices are all needed to continually reduce dental caries rates in the United States.¹⁷

Biochemistry of Dental Resin

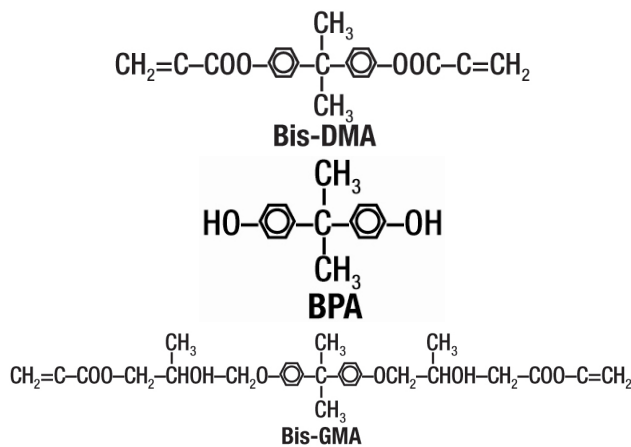
Most dental sealants contain an organic resin matrix only, and therefore differ from resin composite material. The commonly used resin matrix bis-GMA has a foundational structure similar to an epoxy resin with a methacrylate group attached to each end of the carbon molecule, thus, bis-GMA is known as a dimethacrylate.¹⁸ Monomers are added to dilute the viscous bis-GMA and enhance flow and mixing abilities.⁴ These

lower-molecular weight monomers may include triethylene glycol dimethacrylate (TEGDMA), ethylene glycol dimethacrylate (EGDMA) and BPA. If additional BPA is added to bis-GMA, dimethacrylate (BPDMA, bis-DMA) is created.¹⁹ All monomers, including BPA, which are added to bis-GMA are based on carbon-carbon double bonds and react by polymerization, which links compatible monomers into a larger molecule called a polymer (Figure 1). As a result of the process of polymerization (curing), a chemical by-product is produced following the hardening of dental sealants.^{18,19} This by-product presents itself as a tacky surface layer which varies in depth with different sealant products.²⁰ This layer of unpolymerized resin has been associated with BPA controversy.

Pharmacokinetics of Bisphenol A

Olea et al⁸ provided one of the earliest cell culture studies regarding the possible estrogenic activity of both bis-GMA resin-based composites and resin-based dental sealants. These bis-GMA materials included 3 different brands of composite resin and 2 batches of a single brand of a dental sealant material. To determine the estrogenicity of bis-GMA-based resins used in this study, experiments were conducted both *in-vivo* and *in-vitro*. The cell yield obtained with a 5 µg/ml sealant sample was 6-fold greater than in the control cultures, and demonstrated proliferative effects equal to the most potent estrogen hormone, estradiol. In contrast to the sealant sample, cell proliferation did not occur when exposed to the 3 resin-based composites at a maximum concentration of 1 mg/ml.

Figure 1. Chemical structure of bisphenol A and related compounds



The authors attributed these low cell proliferation rates of the composite resin to the high proportion of inorganic filler used in the formulation. As confirmed by the analysis profile, BPA and BPA dimethacrylate were present in all sealant samples. The study by Olea et al⁸ initiated concern in the dental community and prompted dental researchers to examine the xenoestrogen threat from bis-GMA-based materials used in dentistry.

At the inception of the current study, only 1 other *in vivo* research project had been reported. The study was conducted by Fung et al,⁹ and it evaluated whether BPA was being released from a particular dental sealant product. BPA in saliva was detected at a concentration of 5.8 to 105.6 ppb, collected at 1 and 3 hours after sealant application. This rate differed from findings of Olea et al, which revealed 3,300 to 30,000 ppb.⁸ Fung did not detect BPA in saliva after 3 hours, and was not able to detect the compound in blood specimens.

A recent study evaluating the release of BPA following the placement of dental sealants was published in 2006 by Joskow et al.¹¹ This study examined 3 different brands of sealant material. The researchers examined BPA in saliva and urine samples at varying time intervals. One of the sealant material released more BPA than the other brands of dental seal-

ant material used in this study. The authors emphasized that the American Dental Association (ADA) only grants its Seal of Acceptance to those materials that do not release detectable levels of BPA (>5 ng/mL) prior to the cessation of this program.^{11,21,22} The ADA recognizes that laboratory research demonstrates a xenoestrogenic effect from BPA that may affect reproduction and development. Based on present research evidence, BPA has no effects observed in humans. Although BPA is present in biological specimens after the placement of dental sealant, the ADA states that mere presence of this xenoestrogen in the environment or in human blood or urine samples “does not mean that the substance is necessarily causing harm.”²²

Effects of xenoestrogens and fluctuations in estrogen exposure have become the focus of current research. Scientific data suggests that changes in the fetal environment may predispose individuals to disease and/or organ dysfunction, which may not become evident until adulthood.^{13-15,23} Scientists hypothesize that fetal exposure to environmental estrogens may be the underlying cause in an increased incidence rate of some cancers, including breast and testicular cancer.^{2,23} For example, women aged 40 years and older who were exposed in utero to diethylstilbestrol (DES) have a 2.5-fold increase in the incidence of breast cancer when compared to unexposed women of the same age.^{24,25}

BPA is present in the environment and is the principal monomer used to manufacture polycarbonate plastic at a rate of 6.4 billion lbs/year.²⁶ Heat and contact with acidic and basic compounds accelerate hydrolysis and degradation of the ester bond linking BPA molecules in polycarbonate and resins. Heating of cans to sterilize food and repeated washings of polycarbonate products have all been shown to increase exposure to BPA.²⁵ A daily ingestion value can be estimated at <1 µg BPA/kgBW/day, and is believed to be the main source of human exposure.^{27,30} The U.S. En-

vironmental Protection Agency estimates a safe dose calculated at 50 µg BPA/kgBW/day.³⁰

Methodology

Prior to the start of this investigation, the protocol was reviewed and approved by the Old Dominion University Institutional Review Board ensuring the protection of human subjects. Participants comprised a convenience sample of 16 females and 14 males, 18 to 40 years of age with no history of dental sealants or composite material placement, and no previous exposure to BPA in its raw form. All data collection occurred at the Old Dominion University Dental Hygiene Research Center.

The researcher selected a widely used, commercially available light cured sealant material (Delton® Pit & Fissure Sealant – Light Cure Opaque, DENTSPLY Professional, York, PA). This sealant material uniquely contained 91.2% aromatic and aliphatic dimethacrylate monomers, 1.5% titanium dioxide (colloidal), 5.4% silica (colloidal), 1.0% ethyl-p-dimethylaminobenzoate and <1.0% light activators as described in the Material Safety Data Sheet.³²

The participants were randomly divided into 2 groups a high-dose group and low-dose group. Participants were selected for each group based on the availability and eligibility of surfaces. Subjects in the low-dose group received 1 occlusal sealant only. Subjects in the high-dose group received 4 occlusal sealants. The amount of sealant material placed was reflective of clinical relevance and was applied according to the manufacturer’s instructions.

This study utilized a BPA Enzyme Linked ImmunoSorbent Assay (ELISA) (Abraxis LLC, Warminster, Pa) to detect and quantitate levels of BPA in saliva and serum prior to and after placement of dental sealants in adults. The direct competitive ELISA protocol was used on the recognition of BPA by specific monoclonal antibodies. Distinctively, the BPA ELISA used in this study pro-

vided a high level of sensitivity, with a detection range from 0.05 µg/L to 10 µg/L and coefficient of variation less than 10%.

Quality control measures included performing sample titrations and spike recovery tests. Results of the sample titration produced 82% of maximum binding. Spike recovery tests were performed on 3 BPA ELISA plates to establish reliability and instrument validity. The average recovery rates were 86.83% and 80.18% for the spiked serum and saliva samples, respectfully.

Statistical Analysis

Parametric tests were chosen to allow testing of multiple variables and their interaction. Data collected were analyzed using a 2 way ANOVA for repeated measures. Dosage was used as the grouping factor and time was the repeated factor.

T-tests provided an indication of significance and direction (positive or negative) of sample differences. Statistical analysis for all data was accomplished using the Statistical Analysis System, SAS® version 9.1.

Results

The repeated measures of ANOVA for within subject effects revealed a statistically significant effect of time on salivary BPA levels of all samples (Table I). Further, the t-test revealed a statistically significant difference in the salivary BPA levels between the 1 hour pre- and 1 hour post-dental sealant placement (Table II). BPA was detected in all baseline saliva samples, and there was an increase in salivary BPA levels after placement of the dental sealant in all samples. The increase in BPA concentration readings 1 hour after sealant application suggests that BPA was released from the dental sealant material.

Although only slight, the t-test revealed a statistically significant difference in the salivary BPA levels between the 1 hour prior and 24 hours post (Table 2, Figure 1). A statistically significant difference at the 0.05 level established a difference in the amount of BPA detected in saliva

samples 1 hour prior to and 1 hour, 3 hours and 24 hours post-dental sealant placement in all samples. The serum BPA levels were below the limit of quantitation (<0.05 ng/mL).

The repeated measures of ANOVA for between subjects effects revealed a statistically significant effect of dose on salivary BPA levels ($F=11.12$, $p<.0001$) (Table III). A post-hoc test (Tukey's) revealed a statistically significant difference between the mean salivary BPA concentration levels in the low-dose and high-dose groups at both the 1 hour ($p<.0001$) and 3 hours post ($p=0.0027$) time periods. No statistically significant difference was revealed between the mean salivary BPA concentration levels in the low-dose and high-dose groups at either 1 hour prior ($p=0.4328$) or 24 hours post ($p=0.3283$) time periods. Figure 1 displays the mean BPA levels for both low and high-dose groups at all time periods.

Discussion

This exploratory investigation replicated the in vivo study conducted by Fung, et al.⁹ Analysis of saliva samples at baseline revealed detectible levels of BPA (0.06 to 4.02 ng/mL) in each of the 30 samples measured 1 hour prior to dental sealant placement. The detection of salivary BPA in every participant suggests either short term or long term exposure levels. Detection of BPA in baseline readings from the current study differed from the findings of Fung et al, which did not detect salivary levels of BPA in any baseline samples.⁹

In vivo results from the current study, Fung et al⁹ and Joscow et al¹¹ consistently reveal detectible levels of BPA in saliva at the 1 hour and 3 hours post-sealant application collection times, thus indicating the release of unpolymerized or leachable BPA from the same dental sealant material used in all 4 studies.^{8,9,11} The dental sealant material used in all 4 studies contained bis-GMA combined with an additional monomer, BPA, result-

Table I. ANOVA Test for Saliva 1 hour pre- and 1 hour post-Sealant Placement

Source (n=30)	Sums of Squares	Degrees of Freedom	Mean Squares	F-Statistic	P-Value
Time	193.69	3	64.56	90.32	<.0001

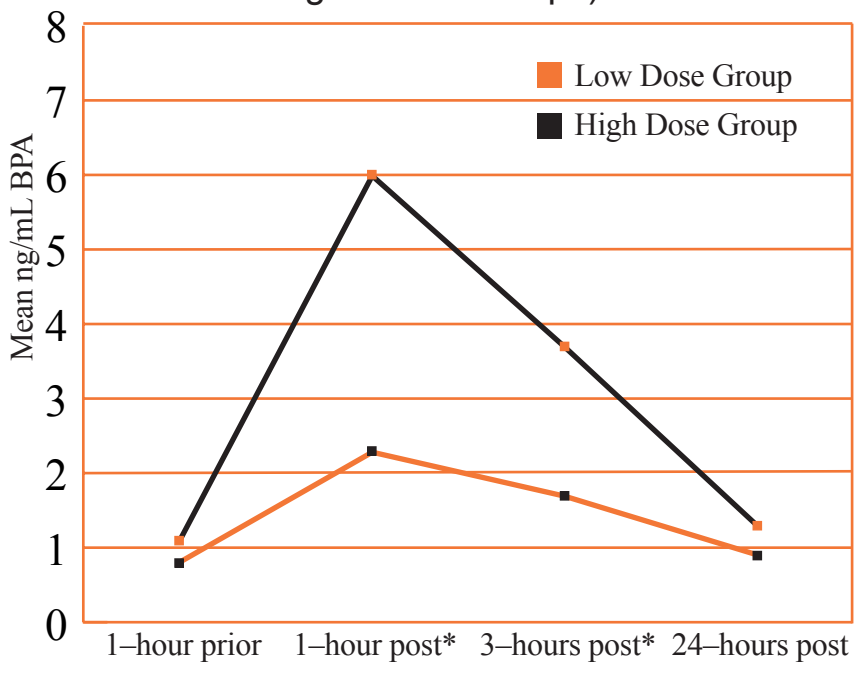
Table II. T-Test Results for Salivary BPA at the 1 hour Prior, 1 hour, 3 hours and 24 hours Post Time Periods

Difference (n=30)	Degrees of Freedom	t-Value	p-Value
1-hr prior 1-hr post	29	7.20	<.0001
1-hr prior 3-hrs post	29	6.34	<.0001
1-hr prior 24-hrs post	29	3.93	0.0005

Table III. Analysis of Variance Test for Salivary BPA Levels Between Low-Dose and High-Dose Groups

Source n=30	Sums of Squares	Degrees of Freedom	Mean Squares	F-Statistic	P-Value
Low-Dose and High-Dose Groups	84.38	1	84.38	11.12	<.0001

Figure 2. Overall Salivary BPA Concentration (*Indicates statistical Significant Difference Between Low-Dose and High-Dose Groups)



ing in bisphenol A dimethacrylate (BPDMA, bis-DMA), however, the manufacturer's of these dental sealant products maintain the specific proprietary chemical formulation.

Salivary BPA levels demonstrate a peak level between the 1 hour and 3 hour post-application collection time. BPA levels began to reduce in concentration between the 3 hour and 24 hour post-application collection times, almost equating baseline with the final collection.

There was no BPA detected in the serum samples collected at the 1 hour and 3 hour post sealant application times. These findings are similar to those of Fung et al,⁸ who did not detect BPA in any of the serum specimens examined in the study.⁹ Implications from the findings of this study are that BPA is not absorbed into the blood circulatory system after exposure has occurred from the dental sealant formula used in this and previous studies.

Although not statistically significant, salivary BPA levels were different between the low-dose and high-dose groups when measured at baseline. This finding indicates a possible influence from an extraneous variable such as age. Additional extraneous variables that may have influenced differing salivary BPA levels between the low-dose and high-dose groups include gender, ethnicity and other demographic variables.

The specific molecular formulation of dental sealant material differs between manufactured brands of dental sealants, depending on chemical additives to bis-GMA⁴ and may not be recognized by dental professionals. The amount of BPA exposure encountered in

this study is below the allowable safe dosage/day as established by the U.S. Environmental Protection Agency, calculated at 50 µg BPA/kgBW/day.^{31,33,34} It has not been established that this level of short-term (24-hour) exposure causes irreversible damage or poses a true health threat. Further research is needed to establish health effects of this short-term exposure.

National data confirms that 80% of dental caries risk is among children and the adolescent population, known as a focus group of dental sealants.¹⁷ Considering sealants are applied during these adolescent, pubescent years of development, all precautions to reduce BPA exposure should be taken. It is important that dental professionals still apply dental sealants in the nation-wide effort to reduce dental decay.

Dental sealant formulations differ among manufacturers and may not directly specify if BPA was a precursor included in the foundational bis-GMA product, or if it was added as a monomer. BPA may not be indicated as a direct ingredient indicated on the Material Safety Data Sheet. Therefore, dental professionals should consult with each individual sealant material manufacturer and frequently review each product among the evidence-based clinical and laboratory research. To further reduce BPA exposure after sealant application, a pumice wash should be delivered to the surface with a prophylactic rubber cup and followed by a water rinse.^{35,36}

Conclusion

Dental professionals should reduce BPA exposure to patients from dental sealants by using products that have the ADA Seal of Accep-

tance and adopt the accepted protocols for use. Future research should investigate the cumulative exposure effects of BPA on humans from dental sealants considering environmental influences. Studies should also analyze existing demographic data generated in this study to determine if there is a relationship between salivary BPA concentration levels and gender, ethnicity, age, marital status or household income. Replication of this study is recommended, using a larger sample population and analysis of biological specimens including semen, vaginal fluid, dental pulp tissue and hair follicles.

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