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REVIEW ARTICLE

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An integrative review on the toxicity of Bisphenol A (BPA) released from resin composites used in dentistry

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Abstract

The main aim of this study was to perform an integrative review on the release of bisphenol A (BPA) from resin-matrix composites and potential toxic effects. A bibliographic search was performed on the PubMed platform using the following keywords: "Bisphenol A" OR "BPA" AND "resin composite" OR "composite resin" AND "toxicity" OR "cytotoxicity" OR "release". Inclusion criteria involved *in vitro* and *in vivo* studies on the release and toxicity of BPA. Results highlighted the release of BPA from resin-matrix composites due to insufficient polymerization and/or degradation of the polymeric matrix. BPA is part of the organic matrix of resin-matrix composites and may be hydrolysed in human saliva, although studies report that low doses might not be detected by traditional chemical analysis. Studies exposing zebrafish embryos to different concentrations of Bis-GMA, showed 55% mortality at 10 μM Bis-GMA while 30% mortality was recorded at 1 μM Bis-GMA. In patients, a BPA concentration of around 2.09×10^{-2} μg/ml was found in the saliva after placement of lingual orthodontic retainers with resin-matrix composites. Also, the BPA molecule can be swallowed and absorbed by the oral/gastrointestinal mucosa, which might result in systemic toxicity. The degradation of resin-matrix composites and release of BPA in oral environment are dependent on the organic matrix content and on the polymerization method. A increased release of BPA can lead to the absorption into oral and gastrointestinal mucosa with high risks of local and systemic toxicity.

KEYWORDS
bisphenol A, BPA, cytotoxicity, resin composite, toxicity

1 | INTRODUCTION

Bisphenol A (BPA) is an organic compound, which is used in the synthesis of polycarbonate-, epoxy-, and methacrylate-matrix materials utilized in several industrial and health fields.[1,2] Resin-matrix composites used in dentistry have BPA derivatives 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 20wt% while Bis-EMA can range from 1 up to 5wt% and Bis-MPEPP between 5 and 10wt%.[4,5] Although the popularity of resin-matrix composites has increased in recent years, a concern on the release of toxic molecules such as BPA and its derivatives has gathered attention by scientists, clinicians, and patients.[2,4,5]

After light curing, the release of methacrylate monomers from resin-matrix composites occurs mainly within the first 24 h and may

continue to have effects over time due to degradation by chemical and mechanical processes.[6,7] Chemical degradation occurs due to a hydrolysis and enzyme-induced catalysis by human saliva esterase and oral fluids from dietary.[7-9] On the mechanical phenomena, tooth brushing, occlusal sliding, and abrasion are the dominant wear pathways on resin-matrix composites. Additionally, the unproper polymerization of the resin-matrix 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-matrix composites.

BPA and its derivatives have been identified as an endocrine disrupter effective in binding and activating the estrogenic receptor.[11] BPA may be ingested and absorbed by the oral and gastrointestinal mucosa leading to localized and systemic toxicity.[12-14] As a consequence, BPA is classified in category 3 within the Globally Harmonized System Hazard Classification, on human fertility (risk phrases: R62 or R63).[15] *In vivo* studies have shown that the administration of low content of BPA, both pre- and post-natal, 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, decreased sperm production, neurologic system disturb, immune system disturb, insulin sensitivity, and lack of lipid metabolism.[14,16,17] Besides, there is the possibility to induce adverse effects in the brain, cardiovascular system, thyroid, intestine, and prostate.[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 an integrative review on the release of BPA from resin-matrix composites and the resultant adverse biological effects. It was hypothesized that BPA may be released from resin-matrix composites at different amounts depending on the chemical composition of the restorative materials and the 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

2.1 | Information sources and search strategy

A literature search was carried out on PubMed (via National Library of Medicine) considering such database includes the major articles in the field of dentistry and biomaterials. The following combination of search terms was used in this study: *"Bisphenol A"* OR *"BPA"* AND *"Resin Composite"* OR *"Composite Resin"* AND *"Toxicity"* OR *"Cytotoxicity"* AND *"Release"*. The inclusion criteria involved articles published in the English language, up to January 2020, regarding the release and toxicity of bisphenol A (BPA) molecule from resin-matrix composites used in dentistry. The eligibility inclusion criteria used for article searches also involved: experimental studies, meta-analyses, randomized controlled trials, and prospective cohort studies. The exclusion criteria were the following: articles without abstract; case report with short follow-up period; release of BPA from resin-matrix materials used in other fields. Also, a hand-search was performed on the reference lists of all primary sources and eligible studies of this systematic search for additional relevant publications. Studies based on publication date were not restricted during the search process. The present search of studies was carried out in accordance with previous integrative or systematic review articles.[20-22]

2.2 | Study selection and data collection process

The articles retrieved by the search process were evaluated in three steps. At first, the total of articles was compiled for each combination of key terms and the duplicates were removed using Mendeley citation manager (Elsevier B.V.). Studies were primarily scanned for relevance by title, and the abstracts of those that were not excluded at this step were assessed. The second step comprised the evaluation of the abstracts and non-excluded articles, according to the eligibility criteria on the abstract review. Three of the authors (J.C.M.S.; L.L-R.; L.R-G.) independently evaluated the titles and abstracts of potentially pertinent articles. An initial evaluation of the abstracts was carried out to establish whether the articles met the main aim of this study. Then, selected articles were individually read and analyzed concerning the purpose and hypotheses of this study. At last, the eligible articles received a study nomenclature label, combining first authors' names and year of publication. Two reviewers independently collected and catalogued data, such as authors' names; 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. Data of the reports were harvested directly into a specific data-collection form to avoid multiple data regarding various reports within the same study (e.g., reports with different set-ups). This evaluation was individually carried out by two researchers, followed by a joint discussion to select the relevant studies.

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 studies 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 studies were included in the present integrative review.

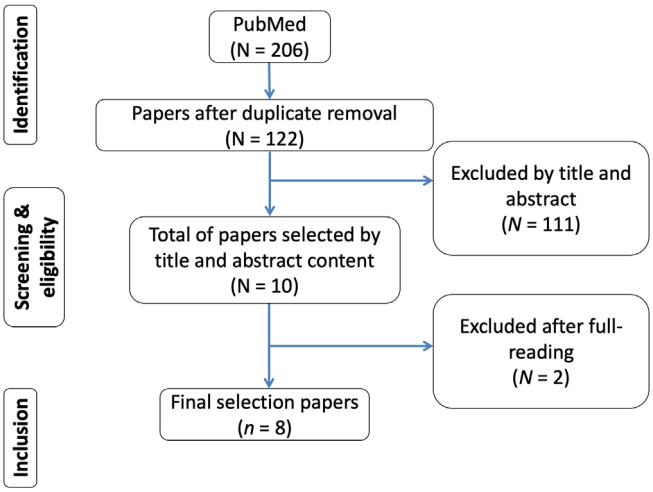

FIGURE 1 Flow diagram of the search strategy used in this study

Of the eight selected studies, one study compared the chemical composition of different resin-matrix composites commercially available concerning the presence of BPA while 3 (37.5%) articles measured the *in vitro* release of BPA or Bis-GMA monomers.[3,5,23.] 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.[24,25] 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 lingual orthodontic retainers.[2] The major findings are shown in Table 1 and drawn as follow:

- A total of 160 different resin-matrix composites from 31 manufacturers were assessed regarding the chemical composition of the methacrylate-based matrix. Approximately 81% resin-matrix composites comprised BPA derivatives in their organic matrix of which Bis-GMA corresponded to the main (74%) methacrylate-based monomer. Twenty three resin-matrix 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 lingual orthodontic retainers. BPA levels detected in urine did not appear to have any link to the placement of the lingual orthodontic 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[26];
- *In vitro* analysis detected significant amounts of BPA, TEGDMA, and other methacrylate-based monomers released from orthodontic adhesives used in daily clinical practice. The content of BPA at around 12.54% was significantly high for inducing localized or systemic toxicity regarding the previous studies.[4]

4 | DISCUSSION

4.1 | Resin composites

Nowadays, the esthetic outcomes have been getting attention and the development of resin-matrix composites provide a variety of clinical applications in dental restorations, prosthetic cementation, occlusal fissure sealing, and orthodontic adhesion, and retaining.[27,28] The chemical composition of resin-matrix composites varies according to the clinical applications and manufacturers. The organic matrix often involves monomers such as Bis-GMA, TEGDMA, UDMA, Bis-EMA, and photoinitiators, as seen in Table 1 and Figure 2. The inorganic content can reach up to 90wt% 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.[27,30,31] The balance in the percentage of the organic matrix and inorganic fillers determine the physicochemical properties of the resin-matrix composites.[8,29-32]

The polymerization reaction of methacrylate-based materials is accomplished due to the presence of 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.[8,32,33] Nowadays, a light-emitting diode 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.[8,29] The degree of conversion during the polymerization of the monomers ranges from 50 up to 70%,[34,35] although the maximum degree of conversion 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 leading to a susceptibility to degradation in the oral cavity.[36,37] Then, BPA can be released from the organic matrix of resin-matrix to the surrounding environment. An unproper polymerization and the degradation of the resin-matrix composites can lead to a high release of monomers to the saliva and oral tissues, which include BPA and its derivatives[6] (Figure 2). BPA molecules and their derivatives can be absorbed locally by immune response cells and tissues in the gingival margins, as illustrated in 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 the saliva and absorbed by the gastrointestinal mucosa leading to systemic toxicity if not excreted by urine.[13,38] The localized and systemic pathways for BPA release from resin-matrix composites are illustrated in Figure 3.[39,40]

4.2 | Release of BPA from resin-matrix composites

Previous studies have shown a high release of Bis-GMA since that monomer is often existing in the chemical composition of conventional resin-matrix composites. Also, a lower degree of conversion has been associated with a high content of Bis-GMA.[41-43] However,

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)[24]	Identification of the formation of the tetrahydroxylated metabolites of BFDGE and Bis-GMA after exposure of each monomer to the liver S9 fractions *in vitro*. Evaluation of the biocompatibility of the hydroxylated metabolites in relation to their parent compounds.	-*In vitro*. Rat or human liver S9 fractions. - Liquid chromatography/mass spectrometry (LC/MS)	Bis-GMA was obtained from 3 M-ESPE. BFDGE, BDAPE-40H and BFDPE-40H were obtained from Fluka chemical company.	After 10 min, more than 90% of the initial Bis-GMA and BFDGE concentrations had disappeared.	Cytotoxicity against L929 cells showed that the metabolites were significantly ($p < 0.05$) lesser cytotoxic than the parent monomers.
Al-Hiyasat et al. (2005)[25]	Determination of the cytotoxicity of commercially available flowable resin-matrix materials with different structures in comparison with those of their traditional resin-matrix composites, and to determine their leachable products.	-*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 (HPLC)	UDMA, Bis-GMA, TEGDMA, photonitiator/activation system, silane-treated fillers (Admira, VOCO, Germany) UDMA, Bis-GMA, TEGDMA, photonitiator/activation system, silane-treated fillers (Admira Flow, VOCO, Germany) UDMA, Bis-GMA, Bis-EMA, photonitiator/activation system, silane-treated fillers (Filtek Z250, 3M ESPE, USA) Bis-GMA, TEGDMA, photonitiator/activation system, silane-treated fillers (Filtek Flow, 3M ESPE, USA) UDMA, Bis-GMA, TEGDMA, photonitiator/activation system, silane-treated fillers (Tetric Ceram, Ivoclar Vivadent, Liechtenstein) UDMA, Bis-GMA, TEGDMA, photonitiator/activation system, silane-treated fillers (Tetric Flow, Ivoclar Vivadent, Liechtenstein)	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 resin-matrix composites compared with traditional resin-matrix composite. TEGDMA has been reported to be toxic in different cell lines.
Koin et al. (2008)[23]	Evaluation of the degradation of this model overlayer, after being aged in water, by LC/MS. A two-week aging period was chosen, since this was found to be the interval of maximum release of Bis-GMA from commercial dental composites.	-*In vitro*. Degradation of dental composites was studied in a simplified overlayer model in which Bis-GMA was bonded to a porous silica surface. - Liquid chromatography/mass spectrometry (LC/MS)	Bis-GMA and silane-treated silca fillers.	Bis-GMA: 0 μg/ml	N/A
Kang et al. (2011)[2]	Examination of the amounts of salivary and urinary BPA to assess the amount of BPA released from	-*In vivo*, 22 human participants: 10 male patients (range, 13–25 years)	Flowable resin composite containing Bis-GMA and TEGDMA (Filtek Flow, 3M ESPE, USA).	The saliva samples collected immediately after lingual retainer placement showed a significant	The amount of BPA leaching from Bis-GMA–based composite used for bonding orthodontic lingual

TABLE 1 (Continued)

Author (year)	Purpose	Study design	Chemical composition	BPA release (μg/ml)	Biological response
	the resin-matrix composite used in a lingual orthodontic retainer during the first month of placement.	and 12 female patients (range, 13–32 years). - Liquid chromatography/mass spectrometry (LC/MS)	Hybrid resin composite containing Bis-GMA, UDMA and Bis-DEMA (Filtek Z250, 3M ESPE, USA).	increase in BPA compared with the baseline samples (before placing the lingual 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 8.53 $x10^{-4}$ and 2.0889 $x10^{-2}$ μg/ml. The urine samples contained BPA at various times but without an association with the time point.	retainers was detected at low concentration and far below the reference doses for daily uptake.
Bationo et al. (2016)[4]	Characterization of monomers released from orthodontic adhesives.	-*In vitro*. - samples of orthodontic adhesives by associating 2 techniques: GC /MS.	Transbond XT (3M ESPE, USA) : 10-20 wt% Bis-GMA BPA Bis 2-hydroxyethyl ether dimethacrylate: 5-10wt%; Silane-treated quartz: 70-80wt%; Silane-treated silica: < 2wt%; Diphenyliodonium hexafluorophosphate: < 0.2wt%. Transbond supreme LV (3M ESPE, USA): Bis-GMA: 10-15wt%; TEGDMA: 10-15wt%, Bis-EMA: 1-5wt%; Silane treated ceramic: 52-60wt%, Silane-treated zirconium oxide: 3-11wt%, Silane-treated silica: 3-11wt%, Functionalized dimethacrylate polymer: 1-5wt%. Blugloo (Ormco Corp, USA): Glycidyl methacrylate: 3-5wt%, Inert fillers and pigments, MonoLok 2 light-activated bonding system: Monomers of aromatic and aliphatic dimethacrylates; methacrylate	BPA: 0 μg/ml	N/A

(Continues)

TABLE 1 (Continued)

Author (year)	Purpose	Study design	Chemical composition	BPA release (μg/ml)	Biological response
			monomers; Camphorquinone; Tertiary amine.		
Dursun et al. (2016)[3]	Reporting an exhaustive list of resin-matrix composites commercially available in Europe focusing on their chemical composition. Estimation of the number of resin-matrix composites containing BPA or its derivatives (Bis-GMA, Bis-DMA, Bis-EMA, Bis-MPEPP, PC Bis-GMA).	-Case report -MSDS	160 different brands of resin-matrix composites.	N/A	N/A
Krammer et al. (2016)[26]	Evaluation of 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% DMSO for the control group. A solution of Bis-GMA (Sigma-Aldrich, USA) dissolved in DMSO for test group.	N/A	Exposure to 1 and 10 μM Bis-GMA in *Danio rerio* embryos resulted in increased mortality of approximately 30% and 55%, respectively. Changes in morphologic efatures, mainly craniofacial abnormalities, were seen at concentrations as low as 10 nM BisGMA.
Pelourde et al. (2018)[5]	Evaluation of the *in vitro* release of monomers from resin-matrix orthodontic retainers.	-*In vitro*. -Samples of each resin composite. - Gas chromatography/mass spectrometry (GC/MS)	Transbond LR (3M ESPE, USA): Silane treated quartz: 75-85%, TEGDMA: 5-15wt%, Bis-GMA: 5-15wt%, Dichloromethylsilane reaction product with silica: < 2wt%, N,N-dimethylbenzocaïne: < 0.3wt%, Diphenyliodonium hexafluorophosphate: < 0.1wt%, Transbond XT (3M ESPE, USA): Silane treated quartz: 70-80wt%; Bis-GMA: 10-20wt%, Bisphenol A Bis (2-hydroxyethyl ether) dimethacrylate: 5-10wt%, Silane-treated silica, < 2%; Diphenyliodonium hexafluorophosphate: < 0.2wt%.	BPA: 0 μg /ml TEGDMA: 31.7 μg/ml (Transbond LR) / 13.2 μg/ml (Transbond XT)	N/A

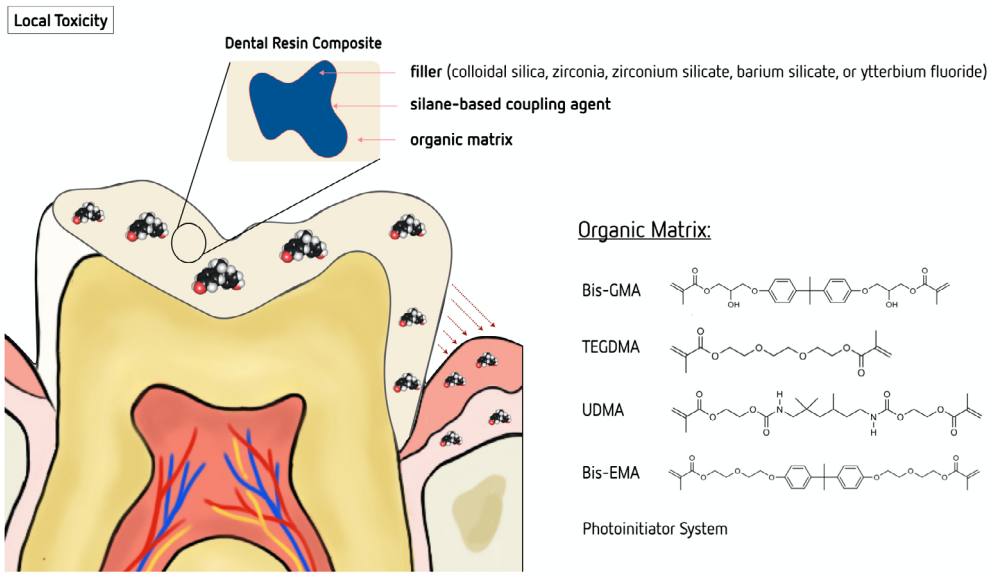

FIGURE 2 Schematics on the chemical composition of the resin-matrix composites used in dental restorations

Bis-EMA has been used to replace Bis-GMA to control the physical properties of recent resin-matrix composites.[23] Thus, BPA is formed from the degradation of Bis-GMA or Bis-EMA and released at varied concentrations depending on the chemical and mechanical processes occuring in the oral cavity. The degradation phenomenon of resin-matrix composites can occur due to chemical reactions and wear (e.g., abrasion) 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 Figures 3 and 4.[44] That results in a faster chemical degradation by hydrolysis of the organic matrix of the resin composites.[7,44] The mechanical factor is linked to the abrasion wear of occlusal surfaces of resin-matrix composite restorations leading to a material loss and ejection of polymeric and monomeric debris to the surrounding tissues and human saliva (Figure 2).[7] The wear of restorative surfaces depends on the polymerization and resultant physical properties of the resin-matrix composites.[7,27,45]

A previous study reported an exhaustive list of commercially available resin-matrix composites in Europe and data regarding the presence of BPA and its derivates.[3] The authors identified a total of 160 types of resin-matrix composites from 31 different manufacturers, but only 23 manufacturers responded to the survey. The detailed chemical composition of 130 different resin-matrix composites was therefore listed, of which 112 had BPA derivatives in their chemical composition, while 97 contained Bis-GMA and 43 Bis-GMA and UDMA. However, 17 resin-matrix composites contained monomers not derived from BPA or Bis-GMA (UDMA or sometimes TEGDMA) in their chemical composition while 6 had only UDMA in their chemical composition and only 1 did not contain any BPA neither UDMA or TEGDMA derivatives.[3]

Studies indicate that ordinary resin-matrix composites reveal regularly lower cytotoxic when compared to flowable resin-matrix composites. However, nanohybrid Organically Modified Ceramics (ORMOCER) flowable composite showed lesser cytotoxicity than those recorded on traditional nanohybrid ORMOCER composites. The monomers released from the test resin-matrix 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. In culture medium, a small concentration of 0.64 BPA μg/ml was detected from microhybrid resin-matrix composites while 1.65 μg/ml BPA was detected from flowable nano-structured resin-matrix composites.[25] Another *in vitro* study reported the degradation of resin-matrix composites using a simplified overlay model in which Bis-GMA was bonded to a porous silicon oxide surface.[23] 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.[23]

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-matrix composites.[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 orthodontic retainer at higher values of around 31.7 mg/ml when compared to aligner attachments (13.12 mg/ml). Other toxic components have been detected, such as, iodobenzene, iodobiphenyl, triphenyl stibine, among others. Thus, toxic and carcinogenic molecules not mentioned in the safety data sheets were detected.[5]

A few studies reported that the release of BPA and its derivative is hardly measurable.[4,5,23] 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.[46] In the oral environment, the overall degradation of resin-matrix composites can be progressive,

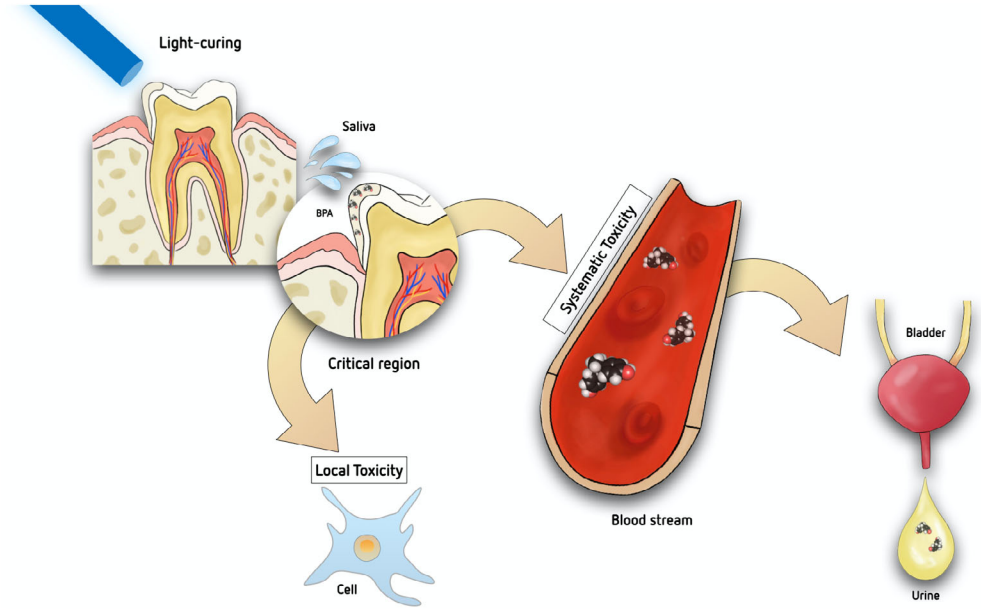

FIGURE 3 Schematics of the localized and systemic toxicity pathways

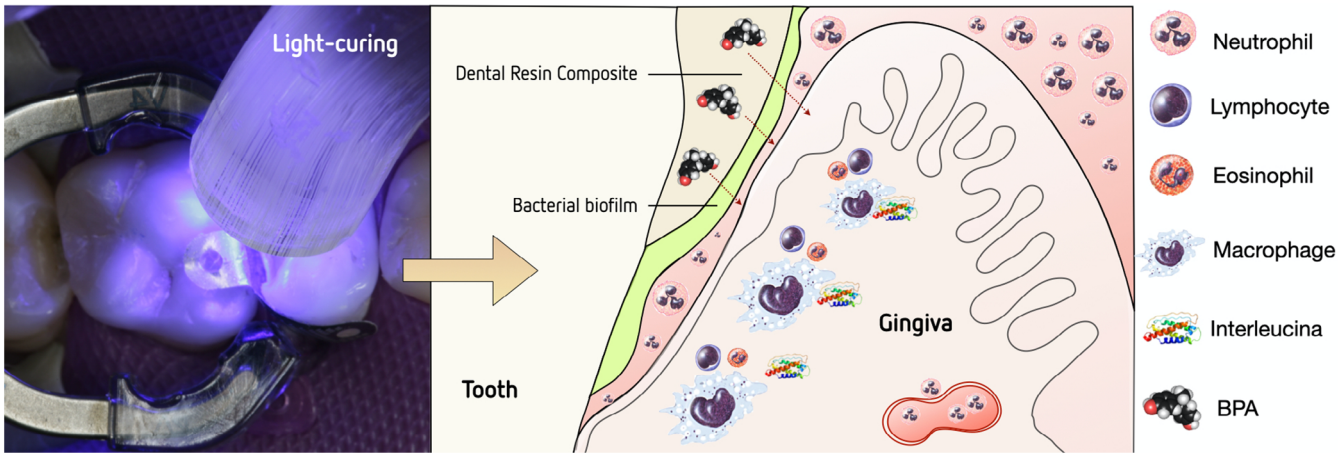

FIGURE 4 Schematics on the release of BPA and its derivatives from resin-matrix composites to the surrounding soft tissues and chemical interaction with cells

and the saturation could never be reached due to the continuous removal of the monomers by the human saliva flow.[47] The retrieval of the saliva medium should be performed at standard time points to avoid the saturation by the leached products.[38,46] Therefore, margins of resin-matrix composite restorations have not been examined concerning the release of BPA and its derivatives, as illustrated in Figure 4. It must be emphasized that the intimacy of the resin-matrix composite surfaces can increase the amount of BPA in the connective tissues. In fact, the release of BPA from resin-matrix composites 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 toward the human saliva.

One study stated that the color of the resin-matrix composite and the method of light curing show some correlations with the cytotoxicity.[43] Resin-matrix composites with a higher chroma exhibited higher cytotoxicity, even though changing the light curing method.[43] Such *in vitro* study used human gingival fibroblasts to validate the decrease in cytotoxicity when applying a light curing method with a high power intensity over a short period of time.[48] In fact, dental clinicians should take into consideration the following factors: (i) distance between the light-curing unit tip and the resin-matrix composite; (ii) spectra wavelength; (iii) light intensity and energy delivered to polymerization; (iv) period of time; (v) the compatibility between the organic matrix photoinitiator and wavelength.[41-43,49,60]

The release of monomers and products from the organic matrix of resin-matrix composites has also been reported in previous *in vivo* studies.[38,50] In clinical studies involving human participants, one study revealed data on the content of BPA in saliva and urine as a result of the degradation of resin-matrix 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 of orthodontic retainer.

However, the salivary BPA values detected were lower than those recorded for 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 the 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 | Localized and systemic toxicity

In vitro studies 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 4. A previous study reported the cytotoxic, mutagenic, and estrogenic effects of BFDGE e Bis-GMA in contact with MCF-7 human breast cancer cells, L929 mouse fibroblast, or S9 rat hepatic cells.[24] Tetrahydroxy and methacrylic acid metabolites from Bis-GMA and BFDGE revealed a low cytotoxicity.[24] Another *in vitro* study compared the cytotoxicity of three different types of conventional resin-matrix composites which contained Bis-GMA.[25] 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.[25] The release of Bis-GMA from the resin-matrix composites revealed a significant cytotoxicity in contact with fibroblasts. The increase of the cytotoxicity was also noted in function of the content of Bis-GMA into the culture medium.[25]

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. After fertilization (hpf), different content of Bis-GMA (10 nM, 100 nM, 1 μM and 10 μM) were added for 12 h post fertilization (hpf) in the surrounding culture environment for 12 hr and their toxicity and mortality was daily evaluated.[26] The concentrations of Bis-GMA were chosen from previous studies on the release of BPA.[51] An increase in zebrafish mortality of approximately 30% was recorded at 1 μM Bis-GMA that increased up to 55% at 10 μM Bis-GMA.[26] The mortality was significant low (as the baseline) when the zebrafish was exposed to 10 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.[26] The severity of all these morphological changes was dependent on the monomer concentration, denoting the most severe defects at 10 μM Bis-GMA.[26]

Literature has shown that the release of monomers and other additives can be dangerous for the human body and environment.[39,40] Possible routes for systemic ingestion of monomers released by the resin-matrix composites can be established through the oral mucosa, dentin-pulp complex, lungs by breathing, and the gastrointestinal tract ingestion.[38,52,53] Such routes are illustrated in Figures 3 and 4.

In vivo studies have found that concentrations of BPA in saliva and urine have increased after performing a resin-based dental restoration containing BPA.[13,54-57] It should be emphasized that the expiry date of the resin-matrix composites may influence their cytotoxicity. A study reported a statistically significant difference in cell viability between groups of resin-matrix composites regarding the expiry date, once the expired date materials revealed the lowest cell viability (L929 mouse fibroblasts culture) for 2, 5, and 7 days.[58] Human salivary flow rate, intestinal absorption, and metabolic purification are physiological conditions that should also be analyzed when assessing the toxic potential of substances.[59] BPA is an endocrine disruptor and an agonist of the estrogen receptor that can cause toxicity as validated in previous studies. BPA derivatives also induce a similar endocrine disrupter effect.[14] 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 contain BPA-derivatives such as Bis-GMA and Bis-EMA. Other monomers like Bis-DMA can be hydrolyzed into BPA in human saliva. Flowable resin-matrix composites showed a higher proportion of organic matrix when compared to traditional resin-matrix 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 depending 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-matrix composites due to the limitations of the physicochemical methods. Although small content of BPA could not be measured by some *in vivo* or *in vitro* studies due to experimental limitations, it should not be excluded that BPA might be toxic when released at low concentrations below 0.02 ppm;
- The cytotoxiciy of Bis-GMA from the resin-matrix composites in contact with fibroblasts increased in function of the content of Bis-GMA into the culture medium. The cytotoxicity of the resin-matrix composites decreased under light-curing methods using a high-power intensity over a short period of time;

- Clinicians should pay attention to the proper use of resin-matrix composites considering light-curing parameters and equipment for the polymerization and decrease of residual toxic monomers. Further *in vivo* studies are required to validate the localized and systemic toxicity of BPA and their derivatives released from resin-matrix composites.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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