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A Scoping Review on the Toxicity of Bisphenol A released from Resin Composites used in Dentistry

Lara Ribeiro Gonçalves

Dissertação conducente ao Grau de Mestre em Medicina Dentária (Ciclo Integrado)

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Trabalho realizado sob a Orientação do Professor Doutor Júlio C. M. Souza (PhD, MSc) e Co-Orientação da Mestre Lígia Rocha (MSc)

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"Andrà tutto bene"

RESUMO

Objetivo: O objetivo deste trabalho foi realizar uma revisão sistemática integrativa sobre a libertação do Bisfenol A das resinas compostas e as suas consequências quanto à toxicidade local e sistémica, em pacientes.

Método: Efetuou-se uma pesquisa bibliográfica na plataforma PUBMED utilizando as palavras-chave: *"Bisphenol A"* OR *"BPA"; "Resin Composite"* OR *"Composite Resin"; "Toxicity"* OR *"Cytotoxicity"; "Release".* Critérios de inclusão envolveram trabalhos *in vitro* e *in vivo* sobre a libertação e toxicidade do BPA.

Resultados: Resultados mostram que a libertação do BPA é devida a uma insuficiente polimerização e/ou degradação das resinas compostas. O BPA está presente na matriz orgânica das resinas compostas e pode ser hidrolisado na saliva humana, embora estudos reportem que doses baixas possam não ser detetados pelas análises químicas convencionais. Estudos expondo embriões de peixe-zebra a diferentes concentrações de Bis-GMA, demonstraram mortalidade de 55%, com uma concentração de 10 μM e 30% de mortalidade, com uma concentração de 1 μM. Em pacientes encontrou-se uma concentração de aproximadamente 2.09 x 10^{-2}μg/mL de BPA na saliva após inserção da retenção lingual, com resina composta. De facto, a molécula de BPA pode ser ingerida por deglutição e absorvida pela mucosa oral/gastrointestinal, podendo resultar numa toxicidade sistémica.

Conclusões: A degradação das resinas compostas e libertação do BPA em meio oral são dependentes da proporção da matriz orgânica e polimerização das resinas compostas. Uma maior libertação de BPA aumenta a possibilidade de absorção pelos tecidos da mucosa oral e trato gastro-intestinal com riscos de toxicidade local e sistémica, em pacientes.

KEY TERMS: *"Bisphenol A"; "BPA"; "Toxicity"; "Resin Composite"*

ABSTRACT

Objective: The main aim of this study was to perform a systematic integrative review on the release of Bisphenol A from resin composites and its consequences on local and systemic toxicity in patients.

Method: A bibliographic search was performed on the PUBMED platform using the keywords: "*Bisphenol A*" OR *"BPA"; "Resin Composite"* OR *"Composite Resin"; "Toxicity"* OR *"Cytotoxicity"; "Release".* Inclusion criteria involved *in vitro* and *in vivo* work on the release and toxicity of BPA.

Results: Results indicate the release of BPA from resin composites due to insufficient polymerization and/or degradation of resin composites. BPA is contained in the organic matrix of resin composites and may be hydrolysed in human saliva, although studies report that low doses might not be detected by conventional chemical analysis. Studies exposing zebrafish embryos to different concentrations of Bis-GMA, showed 55% mortality within 10 μM Bis-GMA and 30% mortality on 1 μM Bis-GMA. In patients, a BPA concentration of about 2.09×10^{-2} μg/mL was found in the saliva after placement of lingual retainers with resin composites. Thus, the BPA molecule can be swallowed and absorbed by the oral/gastrointestinal mucosa, which might result in systemic toxicity.

Conclusions: The degradation of resin composites and release of BPA in oral environment are dependent on the proportion of the organic matrix and polymerization of resin composites. Increased release of BPA can lead to the absorption into oral mucosal and gastrointestinal mucosa with high risks of local and systemic toxicity.

KEY TERMS: *"Bisphenol A"; "BPA"; "Toxicity"; "Resin Composite"*

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LIST OF ABBREVIATIONS:

BADGE: Bisphenol A Diglycidyl-Ether

BFDGE: Bisphenol F Diglycidyl-Ether

Bis-DEMA: Bisphenol A-Polyethylene Glycol Diether Dimethacrylate

Bis-DMA: Bisphenol A-Dimethacrylate

Bis-EMA: Ethoxylated Bisphenol A Dimethacrylate

Bis-GMA: Bisphenol A-Diglycidyl Methacrylate

Bis-MPEPP: Bisphenol A Polyethoxy Methacrylate

Bis-PMA: Propoxylated Bisphenol A-Dimethacrylate

BPA: Bisphenol A

DMSO: Dimethyl Sulfoxide

EFSA: European Food Safety Agent

ELISA: Enzyme-Linked Immunosorbent Assay

GC/MS: Gas Chromatography / Mass Spectrometry

hpf: hours post fertilization

HPLC: High Performance Liquid Chromatography

LC/MS: Liquid Chromatography / Mass Spectrometry

LED: Light-Emitting Diode

MCF-7: Michigan Cancer Foundation-7

mg/mL: milligrams /milliLitre

MSDS: Material Safety Data Sheet

Nm: Nanometers

nM: nanoMolar

ORMOCER: Organically Modified Ceramics

PC Bis-GMA: Polycarbonate-modified Bis-GMA

ppm: parts per million

TDI: Tolerable Daily Intake

TEGDMA: Triethylene Glycol Dimethacrylate

UDMA: Urethane Dimethacrylate

µg/Kg: microgram / Kilogram

µg/mL: microgram /milliLitre

µM: microMolar

1. INTRODUCTION

Bisphenol A (BPA) is an organic compound which is used in the synthesis of polycarbonate-, epoxy-, and methacrylate-matrix materials that are applied in several industrial and medical fields (1,2). Resin composites used in dentistry have BPA derivates in their organic-matrix composition such as Bisphenol A-Diglycidyl Methacrylate (Bis-GMA), Bisphenol A-Dimethacrylate (Bis-DMA), Ethoxylated Bisphenol A Glycol Methacrylate (Bis-EMA), Propoxylated Bisphenol A-Dimethacrylate (Bis-PMA), Bisphenol A Diglycidil-Ether (BADGE), Polycarbonate-modified Bis-GMA (PC Bis-GMA), and Bisphenol A Polyethoxy Methacrylate (Bis-MPEPP) (3). The content of Bis-GMA can range from 5 up to 20 % while Bis-EMA can range from 1 up to 5 % and Bis-MPEPP between 5 and 10 % (4,5). Although the popularity of resin composites has increased in recent years, the concern on the release of toxic molecules such as BPA has gathered attention by scientists, clinicians, and patients (2,4,5).

After light curing, the release of methacrylate monomers from resin composites occurs mainly within the first 24 h and may continue to have effects over time due to chemical and mechanical wear (6,7). Chemical degradation occurs due to hydrolysis and enzyme catalysis through human saliva esterase and oral fluids from dietary (7–9). On the other hand, tooth brushing, occlusal sliding, and abrasion are the dominant wear pathways on resin composites. Additionally, the unproper polymerization of the resin composites can enhance those adverse physicochemical effects (10). In fact, the release of monomers and BPA is dependent on the chemical composition and amount of organic matrix in the resin composites.

Bisphenol A has been identified as an endocrine disrupter effective in binding and activating the estrogenic receptor (11). Bisphenol A may be ingested and absorbed by the oral and gastrointestinal mucosa leading to local and systemic toxicity. As a consequence, BPA is classified in category 3 within a scale Globally Harmonized System Hazard Classification, on human fertility (risk phrases: R62 or R63) (12–14). *In vivo* studies have shown that the administration of low content of BPA, both pre-and postnatal, have implications for the male and female reproductive system and the overall human health state. The following pathologic alterations have been reported: endometrial hyperplasia, increased presence of ovarian cysts, breast hyperplasia, premature puberty and decreased sperm production, neurologic system, immunologic system, sensibility to insulin, and lipid

metabolism (15–17). Besides, there is the possibility to induce adverse effects in the brain, cardiovascular system, thyroid, intestine, prostate, and breast (18,19). Further studies are required to validate the findings, because the previous data have shown epidemiologic heterogenicity.

Thus, the main aim of this study was to perform a scoping review on the release of BPA from resin composites and the resultant adverse biological effects to the patients. It was hypothesized that BPA may be released from dental resin composites at different amounts depending on the chemical composition of the restorative materials and complexity of the oral environment. Bisphenol A molecule can be locally absorbed by the surrounding tissues (e.g., gingival margin) and diffused into the bloodstream resulting in systemic toxicity.

2. METHOD

A literature search was carried out on PUBMED (via National Library of Medicine), using the following combination of search terms: *"Bisphenol A"* OR *"BPA"*; *"Resin Composite"* OR *"Composite Resin"*; *"Toxicity"* OR *"Cytotoxicity"*; *"Release"*. The inclusion criteria involved articles published in the English language, up to January 2020, regarding *in vitro* and *in vivo* studies on the release and toxicity of the Bisphenol A molecule from resin composites used in dental restorations. Also, meta-analyses, randomized controlled trials, and prospective cohort studies were included in the search strategy. The total of articles was compiled for each combination of key terms and therefore the duplicates were removed using Mendeley citation manager (Elsevier B.V.). Three of the authors (J.C.M.S.; L.R.; L.G.) independently evaluated the titles and abstracts of potentially pertinent articles. Selected articles were individually read and analyzed concerning the main aim of this study. The following factors were taken into consideration for the present study: author's name; journal; publication year; purpose of the study; BPA release in human saliva; BPA release in stock solutions; BPA diffusion into the bloodstream (systemic pathway); BPA uptake by swallowing and BPA absorption through the gastrointestinal mucosa.

3. RESULTS

The literature search on PUBMED identified a total of 206 articles although 84 duplicates were removed, as seen in Figure 1. After a preliminary evaluation of the titles and abstracts of the articles, 108 were excluded because they did not assemble to the inclusion criteria. The remnant 14 potentially relevant studies were selected for full reading. However, 6 studies were excluded due to the lack of relevant information according to the purpose of this study. At last, 8 articles were included in the present scoping review.

Of the eight selected studies, one study compared the chemical composition of different resin composites commercially available concerning the presence of BPA while 3 (37.5 %) articles measured the *in vitro* release of BPA or Bis-GMA monomers (3–5,20). Regarding cytocompatibility assays, one study reported the toxic effect of derivatives molecules from Bis-GMA and Bisphenol F Diglycidyl-Ether (BFDGE) in rat or human hepatocytes while another study evaluated the effect of BPA and its derivatives in contact with fibroblasts (21,22). On *in vivo* assessment, a previous study evaluated the effects of Bis-GMA on the development of craniofacial chondrogenesis in zebrafish. Only one study reported the changes in BPA levels in saliva and urine of human participants after the placement of orthodontic lingual retainers (2,23). The major findings are shown in Table 1 and drawn as follow:

- A total of 160 different resin composites from 31 manufacturers were assessed regarding the chemical composition of the methacrylate-based matrix, but only 23 manufacturers responded to the survey. Approximately 112 (86 %) resin composites comprised BPA derivatives in their organic matrix in which Bis-GMA corresponded to the main (74 %) methacrylate-based monomer. Seventeen resin composites (13 %) were free of BPA once Urethane Dimethacrylate (UDMA) or UDMA/ Triethylene Glycol Dimethacrylate (TEGDMA) replaced Bis-GMA (3);
- On *in vivo* assessment including 22 human participants, the maximum concentration of BPA in saliva was around 2.0889×10^{-2} µg/mL after placement of the lingual bonded retainer. BPA levels detected in urine did not appear to have any relevance to the placement of the lingual bonded retainers (2);

- The exposure of different content of Bis-GMA to zebrafish embryos resulted in mortality of 55 % embryos at 10 µM Bis-GMA and 30 % embryos at 1 µM Bis-GMA (23);
- *In vitro* analysis detected significant amounts of BPA, TEGDMA, and other methacrylate-based monomers released from orthodontic adhesives used in daily practice. The content of BPA at around 12.54 % was significantly high for inducing local or systemic toxicity regarding the previous studies (4).

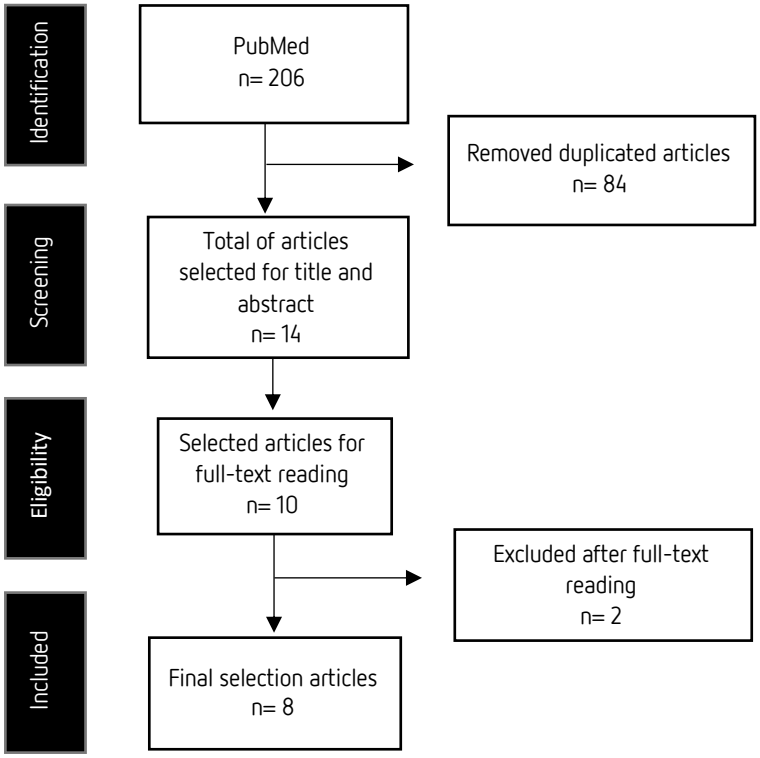

Figure 1: Flow diagram of the search strategy used in this study.

Table 1: Relevant data of the studies selected.

Author (year)	Purpose	Study design	Chemical composition	BPA release (µg/ml)	Biological response
Kostoryz *et al.* (2003) (21)	Identify the formation of the tetrahydroxylated metabolites of BFDGE and Bis-GMA after exposure of each monomer to the liver S9 fractions *in vitro* and evaluate the biocompatibility of the hydroxylated metabolites in relation to their parent compounds.	-*In vitro.* Rat or human liver S9 fractions. -Liquid Chromatography / Mass Spectrometry	Bis-GMA was obtained from 3M-ESPE and BFDGE, BDAPE-40H and BFDPE-40H were obtained from Fluka Chemical Company.	By 10 minutes, greater than 90 wt% of the initial Bis-GMA and BFDGE concentrations had disappeared.	Cytotoxicity against L929 cells showed that the metabolites were significantly ($p < 0.05$) less cytotoxic than the parent monomers.
Al-Hiyasat *et al.* (2005) (22)	Determine the cytotoxicity of commercially available flowable dental resin materials with different structures, to compare the results with those of their traditional resin composites, and to determine their leachable components.	-*In vitro.* Balb/c 3T3 fibroblasts by measuring cellular metabolic activity (3{4,5-dimethylthiazol-2-yl}-2,5-diphenyltetrazolium bromide [MTT] assay). -High Performance Liquid Chromatography	Admira: UDMA, Bis-GMA, TEGDMA. Admira Flow: UDMA, Bis-GMA, TEGDMA. Filtek Z250: UDMA, Bis-GMA, Bis-EMA. Filtek Flow: Bis-GMA, TEGDMA. Tetric Ceram: UDMA, Bis-GMA, TEGDMA. Tetric Flow: UDMA, Bis-GMA, TEGDMA.	A relatively small amount of BPA was found in the medium extracts of Z250 and Tetric Flow.	The cytotoxicity of the materials could be related to the amount of TEGDMA that was leached from the flowable composites compared with their non-flowable traditional composite. Indeed, TEGDMA has been reported to be toxic in different cell lines.
Koin *et al.* (2008) (20)	To study the degradation of this model overlayer, after being aged in water, by Liquid Chromatography / Mass Spectrometry. A two-	-*In vitro.* Degradation of dental composites was studied in a simplified overlayer model in which Bis-GMA was covalently bound to	Bis-GMA and silicon oxide.	Bis-GMA: 0 µg/ml	N/A

	week aging period was chosen, since this was found to be the interval of maximum release of Bis-GMA from commercial dental composites.	a porous silicon oxide surface. -Liquid Chromatography / Mass Spectrometry			
Kang *et al.* (2011) (2)	Examine the amounts of salivary and urinary BPA to assess the amount of BPA released from the resin composite used in a lingual bonded retainer during the first month of placement as part of a larger effort to confirm the safety of Bis-GMA–based dental resin composite.	*-In vivo,* 22 volunteers: 10 male patients (range, 13-25 years) and 12 female patients (range, 13-32 years). -Liquid Chromatography / Mass Spectrometry	Flowable resin composite (Filtek Flow): Bis-GMA and TEGDMA. Hybrid resin composite (Filtek Z250): Bis-GMA, UDMA and Bis-DEMA.	The saliva samples collected immediately after lingual bonded retainer placement showed a significant increase in BPA compared with the baseline samples (before placing the lingual bonded retainer). However, the samples taken after 1 day, 1 week, and 1 month showed similar levels to the baseline. All baseline saliva samples except for 2 showed undetectable levels of BPA, and 17 of 20 saliva samples collected immediately after retainer bonding contained BPA levels ranging from 8.53x 10^{-4} and 2.0889 x 10^{-2} µg/ml. The urine samples contained BPA at various times but without an association with the time point.	The amount of BPA leaching from Bis-GMA–based resin composite used for bonding orthodontic lingual retainers was low and far below the reference doses for daily uptake.
Bationo *et al.* (2016) (4)	To characterize monomers released from orthodontic adhesives.	*-In vitro.* -Samples of orthodontic adhesives by associating 2 techniques: Gas Chromatography / Mass Spectrometry	Transbond XT: Bis-GMA: 10-20 wt%; BPA Bis 2-hydroxyethyl ether dimethacrylate: 5-10 wt%; Silane treated quartz: 70-80 wt%; Silane treated silica: < 2 wt%; Diphenyliodonium hexafluorophosphate: < 0.2 wt%.	BPA: 0 µg/mL	N/A

			Transbond Supreme LV: Bis-GMA: 10-15 wt%; TEGDMA: 10-15 wt%; Bis-EMA: 1-5 wt%; Silane treated ceramic: 52-60 wt%; Silane treated zirconium oxide: 3-11 wt%; Silane treated silica: 3-11 wt%; Functionalized dimethacrylate polymer: 1-5 wt%. Blugloo: Glycidyl methacrylate: 3-5 wt%; Inert fillers and pigments. MonoLok 2 light-activated bonding system: Monomers of aromatic and aliphatic dimethacrylates; Methacrylate monomers; Camphorquinone; Tertiary amine.		
Dursun *et al.* (2016) (3)	Establish an exhaustive list of resin composites marketed in Europe and detail their composition, and second, to estimate the number of resin composites using BPA or BPA derivates (Bis-GMA, Bis-DMA, Bis-EMA, Bis-MPEPP, PC Bis-GMA) in their manufacturing.	-Case report -Material Safety Data Sheet	160 different brands of resin composite.	N/A	N/A

Krammer *et al.* (2016) (23)	To quantitatively assesse the effects of Bis-GMA on vertebrate development and its effects on craniofacial chondrogenesis in the viscerocranium of zebrafish larvae.	-*In vivo*. Adult zebrafish and Zebrafish embryos (*Danio rerio*). -Olympus FSX100 fluorescent microscope using Tg (sox10: gfp)	0.1 wt% DMSO for the control and a solution of Bis-GMA, Sigma dissolved in DMSO.	N/A	Exposure to 1 µM and 10 µM Bis-GMA in *Danio rerio* embryos results in increased mortality of approximately 30 wt% and 55 wt% respectively. Changes to gross morphology, specifically craniofacial abnormalities, were seen at concentrations as low as 10 nM.
Pelourde *et al.* (2018) (5)	To evaluate *in vitro* the release of monomers from orthodontic bonded retentions.	-*In vitro*. - To better simulate the quantity of adhesive applied to an orthodontic retainer, a sample of each composite (Transbond XT, Transbond LR; 3M Unitek, Monrovia, Calif). - Gas Chromatography / Mass Spectrometry Analysis	<u>Transbond LR</u>: Silane treated quartz: 75-85 wt%; TEGDMA: 5-15 wt%; Bis-GMA: 5-15 wt%; Dichloromethylsilane reaction product with silica: < 2 wt%; N,N-dimethylbenzocaïne: < 0.3 wt%; Diphenyliodonium hexafluorophosphate: < 0.1 wt%. <u>Transbond XT:</u> Silane treated quartz: 70-80 wt%; Bis-GMA: 10-20 wt%; Bisphenol A Bis (2-hydroxyethyl ether) dimethacrylate: 5-10 wt%; Silane treated silica: < 2 wt%; Diphenyliodonium hexafluorophosphate: < 0.2 wt%.	BPA: 0 µg / mL TEGDMA: 31.7 µg / mL (Transbond LR) / 13.2 µg/ mL (Transbond XT)	N/A

4. DISCUSSION

4.1. RESIN COMPOSITES

Nowadays, the esthetic outcomes have been getting attention and the development of resin composites provide a variety of clinical applications in the case of dental restorations, prosthetic cementation, occlusal fissure sealing, orthodontic adhesion, and retaining (24,25). The chemical composition of resin composites varies according to the clinical applications and manufacturers. The organic matrix involves monomers such as Bis-GMA, TEGDMA, UDMA, Bis-EMA, and photoinitiators. The inorganic content can reach up to 90 % resin-matrix composite that can include one or two types of silanized ceramic or glass-ceramic fillers such as colloidal silica, zirconia, zirconium silicate, barium silicate, or ytterbium fluoride (24). The balance in the percentage of the organic matrix and inorganic fillers determine the physicochemical properties of the resin composites (8,26–29).

The polymerization reaction of methacrylate-based resins is accomplished using a photoinitiator, mainly camphorquinone, which is stimulated by visible light at a wavelength of around 470 nm. A co-initiator (e.g., tertiary amina) is required to interact with the activated photoinitiator and provide free radicals to binding the methacrylate chains (8,29,30). Nowadays, a Light-Emitting Diode (LED) at wavelength range between 400-500 nm is used in different intensities, time, and mode to provide the energy required for the light-curing of resin composites (8,26). The degree of conversion during the polymerization of the monomers ranges from 50 and 70 % (31,32) although the maximum conversion degree is only achieved over a period of 24 h from the light-curing. In the first hour, the degree of conversion is quite low (~40%) that indicates the instability of the polymeric bindings and susceptibility to degradation in the oral cavity (33,34). Then, BPA can be released from the organic matrix of resin composites due to the low degree of conversion of resin composites. An inadequate polymerization and the degradation of the resin composites can lead to a high release of monomers into the surrounding environment, which include BPA and its derivatives (6). BPA molecules can be absorbed locally by immune response cells and tissues in the critical region, at gingival margins (Figure 3). Also, BPA can get into the bloodstream and induce a systemic adverse response at different organs and tissues (6). Such reactive molecule can also be ingested by swallowing saliva and absorbed by the gastrointestinal mucosa leading to systemic toxicity if not excreted by urine (35).

The local and systemic pathways for BPA release from resin composites are illustrated in Figure 2 (36,37).

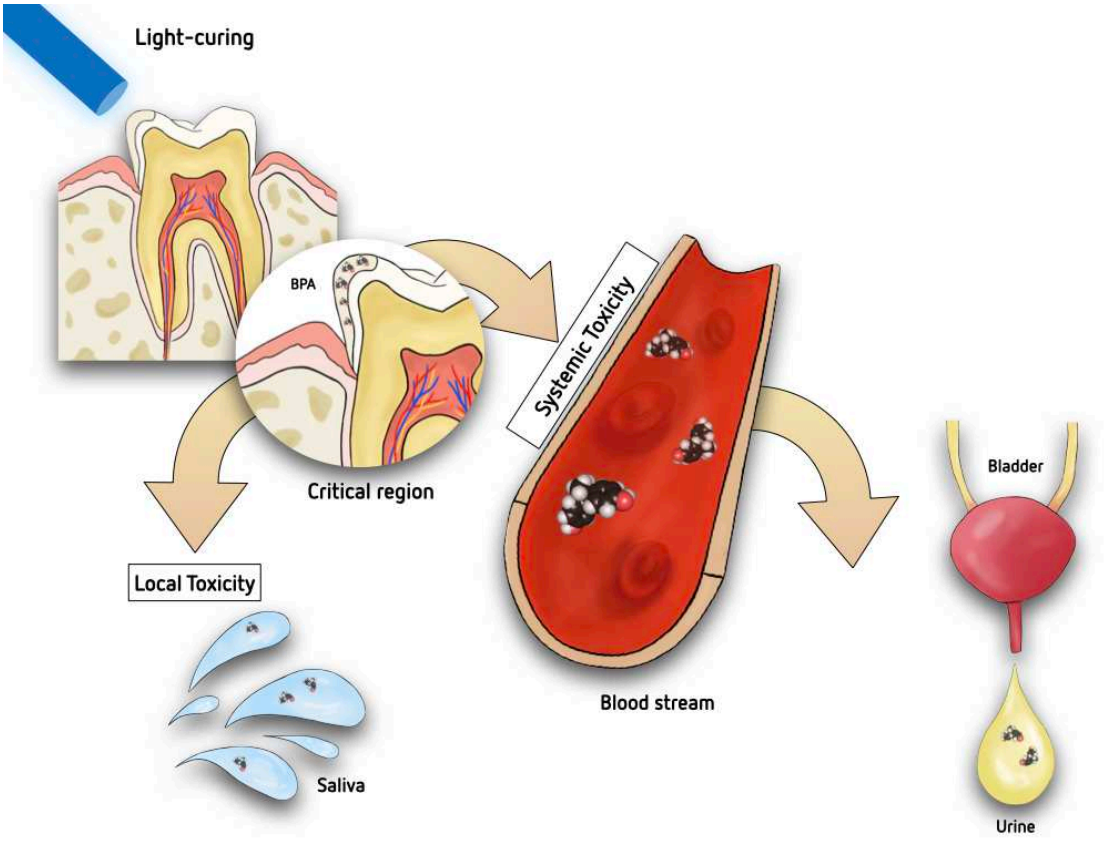

Figure 2: Schematic drawing of the local and systemic toxicity.

4.2. RELEASE OF BPA FROM RESIN COMPOSITES

Previous studies have shown a high release of Bis-GMA since that monomer is often existing in the chemical composition of conventional resin composites. Also, a lower degree of conversion has been associated with a high content of Bis-GMA (38–40). However, Bis-EMA has been used to replace Bis-GMA to control the physical properties of recent resin composites (2,3). Thus, BPA is formed from the degradation of Bis-GMA or Bis-EMA and released at varied concentrations depending on the chemical and mechanical phenomena in the oral cavity. The degradation phenomenon of resin composites can occur due to chemical and mechanical pathways in the oral cavity. Regarding chemical phenomena, a low degree of conversion is the primary cause once monomers out of the polymeric chain can chemically react with several fluids from human saliva such as water, minerals, proteins, and acidic substances, as illustrated in Figure 2 (41). That results in faster chemical degradation by hydrolysis of the organic matrix of the resin composites (7,41). The mechanical factor is linked to the abrasion wear of occlusal surfaces of resin composite restorations leading to a material loss and ejection of polymeric and monomeric debris to surrounding tissues and human saliva (Figure 2) (7). The wear of restorative surfaces depends on the polymerization and resultant physical properties of the resin composites (7,24,42).

Dursun *et al.* have made a previous study reported an exhaustive list of available resin composites in Europe and data regarding the presence of BPA and its derivates (3). The authors identified a total of 160 resins composites from 31 different manufacturers, but only 23 manufacturers responded to the survey. The detailed composition of 130 different resins composites was therefore listed, of which 112 had BPA derivatives in their composition and 97 contained Bis-GMA and 43 Bis-GMA and UDMA. However, 17 resin composites contained monomers not derived from BPA or Bis-GMA (UDMA or sometimes TEGDMA) in their composition while 6 had only UDMA in their composition and only 1 did not contain any BPA or UDMA or TEGDMA derivatives. No resin composite contained solely BPA in the organic matrix (3).

Studies indicate that ordinary resin composites are generally less cytotoxic than flowable resin composites (22). However, nanohybrid Organically Modified Ceramics (ORMOCER) flowable composite showed lesser cytotoxicity than those on ordinary nanohybrid ORMOCER composites (22). The monomers released from the test resin

composites have been quantified by High Performance Liquid Chromatography (HPLC) analysis. In fact, all the test materials released Bis-GMA and TEGDMA which might be absorbed by oral and gastrointestinal mucosa of patients. Only in microhybrid resin composites and nanocomposite flowable culture medium was found a small concentration of 0.64 BPA µg/mL was detected from microhybrid resin composites while 1.65 µg/mL BPA was detected from flowable nano-structured composites (22). Another *in vitro* study reported the degradation of dental resin composites using a simplified overlay model in which Bis-GMA was covalently bonded to a porous silicon oxide surface (20). The chemical structure of the overlay could allow the release of Bis-GMA, BPA and their derivatives when exposed to water. A release of Bis-GMA was detected by Liquid Chromatography/Mass Spectrometry (LC/MS) although BPA could not be detected by using the described method (20).

Another *in vitro* study quantitatively analyzed the release of adhesive monomers used for adhesion of orthodontic retainers. Significant amounts of TEGDMA were detected among other monomers released from aligner attachments adhesives. BPA release was detected from flowable microhybrid resin composites (4). The release of methacrylate monomers and BPA from resin composites for orthodontic retainers (4). BPA was released below the detection threshold of 0.02 ppm although it does not refute the release of BPA in smaller amounts. TEGDMA was detected from lingual retainer adhesive at higher values of around 31.7 mg/mL when compared to aligner attachments adhesive (13.12 mg/mL). Other toxic components have been detected such as: iodobenzene, iodobiphenyl, triphenylstibine, among others. In addition, toxic and carcinogenic molecules not mentioned in the safety data sheets were detected (5).

Thus, a few studies reported that the release of BPA and its derivative is hardly measurable (4,5,20). It might happen due to the kinetic release of monomers from *in vitro* studies that could be influenced by the saturation of the solvent by the monomers (43). In the oral environment, the overall degradation of resin composites can be progressive, and the saturation could never be reached due to the continuous removal of the monomers by the human saliva flow (44). The retrieval of the saliva medium should be performed at standard time intervals to avoid the saturation by the leached products (35,43). Therefore, margins of resin composite restorations have not been examined concerning the release of BPA and its derivatives, as illustrated in Figure 2. It must be emphasized that the intimacy

of the resin composite surfaces can increase the amount of BPA in the connective tissues. In fact, the release of BPA from resin composite structures has been overlooked in clinical studies, as seen in Table 1. *In vitro* studies should also be optimized considering chemical and mechanical factors related to the release of BPA towards to the human saliva.

One study stated that the color of the resin composite and the method of light curing influence cytotoxicity (43). Resin composites with a higher chroma exhibited higher cytotoxicity, even though changing the light curing method (43). This *in vitro* study used human gingival fibroblasts to validate the decrease in cytotoxicity when applying a light curing method with a high power intensity and in a shorter period of time (45). In fact, dental clinicians should take into consideration the following factors: (i) distance between the light-curing unit tip and the resin composite; (ii) spectra wavelength; (iii) light intensity and energy delivered to polymerization; (iv) period of time; (v) the compatibility between resin composite photoinitiator and wavelength (46).

The release of monomers and products from the organic matrix of resin composites has also been reported in previous *in vivo* studies (35,47). In clinical studies involving human participants, one study revealed data on the content of BPA in saliva and urine as a result of degradation of resin composites for orthodontic retainers (2). There was a significant increase in BPA levels in human saliva samples (20 samples) harvested after the lingual orthodontic retainer placement. The baseline was a harvested saliva free orthodontic retainer. However, the salivary BPA values detected were lower than the reference daily intake dose. Seventeen samples collected immediately after the placement showed values between 8.53×10^{-4} and 2.09×10^{-2} µg/mL BPA (2). Furthermore, saliva samples as harvested for 1, 7, and 30 days after the orthodontic retainer placement and that test samples showed similar levels to the baseline (2). Additionally, the urine of the human participants were harvested and analyzed regarding the presence of BPA and the findings were not directly related to the orthodontic retainer placement (2).

4.3. LOCAL AND SYSTEMIC TOXICITY

In vitro assays reported the toxicity of BPA in contact with different animal and human cells such as fibroblasts, mesenchymal, and tumor cells, as seen in Table 1 and Figure 3. A previous study reported the cytotoxic, mutagenic, and estrogenic effects of BFDGE e Bis-GMA in contact with Michigan Cancer Foundation-7 (MCF-7) human breast cancer, L929 mouse fibroblast, or S9 rat hepatic cells (21). Tetrahydroxy and methacrylic acid metabolites from Bis-GMA and BFDGE revealed a low cytotoxicity (21). Another *in vitro* study compared the cytotoxicity of three different types of conventional resin composites which contained Bis-GMA (22). The cell culture was carried out in a medium containing Balb/c 3T3 fibroblasts by using the following methods at different time points: MTT assay, Enzyme-Linked Immunosorbent Assay (ELISA) and HPLC analysis (22). The release of Bis-GMA from the resin composites revealed a significant cytotoxicity in contact with fibroblasts. The increase of the cytotoxicity was noted in function of the content of Bis-GMA into the culture medium (22).

Selected *in vivo* studies also reported toxicity in *Danio rerio* zebrafish embryos or human participants (Table 1). Zebrafish adults were maintained on a 14/10 h light/dark schedule. Different contents of Bis-GMA (10 nM, 100 nM, 1 μM and 10 μM) were added for 12 hours post fertilization (hpf) in the surrounding culture environment and their toxicity and mortality was daily evaluated (23). The concentrations of Bis-GMA were chosen from previous studies on the release of BPA (48). An increase in zebrafish mortality of approximately 30 % was reported and therefore the 10 μM Bis-GMA increased the mortality percentage up to 55 % (23). The mortality was significantly low (as the baseline) when the zebrafish was exposed to 10 nM or 100 nM. Thus, high contents of Bis-GMA revealed an adverse effect to zebrafish embryo since craniofacial abnormalities were noticed such as mandible malformations, decreased eye diameter, increased interocular distance, lack of pigmentation, edema, and incorrect spine shape (23). The severity of all these morphological changes was dependent on the monomer concentration, denoting the most severe defects at 1 μM Bis-GMA (23).

Literature has shown that the release of monomers and other additives can be dangerous for the human body and environment (36,37). Possible routes for systemic ingestion of monomers released by the resin composites can be established through the

oral mucosa, dentin-pulp complex, lungs by breathing, and the gastrointestinal tract ingestion (35,49,50). Such routes are illustrated in Figure 2 and 3.

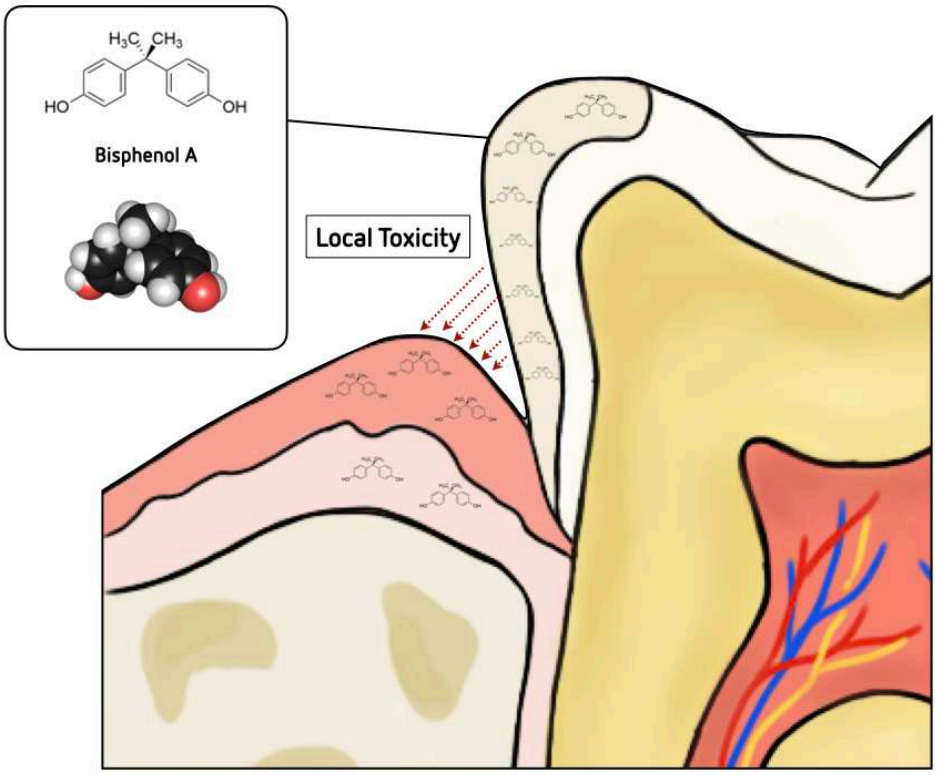

Figure 3: Schematic drawing of the local toxicity.

In vivo studies have found that concentrations of BPA in saliva and urine have increased after performing a resin-based dental restoration containing BPA (51–54). Human salivary flow rate, intestinal absorption, and metabolic purification are physiological conditions that should also be analyzed when assessing the toxic potential of substances (55). BPA is an endocrine disruptor and an agonist of the estrogen receptor that can cause toxicity as validated in previous studies. EFSA (European Food Safety Agent) agreed in 2015 that TDI (Tolerable Daily Intake) of BPA is at 4 µg/kg body weight/day. In this way, the use of BPA in food contact packaging has been banned in many countries in Europe (1,16).

5. CONCLUSIONS

Within the limitations of the *in vitro* and *in vivo* selected studies, the following concluding remarks can be drawn as follow:

- The content and chemical composition of the organic matrix of resin composites influence the availability of BPA derivatives. Most dental materials rarely contain pure BPA rather than BPA-derivatives such as Bis-GMA and Bis-EMA. Other monomers like Bis-DMA can be hydrolyzed into BPA in human saliva. Resin composites such as flowable materials showed a higher proportion of organic matrix when compared to conventional resin composites. Thus, a high content of organic matrix provides a wider surface contact area which is susceptible to erosion and wear in the oral cavity leading to a high release of toxic monomers.
- The light-curing parameters (distance, wavelength, intensity, mode, and time) affected the polymerization of the organic matrix of resin composites and further release of BPA. A high degree of conversion of the organic matrix occurs on optimum light-curing parameters. That results in a low release of monomers and minimum toxicity to the dentin-pulp complex, mucosa, or periodontal tissues.
- The release of BPA was detected at approximately 8.5×10^{-4} and 2.09×10^{-2} µg/ml. However, a few studies could not detect any release of BPA from resin composites due to the limitations of the physicochemical methods. Although small contents of BPA could not be measured by *in vivo* or *in vitro* studies, it should not be excluded that BPA might be released at concentrations below 0.02 ppm which can be toxic.
- The cytotoxicity of Bis-GMA from the resin composites in contact with fibroblasts increased in function of the content of Bis-GMA into the culture medium. The cytotoxicity of the resin composites decreased under light-curing methods using a high-power intensity over a short period of time.
- Dental clinicians should pay attention to the proper use of resin composites considering light-curing parameters (distance, wavelength, intensity, time) and equipment for the polymerization and decrease of residual toxic monomers. Further *in vivo* studies are needed to validate the local and systemic toxicity of BPA and their derivatives released from resin composites.

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