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Minimal invasive and aesthetic rehabilitation of maxillary lateral incisor agenesis: chemical-mechanical behaviour

Lígia Lopes da Rocha

Tese conducente ao **Grau de Doutor** em **Ciências Biomédicas**

—

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Trabalho realizado sob a Orientação de

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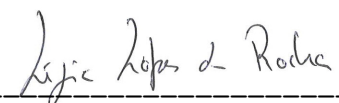

(Lígia Lopes da Rocha)

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Publications resulting from doctoral work (listing)

This work is composed of several research papers, being each chapter structured using one or more papers in sequence. The main goal of such method was the validation of each experimental and numerical contribute being presented. Special care and effort were taken to provide the readers with the necessary experimental and numerical details, as well as avoiding the repetition of information from a paper to another.

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Paper 1: Lopes-Rocha L, Garcez J, Tiritan ME, da Silva LFM, Pinho T. Maxillary lateral incisor agenesis and microdontia: Minimally invasive symmetric and asymmetric esthetic rehabilitation. *Rev Port Estomatol Med Dentária e Cir Maxilofac*. 2022;62(4). doi: 10.24873/j.rpemd.2022.01.857. IF: 0.14; Q4 (Published)

Paper 2: Lopes-Rocha L, Rocha D, Pinho T. A multidisciplinary approach to maxillary lateral incisors agenesis (MLIA): a case report. *Oper Dent.* 2022;47(4):367b-374. doi: 10.2341/21-069-S. IF: 3.02; Q1 (Published)

Paper 3: Lopes-Rocha L, Ribeiro-Gonçalves L, Henriques B, Özcan M, Tiritan ME, Souza JCM. An integrative review on the toxicity of Bisphenol A (BPA) released from resin composites used in dentistry. *J Biomed Mater Res B Appl Biomater.* 2021;109(11):1942-1952. doi:10.1002/jbm.b.34843. IF: 3.37; Q2 (Published)

Paper 4: Lopes-Rocha L, Hernandez C, Gonçalves VMF, Pinho T, Tiritan ME. Analytical methods for determination of BPA and derivatives released from dental resin composites and related materials. *Crit. Rev. Anal. Chem.* 2022;1:1-16. doi: 10.1080/10408347.2022.2093097. IF: 6.66; Q2 (Published)

Paper 5: Lopes-Rocha L, Gonçalves VMF, Cunha S, Garcez J, Pinho T, Tiritan ME. Evaluation of BPA and Bis-GMA release from recent dental composite materials by LC-MS/MS. *Int J Paediatr dent.* IF: 3.26; Q1 (Submitted)

Paper 6: Lopes-Rocha L, Carbas RJC, Borges CSP, Gonçalves VMF, Tiritan ME, Medeiros IS, da Silva LFM, Pinho T. Effect of aging on the mechanical properties among dental resin with and without Bisphenol A. *Strain.* IF: 2.58; Q2 (Submitted)

Paper 7: Lopes-Rocha L, Mendes JM, Garcez J, Sá AG, Pinho T, Souza JCM, Torres O. The Effect of Different Dietary and Therapeutic Solutions on the Color Stability of Resin-Matrix Composites Used in Dentistry: An In Vitro Study. *Materials.* 2021;14(21):6267. doi: 10.3390/ma1421626. IF: 3.62; Q2 (Published)

Resumo

A agenesia dos incisivos laterais maxilares (MLIA) é uma condição que compromete significativamente o sorriso, com particular preocupação nos pacientes mais jovens. Após tratamento ortodôntico com fechamento de espaço, muitas resinas compostas utilizadas para remodelação estética contêm Bisfenol A (BPA) e derivados na sua composição, os quais são considerados monómeros tóxicos e reconhecidos como desreguladores endócrinos. Dados sobre a toxicidade, libertação e quantificação do BPA e seus derivados por parte da resina composta, em estudos *in vitro* e *in vivo,* foram reunidos em duas revisões da literatura relativas à toxicidade e libertação BPA e dos seus derivados,

Uma abordagem conservadora com restaurações diretas com resina composta no sentido de melhorar os resultados clínicos após tratamento ortodôntico (fechamento de espaço), em casos de agenesia dos incisivos laterais superiores (simétrica e assimétrica), assim como em casos de microdontia foi investigada.

Foram realizados dois estudos clínicos nos quais pacientes jovens foram reabilitados com resina composta com e sem BPA na sua composição. Os casos clínicos revelaram que abordagens ortodônticas e restauradoras cuidadosamente planeadas e metodicamente executadas podem alcançar resultados estéticos previsíveis, que melhorarão a autoestima do paciente, com uma solução minimamente invasiva. Além disso, foi estudado a libertação e influência do BPA e dos seus derivados nas resinas compostas e a relação com o seu desempenho químico-mecânico. Em paralelo o grau de libertação de BPA e de metacrilato de bisfenol A-glicidilo (Bis-GMA) em seis resinas compostas foi avaliado em um estudo *in vitro* numa solução etanol/água. O BPA não foi detetado em nenhuma resina composta estudada. No entanto, verificou-se a libertação diária, durante sete dias, entre 33,46 e 0,53 ng/mL de Bis-GMA. Também foi determinado as diferentes propriedades químicas e mecânicas das resinas compostas com e sem BPA, sob as mesmas condições. Concluiu-se que não existem diferenças significativas entre as propriedades mecânicas das

resinas contendo ou não BPA na sua composição. De acordo com todos os parâmetros estudados, a resina Esmalte Plus HRi Bio Function Enamel (BF, Micerium SpA, Avegno, Itália) apresentou o melhor comportamento químico e mecânico, e caracterizou-se por ser uma resina composta isenta de BPA e derivados.

A quantificação de BPA e seus derivados em materiais à base de resinas compostas exige metodologias analíticas adequadas a quantificações residuais. Além disso, a informação completa sobre a composição química dos produtos dentários por parte dos fabricantes é importante para a melhor escolha por parte do corpo clínico. Por outro lado, estes estudos são importantes para o desenvolvimento de novos materiais isentos de toxicidade.

Palavras-chave: Compósito dentário à base de resina, resistência, propriedades mecânicas, metacrilato de bisfenol A-glicidilo , bisfenol A, agenesia dentária

Abstract

Maxillary lateral incisor agenesis (MLIA) is a condition that significantly compromises the smile, with particular concern in younger patients. Following orthodontic treatment with space closure, many composite resins used for esthetic reshaping contain Bisphenol A (BPA) and derivatives in their composition, considered toxic monomers and recognized as endocrine disruptors. Data on the toxicity, release and quantification of BPA and its derivatives from composite resin in in vitro and *in vivo* studies have been gathered in two literature reviews concerning the toxicity and release of BPA and its derivatives.

A conservative approach with direct composite resin restorations to improve clinical outcomes after orthodontic treatment (space closure) in upper lateral incisor agenesis (symmetrical and asymmetrical) and cases of microdontia was investigated.

Two clinical studies were performed in which young patients were rehabilitated with composite resin with and without BPA in its composition. The clinical cases revealed that carefully planned and methodically executed orthodontic and restorative approaches can achieve predictable esthetic results, which will improve the patient's self-esteem with a minimally invasive solution. Furthermore, the release and influence of BPA and its derivatives on composite resins and the relationship with their chemical-mechanical performance were studied. In parallel, the degree of release of BPA and bisphenol A-glycidyl methacrylate (Bis-GMA) in six composite resins was evaluated in an in vitro study in an ethanol/water solution. BPA was not detected in any composite resin studied. However, daily release over seven days was found to be between 33.46 and 0.53 ng/mL of Bis-GMA. It was also determined the different chemical and mechanical properties of the composite resins with and without BPA under the same conditions. It was concluded that there are no significant differences between the mechanical properties of resins containing or not BPA in their composition. According to all the parameters studied, the Esmalte Plus HRi Bio Function Enamel resin (BF, Micerium SpA, Avegno, Italy) presented the best chemical and

mechanical behavior. Therefore, it was characterized as a composite resin free of BPA and derivatives.

BPA quantification and its derivatives in composite resin-based materials require analytical methodologies suitable for residual quantifications. Moreover, manufacturers' complete information on the chemical composition of dental products is important for the best choice by clinical staff. On the other hand, these studies are important for developing new toxicity-free materials.

Keywords: Resin-based dental composite, strength, mechanical properties, bisphenol A-glycidyl methacrylate, bisphenol A, tooth agenesis

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List of acronyms and abbreviations

AF	Admira® fusion
BADGE	Bisphenol a diglycidyl-ether
BE	BRILLIANT Everglow™
BF	Enamel plus Hri bio function
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
DC	Degree conversion
DMSO	Dimethyl sulfoxide
ED	Ips Empress Direct
EFSA	European food safety agent
ELISA	Enzyme-linked immunosorbent assay
FS	Filtek™ Supreme XTE

GC/MS	Gas chromatography / mass spectrometry
hpf	Hours post fertilization
HPLC	High performance liquid chromatography
KNH	Knoop hardness number
LC/MS	Liquid chromatography / mass spectrometry
LED	Light-emitting diode
MCF-7	Michigan cancer foundation-7 (human breast cancer cell line)
MLI	Maxillary lateral incisor
MLIA	Maxillary lateral incisor agenesis
MMA	Methylmethacrylate
MSDS	Material safety data sheet
NC	Experimental resin
ORMOCER	Organically modified ceramics
PC Bis-GMA	Polycarbonate-modified Bis-GMA
ppm	Parts per million
RBFDP	Resin-bonded fixed dental prosthese
TDI	Tolerable daily intake
TEGDMA	Triethylene glycol dimethacrylate
UDMA	Urethane dimethacrylate
EU	European Union

USA	United States of America

List of symbols

E	Young's modulus
E_b	Equivalent bending modulus
E_K	Kinetic energy
E_P	Potential energy
E_x	Longitudinal normal modulus
g	Gravitational acceleration
G	Shear modulus
K	Stress concentration factor
K	Elastic constitutive matrix
K_c	Critical stress concentration factor
m	Equivalent mass
M_∞	The mass uptake at equilibrium
M_1	Conditioned mass prior to immersion in water
M_2	Mass of the specimen at a specific time after immersion in water
M_3	Equilibrium weight of the specimen after desorption
P	Applied load
S	Saturation
t	Thickness

t_n	Cohesive tensile/normal stress
t_n^0	Tensile/normal strength
®	Registered brand
°C	Degrees Celsius
V	Volume
Vol%	Percentage content by volume
w	Width of the specimen
W_i %	Weight gain percentage
W_{sl}	Water solubility
W_{sp}	Water sorption
W_t %	Percentage content by weight
w_t.	Weight
δ	Displacement
δn0	Initial tensile/normal displacement
δnf	Final tensile/normal displacement
δs0	Initial shear displacement
δsf	Final shear displacement
$\dot{\varepsilon}$	Strain rate
εn	Tensile/normal strain
εs	Shear strain

ν	Poisson's ratio

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Chapter I

BACKGROUND AND MOTIVATION

1. Maxillary Lateral Incisor Agenesis (MLIA)

Dental agenesis or hypodontia is the congenital absence of one or more teeth in primary or permanent dentition.[1,2] If it has not erupted in the oral cavity and is not visible from radiography, and has not been extracted or accidentally lost.

Epidemiological studies demonstrated that unilateral or bilateral maxillary lateral incisor agenesis (MLIA) is a frequent clinical condition in permanent dentition, affecting approximately 2% of the population.[3–5] In the Caucasian population, it predominates up to 20% considering all types of agenesis, including the third molars.[6,7] A prevalence of 1.3% was estimated in the Portuguese population, with a slightly higher frequency in females.[4] The type of congenitally missing teeth in the permanent dentition of patients with MLIA can differ from author to author, as well as the demographic and geographic profiles of the patients.[8]

There is evidence that congenital absence of teeth may result from environmental or hereditary causes or even their interaction.[9–11] The development of human dentition concerning structure and organization relies on genetic control and involves several factors, so it is logical to assume that mutations in some genes encoding these factors may affect the normal development of teeth and may eventually cause their absence. The mutations in genes responsible for tooth development are marked as PAX9, MSX1, and AXIN2.[12] Of these, MSX1 is related to congenitally missing first premolars and incisors.[13] Evidence for a genetic cause for non-syndromic hypodontia comes from identifying significant familial aggregation of MLIA. Moreover, it suggests upper lateral incisor microdontia as part of the same phenotype, segregating as an autosomal dominant trait with incomplete penetrance.[14] However, X-chromosome related and polygenic or multifactorial modes of transmission have also been proposed. A family aggregation study of a Portuguese family, consisting of 62 individuals with MLIA and 142 first-degree relatives, showed that the relative risk for a first-degree relative of an individual with MLIA to develop the same type of agenesis was 15 times higher when compared with a relative of an individual without this agenesis. The published results support a significant familiar aggregation of MLIA and show that MLIA

rarely segregates with other agenesis forms. Furthermore, they suggested that maxillary lateral incisor microdontia was part of the same phenotype.[14] However, the previous search for mutations in PAX9 and MSX1 genes, and their potential association with the MLIA phenotype in 12 Portuguese families, did not show a clear association between these genes and the MLIA phenotype.[14–16] Hence, despite recent developments, data concerning the genes responsible for less severe types of hypodontia are still limited and controversial.[17]

There is no consensual opinion on the possible correlation between the changes during maxillary development and tooth agenesis, even though some authors described a potential correlation.[16,18–20] According to Pinho *et al.* [18], MLIA is linked to a shortening of the upper maxilla and a negatively conditioned anterior-superior facial height dimension. There was a significant clinical association between the presence of MLIA (more often unilateral) and the deviation of the maxillary dental midline. MLIA was associated with a Class II malocclusion; this was more frequent on the same side as the agenesis.[21] Thus, various esthetic problems are related to upper lateral incisor agenesis, including median diastema, spacing between permanent incisors and canines, mesial migration of canines, midline shift in case of unilateral missing teeth.[22] Excessive retention of deciduous lateral incisors and maxillary canines, ectopically erupted canines, absence of canine eminence, asymmetric loss of primary teeth, dental asymmetries are significant apparent valuable factors for the diagnosis of the MLIA.[23]

2. Esthetic rehabilitation of patients with MLIA

Patients with MLIA are commonly challenged with functional and esthetic problems at a young age, which may affect their confidence and social relationships.[24–27] Restoring an unbalanced dentition is a challenging process, which demands a multidisciplinary approach that should focus on minimally invasive options to satisfy the expected functional and esthetic objectives.[27–31]

Orthodontically, two treatment options should be primarily considered: 1) space opening with a tooth-supported resin-bonded fixed dental prosthesis (RBFDP) or dental implant; 2)

space closure with canine camouflage.[32,33]

Space recovering through orthodontic therapy aim to provide adequate space for the replacement of missing teeth. The required space can be established by applying golden proportions by measuring Bolton's discrepancy.[34] The missing lateral incisor is replaced with the best of its esthetic and functional demand, using a range of several alternatives (RBFDP or dental implant) depending on the patient's chosen socioeconomic status and age. The main advantages of replacing by an implant are the possibility of obtaining an ideal occlusion, the maintenance of the canine in its natural position, and the clear benefit of avoiding any damage to the adjacent teeth.[35,36] Nonetheless, the placement of implants in the anterior area presents some visible disadvantages, such as bone resorption, infraocclusion of the implant, gingival retraction, the recession of the interdental papillae, gingival changes (including blue staining of the gingiva), and exposure of the abutments.[35,37–40]

Orthodontic space closure involves moving the canines to the missing lateral incisor place and contacting central incisors. In cases where the occlusion and anatomy/dimension of the canine in the lateral incisor position are acceptable to camouflage, orthodontic space closure with canine mesialization provides a satisfactory long-term result both functionally and esthetically.[41,42] The most significant advantage of space closure over other approaches is that adolescents do not have to remain untreated until the facial skeleton matures.[26]

Several treatment options are available for the esthetic and functional rehabilitation in cases of MLIA after space closure, such as a dental crown, ceramic veneers, and direct composite restorations, and these differ in terms of material used and laboratory involvement. The choice of restorative treatment should be based on some well-defined factors, such as, preservation of the vitality of the tooth, absence or minimal reduction of the dental structure, minimal invasion of the gingival area, the esthetic expectation of the patient, cost estimate, and duration of the treatment.[43]

In the past, one of the most commonly indicated treatment options to restore the esthetic features were dental crowns.[44] In dental crown treatment, which includes the removal of healthy dental hard tissue to adapt the physical requirements of the restoration, the

remaining tooth structure is prepared to receive the crown itself. However, the extensive removal of tooth structure carries a high risk of damage to the vital pulp of the tooth and surrounding tissue, like the gingiva.[45] Veneers are another treatment option that involves less tooth structure removal and achieves a comparable result to the conventional crown. Ceramic veneers can be a conservative and long-lasting option.[46–49] An additional restorative option is the direct composite restoration technique offered due to its low cost and conservative concept, which advocates more preservation of the tooth structure.[43,50–52] Also, it can be done by applying dental resin on a minimally or unprepared tooth surface in a single appointment.[53–55]

Composite resin has excellent adhesion to enamel,[53] with a reported overall survival rate of over 88% after 10 years.[54,56] Chipping has been reported to be the most common reason for failure, and shade matching can be challenging.[55] However, an essential advantage of this procedure over other restorative techniques is that it can be repaired and polished intraorally without the need to remove existing restorations, and without modifying the esthetics or mechanical performance.[57] Direct restoration approach can be considered over indirect technique when patients are young patients, or they are financially limited. With the development in dental materials' technology, more resin composite materials with improved handling and physical properties will be produced in the future.[58]

3. Dental resin composites

3.1. Composition

Resin composites have modernized dental care and allowed minimally invasive dentistry to preserve healthy tooth structure and provide natural-looking esthetic results.[59] However, research in dental resin composites is currently undergoing a revamp due to increasing request for low cost, non-toxic, greater longevity, biological functionality, and superior esthetics.[60]

The term "composite" or "composite material" describes a material structure fabricated

from two or more component materials, usually to provide enhanced properties or add more functionality to the original components.[60]

Dental restorative materials have also been developed into several resin-based composite materials, consisting mainly of three components, an organic, inorganic resin matrix, and a coupling agent to chemically bind the resin to the surface filler.[51]

A dental resin is an active organic monomer system in which the base monomer is mixed with the diluent monomer to obtain an optimized viscosity providing acceptable clinical handling properties.[60] The organic matrix consists in methacrylate monomers. The inorganic content can reach up to 90% wt resin-matrix composite, including one or two types of silanized ceramic or glass-ceramic fillers such as colloidal silica, zirconia zirconium silicate, barium silicate, or ytterbium fluoride.[51,61,62] The organic matrix often involves photoinitiators. Bisphenol-A (BPA) and other derivatives 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).[63] The balance in the percentage of the organic matrix and inorganic fillers determines the physicochemical properties of the resin-matrix composites.[61,62,64–66] 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.[67–69]

3.2. Classification

The size of the filling particles incorporated in the resin-matrix and the volume percentage are essential parameters that affect the physical-mechanical properties, enhancing the esthetics and handling properties of commercial dental composites.[70] As a result, the size has systematically decreased from traditional materials to nanocomposites, and the volume percentage has increased.[71]

Resin composites can be defined in many intrinsic filler-related characteristics, including filler size distribution, geometry and composition. Although, they have typically been

classified according to the size of their filler particles.[72,73] Macrofilled particles range from 10-50 µm (single size regime), also called traditional or conventional. Midifilled particles range from 1. 0-10 µm, also called midifil, fine or small particles, provide higher strength than a micro-filled and better polishing than a macro-filled. Finally, minifilled particles range between 0.1-1.0 µm, still have relatively high strength and better polishing than the medium-filled. Moreover, micro-filled particles range between 0.01-0.1 µm (homogeneous or heterogeneous).[74] Unfortunately, this particle size increases the surface area of the fillers. Therefore, only a relatively small amount of filler can be suspended in the monomer, limiting the amount of filler (25%-50% by volume).[74,75]

The majority of materials produced posteriorly have become "hybrids", incorporating both so-called nano- and micronsized particles (particles with 0.6 - 5 µm are coupled with particles with 0.04 µm) to enhance handling (flow) properties and adhesion control.[75] In particular, in hybrid composite minifill, the size ranges between 0.6 to 0.7 µm with micro-filled and volumetric particle percentage is 70% (50%-70% by volume).[75] Nowadays, mini-filled hybrids have become the most popular to attend global applicability, with various shades, translucencies and opacities, superior strength but lower polish ability compared to micro-filled.[71]

In addition to filler characteristics, other properties inherent to the materials must be considered to guide composite selection: filler volume content, thixotropy, and the mat of the surface to handling.[75]

3.3. Toxicity of Bisphenol A and derivatives

A new field of interest in dentistry has emerged, namely endocrine active substances (EAS). BPA was categorized by the European Chemicals Agency (ECHA) as a 'substance of very high concern', because it endocrine disruptor effects with risks for human health (toxic for human reproduction) and for wildlife (ecotoxicity), as determined in Regulation (EC) No 1907/2006.[76] Researchers found that BPA leached into the saliva and found in urine of treated patients may be associated to the estrogenicity of some commercial composites and sealants used in dentistry.[77–80] Furthermore, growing evidence indicates that exposure

to BPA is also associated with an increased risk of developing type 2 diabetes,[81,82] obesity,[83] adverse immune effects,[84] and altered neuroendocrine development.[85,86] As resin-matrix materials are expected to have a shelf life of several years in the mouth, *in vitro* extended storage studies are needed to investigate the long-term release of various compound ingredients.[87,88] For example, it has already been shown *in vitro* that monomers (BPA-based) can elute from resin-matrix composites for up to one year after a weekly extraction solution update protocol (*i.e.*, water, ethanol, and artificial saliva).[89]

Dental resins are composed mainly of BPA derivatives [90] and the most common used BPA derivative in resin-matrix composite is the Bis-GMA. Bis-GMA is a monomer with methyl methacrylate groups attached to hydroxyl groups of BPA. Another BPA derivative used in dentistry is bisphenol A dimethacrylate (Bis-DMA). Bis-GMA hydrolyzes into BPA by salivary esterase due to structural differences between these monomers.[90,91] while Bis-DMA is rarely used in dental materials.[92] BPA itself has no functional role in dental composites, but it exists as an impurity due to an incomplete manufacturing process or as a degradation product.[90]

For this reason, there is an urgent need to develop dental materials free of BPA and its derivatives.[93] In order to prepare Bis-GMA-free dental materials, urethane dimethacrylate (UDMA), which is another typical dimethacrylate monomer applied in dentistry, has been considered the base resin of dental materials.[94] Unfortunately, compared with Bis-GMA-based resin, UDMA-based resin had a critical shortcoming in its higher volumetric shrinkage.[95] The higher volumetric shrinkage of UDMA-based resin might lead to more significant marginal gap between tooth and restorations, resulting in a higher possibility of secondary caries,[96] staining[97], and partial loss or fracture of the restoration.[98] Therefore, alternative monomer compositions of Bis-GMA were introduced to address the limitations of this product in terms of durability and toxicity. One alternative is a hybrid organoceramic to the methacrylate-based resin-matrix composites, known as ORganically MOdified CERamic (ORMOCER®, VOCO, Germany), which is an Organically Modified SILicate (ORMOSIL). The oxygen is replaced by organic groups, resulting in a 3D polymerized material with less organic matrix than the conventional resin-matrix composites ORMOCER®. It provides high biocompatibility due to the absence of residual monomers, lesser

polymerization shrinkage, high wear resistance, increased opacity, and improved handling characteristics.[99–102]

3.4. Requirements and characteristics of resin-matrix composites

As a measure of the outcome of successful restorative dental treatments, a longevity or survival rate depends significantly on the actual properties of dental composites in physical, mechanical, biological, and practical aspects. However, patient and dentist-related factors such as the patient's occlusion and hygiene habits, the characteristics of the restoration, and the clinical skills and treatment decisions of the dentist are also essential.[103,104]

The chemical composition and content of the organic matrix played a key role in the color stability of the resin-matrix composites. Clinicians should advise their patients about the chemical interaction between dietary substances and different resin-matrix composites.[105]

The elastic modulus and strength of dental resin are essential mechanical properties needed for the restoration's long-term survival. The elastic modulus represents an intrinsic mechanical characteristic of the material and mathematically quantifies a resistance against applied loads (compression or tension) in the elastic deformation region. Therefore, dental composites can be expected to have enough modulus values to resist masticatory forces. It is especially desired that they have higher values in posterior restorations because the occlusal forces on the tooth are higher in the posterior than the anterior ones.[106] Due to the intrinsic characteristics of polymeric resins in dental resin composite, typical modulus values are low compared to enamel. When compared to the modulus of enamel and dentin, which are about 40-90 GPa and 11-20 GPa [107–109], respectively, depending on age, gender, and other factors, dental resin composite have much lower values (*e.g.,* packable: 4-20 GPa, flowable: 2-9 GPa).[110–115] The strength of dental resins with increased filler content up to some limit is stiffer and stronger but with brittle characteristics. Above a critical amount of filler, it will lead to strength degradation with a formation of the weakness of interfacial bonding between the resin and fillers or aggregation/agglomeration of fillers that act as new defects in the composite system.[116] Dental composite systems' elastic modulus and strength can be controlled through resin type, filler shape and size, resin-to-filler ratio, filler

dispersion and orientation, and interfacial adhesion between filler and resin.

Therefore, when considering the development of new dental materials, their combined properties should be enhanced to avoid physical failure and recurrent caries in an aggressive oral environment. Evaluations of materials should be performed in the multiple aspects of inspection and characterization and can provide information to the dentist on which restorative material is most appropriate for a particular situation.[60]

4. Dissertation topic choice

Hypodontia is a significant clinical and public health problem. Patients with permanent tooth loss may suffer reduced chewing ability, inarticulate pronunciation, and unfavourable esthetic appearance. Clinically, early diagnosis of a dental anomaly can alert the clinician to the possible development of other associated dental anomalies in the same patient or family and avoid the possible sequelae. Understanding the patterns of tooth agenesis and their impact on diagnosis prevention and, eventually, therapy has become an integral part of comprehensive dental care. The ideal moment for definitive orthodontic treatment of hypodontia patients is often early adolescence when most of these developed permanent teeth are erupting.[1] Of all the missing teeth, the early missing upper teeth, namely the lateral incisors, most often motivate the request for treatment, probably for esthetic reasons.

Considering young patients with MLIA restoring an unbalanced dentition is a challenging process requiring a multidisciplinary approach that should focus on minimally invasive options to satisfy the expected functional and esthetic objectives. After orthodontic treatment with space closure, the most conservative and esthetic approach is direct restorations with dental resin composite.

Most commercially available dental composites contain BPA and derivatives, a potential endocrine disruptor. Although several studies have shown that low concentrations are released, there was a need to develop products free of such monomers from their

composition. Thus, it is essential to study whether the newly developed composite resins free of BPA and derivatives have the same chemical-mechanical behaviour as conventional resins.

5. Objectives and work plan

The main objective of this research is to study the clinical performance and the *in vitro* chemical and mechanical properties of contemporary dental resin with and without BPA.

We selected six resins for the *in vitro* study divided them into two groups (Table 1)

Table 1. Materials used in this study, their composition, and manufacturers' data.

Group	Resin	Manufacturer	Composition	Filler by weight (%)	Filler dimension (μm)
G1 (Without BPA)	**AF** (Admira® Fusion)	VOCO, Cuxhaven, Germany	ORMOCER® resin, SiO_2 Ba-Al-B-Si-glass fillers	84	2.5 to 3.0
	BF (Enamel Plus HRI BIO Function)	Micerium S.p.A, Avegno, Italy	UDMA, TCDDMA, no co-monomers, and no Bis-GMA glass filler, high dispersion silicon dioxide, fluorine	74	0.2 to 3.0
	NC (Experimental resin)	Coltène-Whaledent, Altäsatten, Switzerland	n.a.	n.a.	n.a.
G2 (With BPA)	**BE** (BRILLIANT EverGlow™)	Coltène-Whaledent, Altäsatten, Switzerland	Bis-GMA*, TEGDMA, Bis-EMA*, ZnO, Amorphous silica fillers	79	0.4 to 0.7
	ED (IPS Empress Direct)	Ivoclar Vivadent, Schaan, Liechtenstein	Bis-GMA*, UDMA, TCDD, Ba-Al-Si-glass, YbF3, SiO_2/ZrO_2, MO, Nanomodifier	78	0.1 to 0.3
	FS (Filtek™ Supreme XTE)	3M ESPE, MN, USA	Bis-GMA*, UDMA, TEGDMA, Bis-EMA*, ZrO_2/SiO_2 cluster SiO_2 nano-scale fillers	72.5	0.6 to 20

Asterisk (*) indicates BPA-based monomers.

Abbreviations: n.a.: not available; Bis-GMA: bisphenol A diglycidyl methacrylate; Bis-EMA: ethoxylated bisphenol A dimethacrylate; TEGDMA: triethylene glycol dimethacrylate; UDMA: urethane dimethacrylate; TCDDMA: tricyclodecane dimethanol dimethacrylate; TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin.

In group 1 (G1), we have three nanohybrid resins characterized by the manufacturer as BPA-free (Admira® Fusion (AF), Enamel Plus HRI BIO Function (BF), and experimental resin (NC)). All are being marketed in Portugal, except the NC resin, as it is an experimental nanohybrid resin kindly provided by Còltene-Whaledent.

In group 2 (G2), three different resins with BPA were in their composition. Brilliant EverGlow™ (BE) is a universal submicron hybrid resin. Empress Direct (ED) is a nanohybrid resin. Finally, Filtek Supreme XT (FS) is a nanofilled resin, being one of the most studied on the parameters of the objectives of this thesis.

To represent the real situation as much as possible, we selected enamel resins for the natural enamel layering technique (BF, ED and FS), or simplified monochromatic systems (AF and BE), because they are the most exposed to the oral environment.

Regarding the *in vivo* study, the resins selected for the rehabilitation of the patients after orthodontic treatment were AF (without BPA) and BE (with BPA). The reason for selecting the AF resin was because when we started the project, it was the only BPA-free resin being marketed in Portugal. On the other hand, BE resin was selected for the restorative technique to be similar and maintain the same protocol between the two patient groups because it has the same restorative system (monochromatic) as AF resin.

The more specific objectives are listed below:

- To devise a minimally invasive rehabilitation of patients affected by MLIA who were treated with orthodontic treatment with space closure, with a monochromatic restoration with recent resin-matrix based composite with BPA (BE) and without BPA (AF). (Chapter II)

- To perform an integrative review on the release of BPA from resin-matrix composites and the resultant adverse biological effects. (Chapter III)

- To review the most appropriate method to analyze the amount of BPA and its derivatives detected in dental materials and biological fluids. (Chapter III)

- To evaluate the elution of BPA and Bis-GMA monomers from six composite resin-matrix composites (three BPA and three without BPA), using a sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS). (Chapter III)

- To evaluate the chemical (water sorption, solubility, and coefficient of diffusion) and mechanical properties (compressive strength, strain, and Young's modulus, and biaxial flexural strength) of six different dental resin-matrix composites (three BPA and three without BPA-Table 1) under the same curing and testing conditions. (Chapter IV)

The Figure 1 represents the workflow of the thesis and how the clinical and experimental sections intertwine to reach the final goal. In addition, the tasks previously stated will be detailed in each chapter in terms of clinical or experimental considerations (type of tests and equipment required).

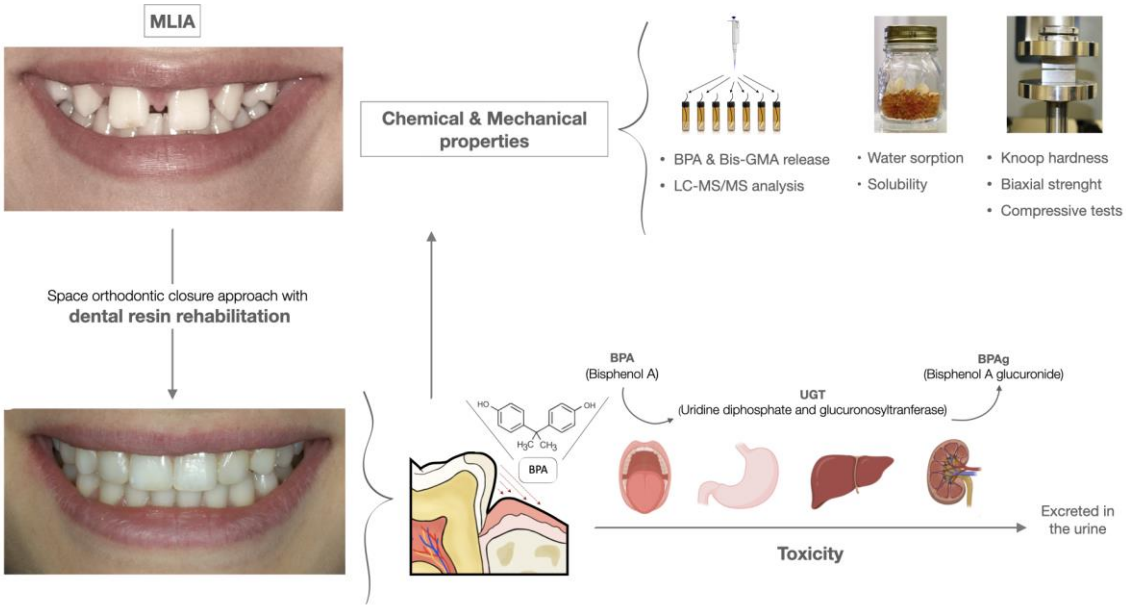

Figure 1. Graphical abstract.

Chapter II

CLINICAL REPORTS OF MLIA

1. Maxillary lateral incisor agenesis and microdontia: Minimally invasive symmetric and asymmetric esthetic rehabilitation.

Lopes-Rocha L, Garcez J, Tiritan ME, da Silva LFM, Pinho T. Maxillary lateral incisor agenesis and microdontia: Minimally invasive symmetric and asymmetric esthetic rehabilitation. *Rev Port Estomatol Med Dentária e Cir Maxilofac.* 2022;62(4). doi: 10.24873/j.rpemd.2022.01.857

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Case Report

Maxillary lateral incisor agenesis and microdontia: Minimally invasive symmetric and asymmetric esthetic rehabilitation

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ABSTRACT

Maxillary lateral incisor agenesis is a condition that significantly compromises smile esthetics, which is particularly worrying in young patients. This article shows how the combination of non-invasive treatments and current restorative options may improve clinical outcomes following orthodontic treatment for symmetric and asymmetric maxillary lateral incisor agenesis and microdontia. Teeth were treated with conservative resin restorations in three separate cases: two of congenitally missing maxillary lateral incisors (i.e., unilateral and bilateral) and one of microdontia. After presenting the clinical results, this article summarizes how bleaching, enameloplasty, and bonding with composite resin can enhance esthetics and functions following orthodontic space closure. The cases reveal that carefully planned, methodically executed orthodontic and restorative approaches can achieve predictable, esthetic outcomes that will improve the patient's self-esteem with a minimally invasive solution. (Rev Port Estomatol Med Dent Cir Maxilofac. 2022;63(1):xxx-xxx)

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Agenesia do incisivo lateral maxilar e microdontia: reabilitação estética minimamente invasiva de casos simétricos e assimétricos

Palavras-chave:
Restaurações conservadoras
Incisivo lateral
Fechamento de espaço ortodôntico
Reconstrução
Agenesia dentária

RESUMO

A agenesia dos incisivos laterais maxilares é uma condição que compromete significativamente a estética do sorriso. Neste artigo, mostramos como a combinação de tratamentos não invasivos e opções restauradoras podem melhorar os resultados clínicos após tratamento ortodôntico, em casos de agenesia simétrica e assimétrica dos incisivos laterais superiores e microdontia. A reabilitação dos três casos clínicos descritos foi realizada com restaurações em resina composta: os dois primeiros casos referem-se a agenesia de incisivos laterais (unilateral e bilateral) e o terceiro a microdontia. Após a apresentação dos resultados clínicos, este artigo resume como o branqueamento, coronoplastia e restaurações com resina podem melhorar a estética e a função após o tratamento ortodôntico. Os casos revelam que a integração de abordagens ortodônticas e restauradoras cuidadosamente planeadas e metodicamente executadas constituem uma solução minimamente invasiva permitindo alcançar resultados estéticos previsíveis. (Rev Port Estomatol Med Dent Cir Maxilofac. 2022;63(1):xxx-xxx)

Introduction

Restoring balance in a patient's dentition often requires a multidisciplinary approach. When the treatment options to replace missing lateral incisors (MLI) involve orthodontic space closure, a minimally invasive approach to canine and premolar remodeling is often preferred over invasive interventions as a way to achieve functional and esthetic objectives.[1-3] The most significant advantage of space closure over other approaches is that adolescents do not have to remain untreated until the facial skeleton matures.[4]

The replacement by an implant is also considered a possible solution. The main advantages are the possibility of obtaining an ideal occlusion, the maintenance of the canine in its natural position, and the clear benefit of avoiding any damage to the adjacent teeth.[5,6] The patient's age is an inexact predictor of dental maturity because young patients develop at different rhythms.[7] Nonetheless, the placement of implants in the anterior area presents some visible disadvantages, such as bone resorption, infra-occlusion of the implant, gingival retraction, recession of the interdental papillae, gingival changes (including blue staining of the gingiva), and exposure of the abutments.[5,8-11]

Unilateral agenesis is frequently associated with the dysmorphia or microdontia of the corresponding contralateral tooth.[12] In particular, the microdontia of maxillary lateral incisors may represent a singular expression of molecular changes that impair the development of the incisors. As such, the condition should be considered in clinical diagnoses and when reviewing the patients' family history as a way to identify agenesis.[13,14] In cases of microdontia, orthodontic planners may need to consider prescribing tooth positioning in all three dimensions if a future restorative treatment with minimal esthetic compromise is planned.[15]

The objective of this clinical report is to illustrate a minimally invasive approach to recovering esthetic disharmony due to bilateral maxillary lateral incisor agenesis (MLIA), unilateral MLIA, and microdontia using dental bleaching and a direct restorative procedure after orthodontic space closure.

Case reports

In three clinical cases, the procedures included clinical photography, radiography, and making impressions to produce study models. Orthodontic treatment involved space closure. Bolton's anterior analysis was performed regarding tooth movement after orthodontic treatment with the six anterior upper and lower teeth.

Before the final restorative phase, at-home dental bleaching was prescribed in a 2-hour daily regimen with 16% carbamide peroxide (VivaStyle 16%, Ivoclar Vivadent AG, Schaan, Liechtenstein). The effectiveness of dental bleaching (dental color change) was evaluated before and after the bleaching treatment in the upper and lower incisors, canines, and premolars using a visual method (Vitapan Classical Shade Guide; Vita Zahnfabrik, Bad Sackingen, Germany), with the results indicating a color improvement to A1.

Four weeks after bleaching, single-shaded restorations were placed on the anterior sextant. After composite shade selection with tooth hydration, rubber dam isolation was applied to ensure an optimally clean and dry operating area. A silicone putty guide was fabricated from a diagnostic wax-up to obtain a favorable esthetic outcome with the addition of composite. Because the restorations were in enamel, we opted firstly to etch the enamel surface with phosphoric acid (Vococid®, VOCO, Cuxhaven, Germany) before applying a universal adhesive bonding agent (Futurabond U®, VOCO, Cuxhaven,

Germany). To prevent the formation of an oxygen-inhibiting layer, glycerin gel was placed on the restoration and polymerized. Finishing and polishing were performed using fine and extra-fine diamond finishing burs, abrasive discs, finishing strips, and felt-coated discs in conjunction with polishing paste (Enamelize, Cosmedent, Chicago, USA) with a felt-coated dental polishing buff (FlexiBuff, Cosmedent, Chicago, USA).

Case 1
Symmetrical bilateral MLIA

An 11-year-old female patient (Figure 1) presented with bilateral MLIA and a 2-mm midline diastema. An intraoral examination revealed healthy dentition and no symptoms or signs of periodontal disease (Figure 2). A panoramic radiograph showed the absence of both maxillary lateral incisors, mesial eruption of the canines, and the presence of deciduous maxillary canines and deciduous right maxillary lateral incisors (Figure 3).

Orthodontic treatment involving space closure was performed. After golden proportion (Figure 4) and Bolton's anterior analysis, the presence of a 77.7% dental discrepancy in the anterior relation with mandibular excess was confirmed (14, 13, 11, 21, 23, 24, / 33, 32, 31, 41, 42, 43). Due to the agenesis of the maxillary lateral incisors and the need to perform esthetic restorations in the upper arch to improve smile harmony, a similar Bolton anterior discrepancy of 76% was obtained after restorations.

After orthodontic treatment, shade selection was accomplished using a Vita Shade Guide (Figure 5), and dental bleaching was performed. Afterward, according to clinical findings and with careful consideration of all treatment options available for rehabilitation, it was decided that the maxillary canines and first premolars would be additively remodeled to the shape of lateral incisors and canines, respectively, using direct composite (Figure 6), which provides advantages in symmetrical cases.

Because the patient had a diastema, the risk of treatment relapse was overcome with long-term fixed retention using a bonded retainer (Figure 7).[16] Meanwhile, the material used for the build-up was a hybrid composite (BRILLIANT EverGlow®,

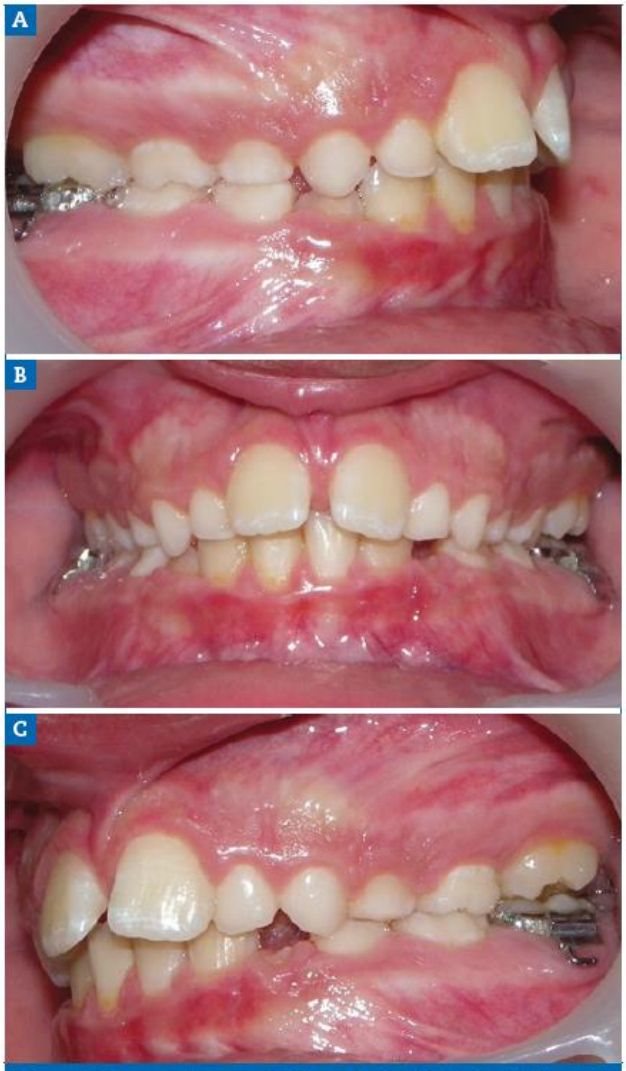

Figure 2. Case 1: Intraoral views of the (A) right, (B) frontal, and (C) left aspects before orthodontic treatment.

Figure 1. Case 1: Initial frontal view before orthodontic treatment, still with deciduous lateral incisors and canines (2014).

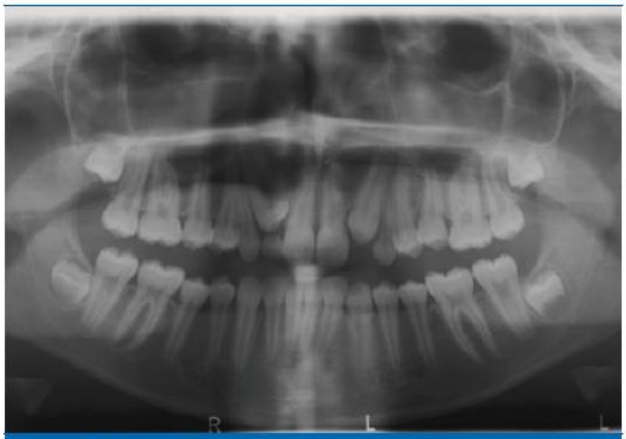

Figure 3. Case 1: Radiological investigation with a panoramic radiograph showing deciduous maxillary canines and right maxillary lateral incisor, as well as mesial eruption of permanent left and right maxillary canines.

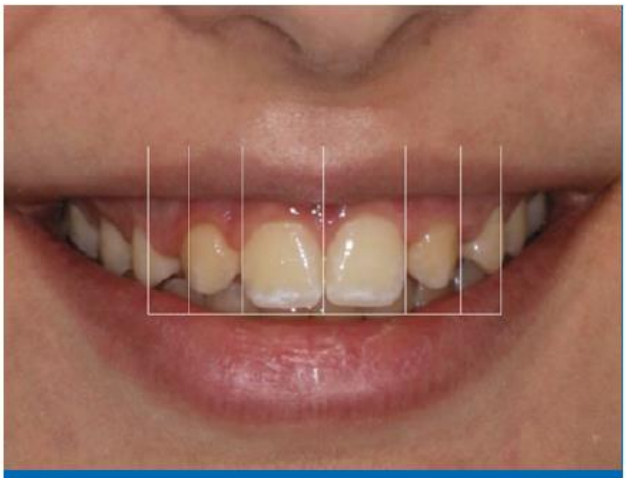

Figure 4. Case 1: Digital smile planning considering gold proportions.

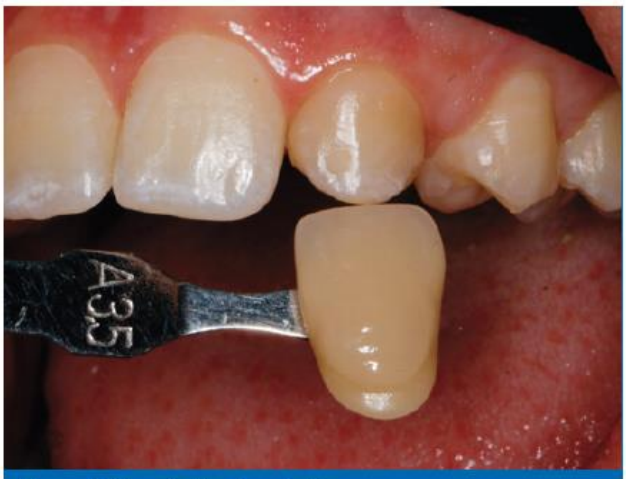

Figure 5. Case 1: Shade selection.

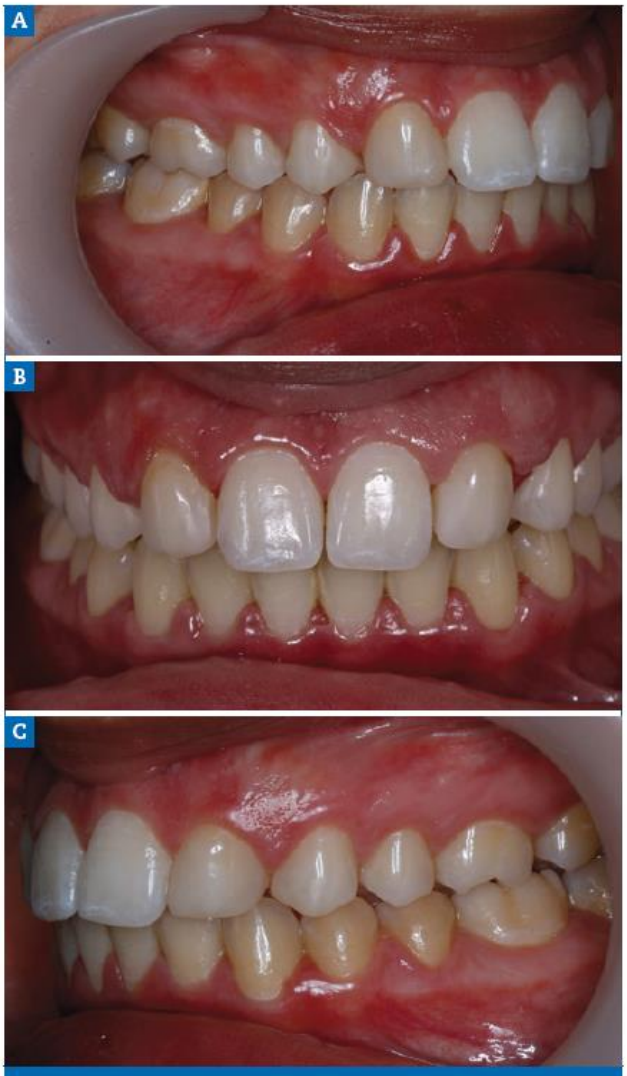

Figure 6. Case 1: Maxillary canines and first premolars additively remodeled to the shape of lateral incisors and canines, respectively, using direct composite ((A) right, (B) frontal, and (C) left aspects).

Coltène-Whaledent, Altstätten, Switzerland). Lastly, occlusal adjustments were performed, and final extra-oral photos (Figure 8) revealed a satisfactory integration of the restorations.

Case 2
Asymmetrical unilateral MLIA

A 15-year-old patient presented with unilateral MLIA and microdontia (Figure 9). The left maxillary canine erupted mesially from its normal position, while the right maxillary lateral incisor was conical. Both central incisors were tipped to the left and associated with occlusal plane canting to the left. There was also an evident Bolton's anterior discrepancy of 74.1 % (13, 12, 11, 21, 23, 24 / 43,42, 81, 71, 32, 33) due to maxillary asymmetric agenesis, a canine in place of the lateral incisor, and a conical contralateral (Figure 10). And due to agenesis of the mandibular central incisors and the presence of correspondent deciduous teeth. After the orthodontic treatment (Figure 11), the complexity of the asymmetric case required digital smile planning (Figure 12)

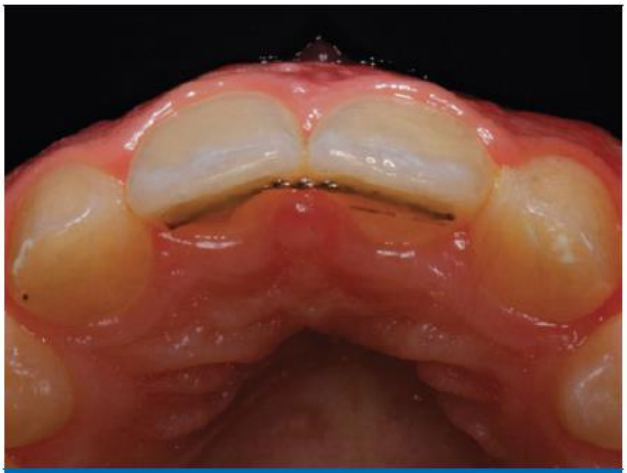

Figure 7. Case 1: Long-term fixed retention.

Figure 8. Case 1: Final frontal view after orthodontic treatment and composite build-ups (8-month recall, 2018).

Figure 9. Case 2: Initial frontal view before orthodontic treatment, showing unilateral MLIA and microdontia.

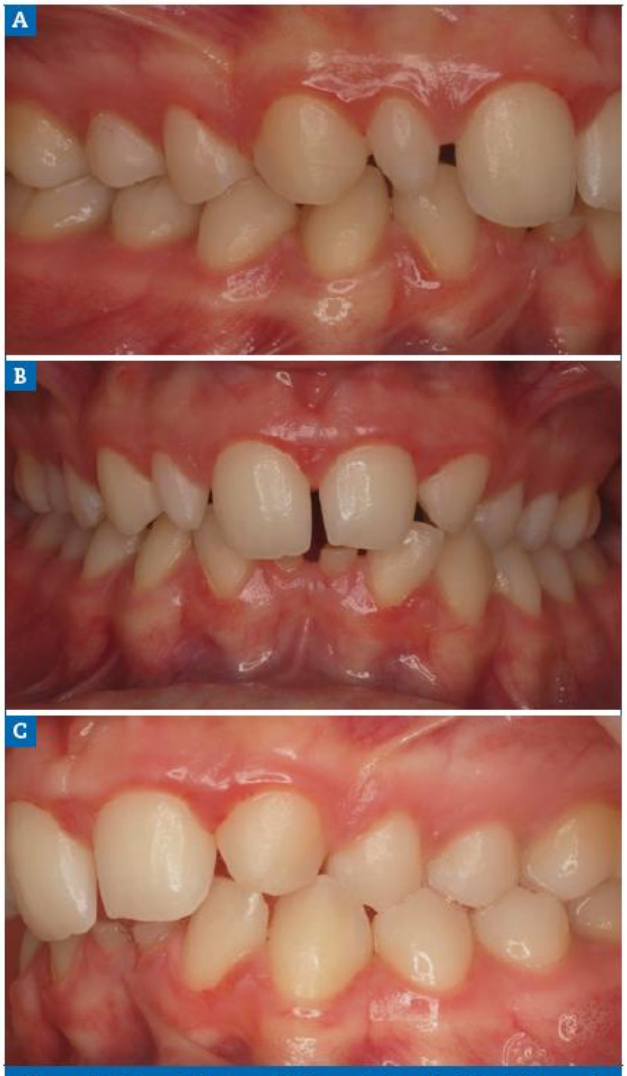

Figure 10. Case 2: Intraoral views of the (A) right, (B) frontal, and (C) left aspects before the orthodontic treatment.

and a diagnosis wax-up (Figure 13) to plan and visually demonstrate the desired result to the patient.

Left maxillary canine contouring procedures (Figures 8 and 9) were performed as indicated by the diagnostic wax-up.[19] First, the canine eminence was reduced on the labial surface, and the tip of the canine was flattened to produce an incisal edge. Second, the distal incisal angle was slightly rounded to reproduce a lateral incisor. Third, ORMOCER® (ORganically-MOdified-CERamic) composite (Admira Fusion, VOCO, Cuxhaven, Germany) without bisphenol A (BPA) was added to the adjacent teeth to improve the distal and mesial dimensions of the left maxillary central incisor and left maxillary first premolar, thereby diminishing the canine and harmonizing the smile line (Figure 14). In addition, because the mandibular primary left central incisor presented mobility due to root resorption, it was extracted, and the mandibular primary right central incisor was restored (Figure 15).

The occlusion was monitored at maximum intercuspation for protrusive and lateral guidance to assess any interference and obtain anterior guidance. Some adjustments were made in the left maxillary central incisor and left maxillary canine to promote optimal occlusion. With all the esthetic and functional limitations of this case with Bolton's anterior discrepancy, a ratio of 70.6% was obtained. A ratio lower than 77.2% indicates anterior maxillary excess. Thus, it was explained to the patient that rehabilitation of the lower arch would be necessary in the future to compensate for the space caused by the deciduous mandibular central incisors' loss and the volume of the mandibular central incisors that present agenesis to reduce the maxillary Bolton's anterior excess. At the 1-year follow-up, the restoration revealed excellent marginal integrity and high polish (Figures 16).

Case 3
Symmetrical microdontia

A 19-year-old female patient presented with primarily esthetic concerns. An intraoral examination revealed a Class I right

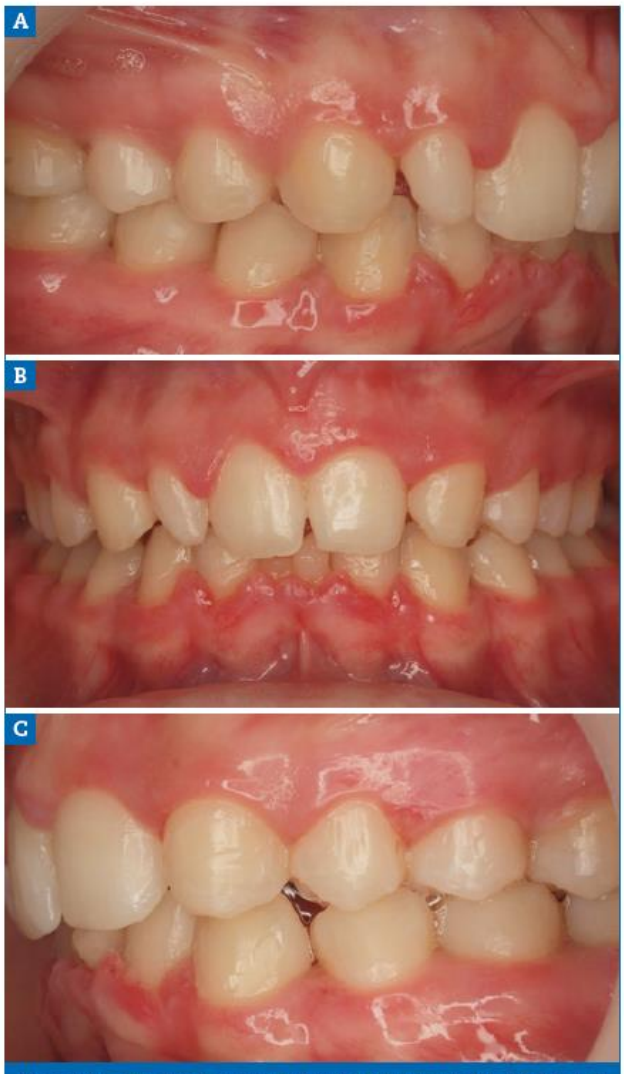

Figure 11. Case 2: Intraoral views of the (A) right, (B) frontal, and (C) left aspects after the orthodontic treatment.

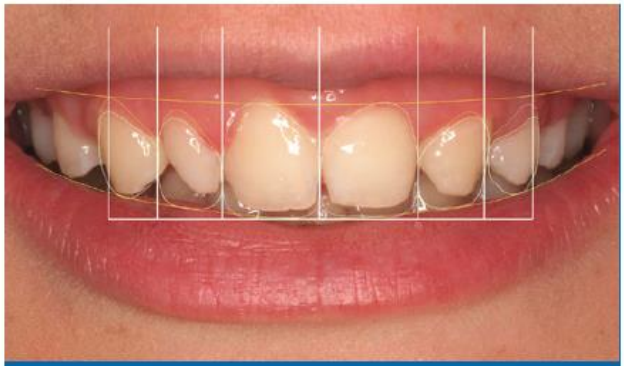

Figure 12. Case 2: Digital smile planning considering gold proportions.

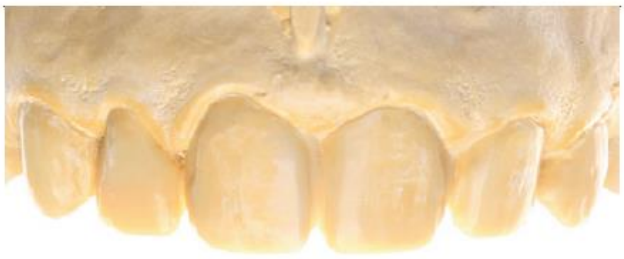

Figure 13. Case 2: Diagnostic wax-up of the superior front teeth.

Figure 14. Case 2 restorative protocol: (A) Preoperative view, (B) Palatal shell in composite based on silicone matrix obtained from the wax-up, (C) One-year recall.

molar, a Class II left molar, and a deep bite (Figure 17). Orthodontic treatment was performed to obtain bilateral Class I molar and canine relationship and, in turn, improve the overbite and remove the traumatic occlusion responsible for the gingival recession on the left mandibular incisor. Bolton's anterior analysis confirmed a dental discrepancy in the anterior sector to mandibular excess with a proportion of 83.1% (13, 12, 11, 21, 22, 23 / 43, 42, 41, 31, 32, 33). It was caused by the

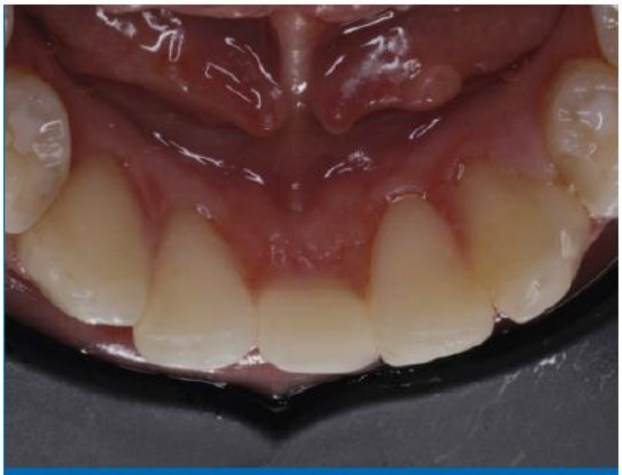

Figure 15. Case 2: Intraoral view after the extraction of the mandibular primary left central incisor and restoration of the mandibular primary right central incisor.

Figure 16. Case 2: Final frontal view after orthodontic treatment and composite build-ups (1-year recall, 2018).

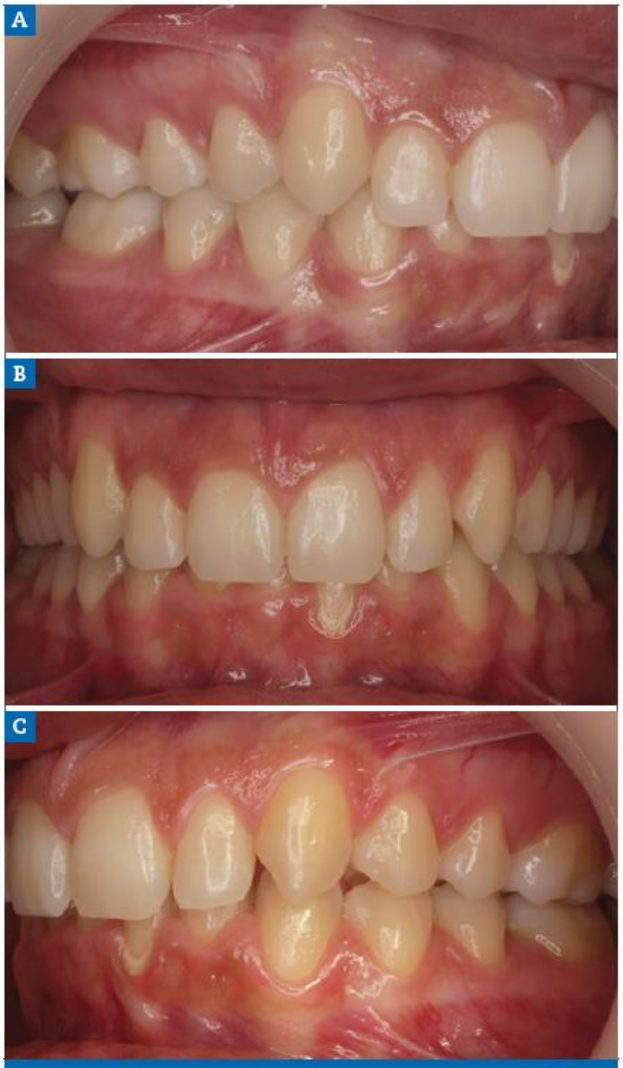

Figure 17. Case 3: Initial intraoral views of the (A) right, (B) frontal, and (C) left aspects before the orthodontic treatment, revealing a Class I right molar, a Class II left molar, and a deep bite.

maxillary dental microdontia and the need to carry out esthetic restorations in the upper arch. A space was created on the distal surface of both microdontic lateral incisors to establish the Bolton's proportion[17] (Figure 18). After orthodontic treatment, shade selection was accomplished using a Vita Shade Guide (Figure 19), and dental bleaching was performed to optimize the harmony of the smile.

Then, to improve the morphology of the microdontic lateral incisors, a nanohybrid ORMOCER-based composite was applied. The right and left maxillary canines were also restored on the mesial face to enhance their anatomical shape (Figure 20A).

This treatment plan made it possible to improve the Bolton discrepancy in the anterior mandibular excess relationship from a value of 83.1% to 79.6%. Diamond impregnated silicone polishers were used after occlusal adjustment (Figure 20B). Cross-polarized photography revealed the monoshade resin color integration with natural teeth (Figure 20C). Intraorally, an optimal overbite and an overjet relationship were established, as was a good buccal occlusion with a Class I molar and canine. The gingival recession on the left mandibular central incisor was also improved. At the 3-year follow-up, the rehabilitation showed good soft-tissue adaptation and excellent esthetic maintenance (Figures 21).

Discussion and conclusions

Beauty is usually associated with harmony, which is one of the chief goals of esthetic rehabilitation. With a multidisciplinary team, cases of symmetrical or asymmetrical MLIA or microdontia can be harmonized.[18]

In treating cases with high esthetic demands such as those presented here, besides the position of teeth, it is important to consider the gingival architecture. In cases of MLIA, the extrusion of the canine and intrusion of the first permanent pre-

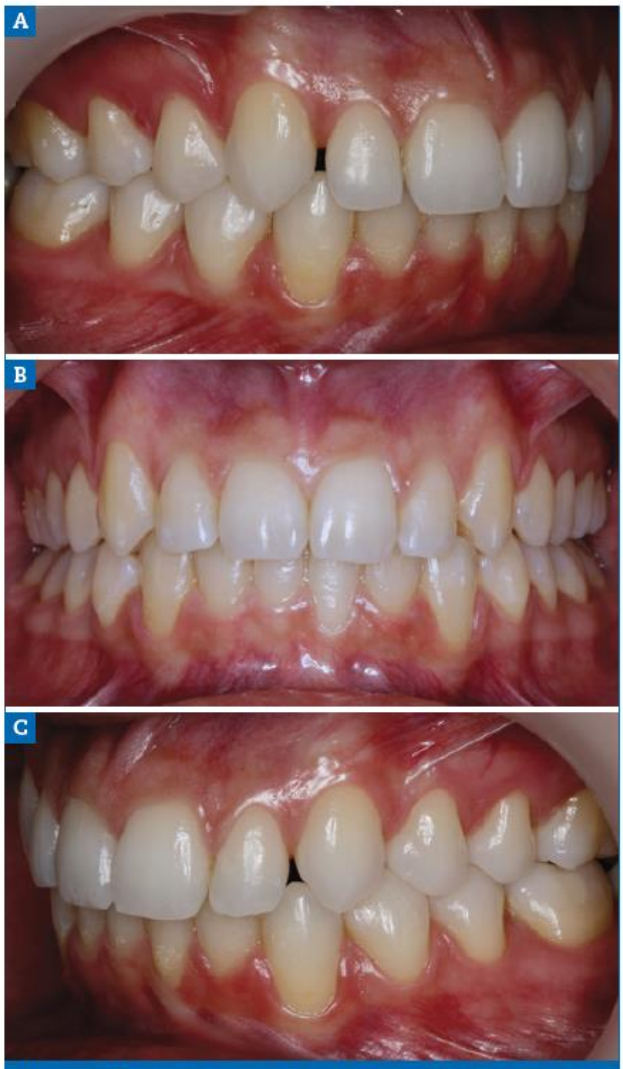

Figure 18. Case 3: Intraoral views of the (A) right, (B) frontal, and (C) left aspects after the orthodontic treatment.

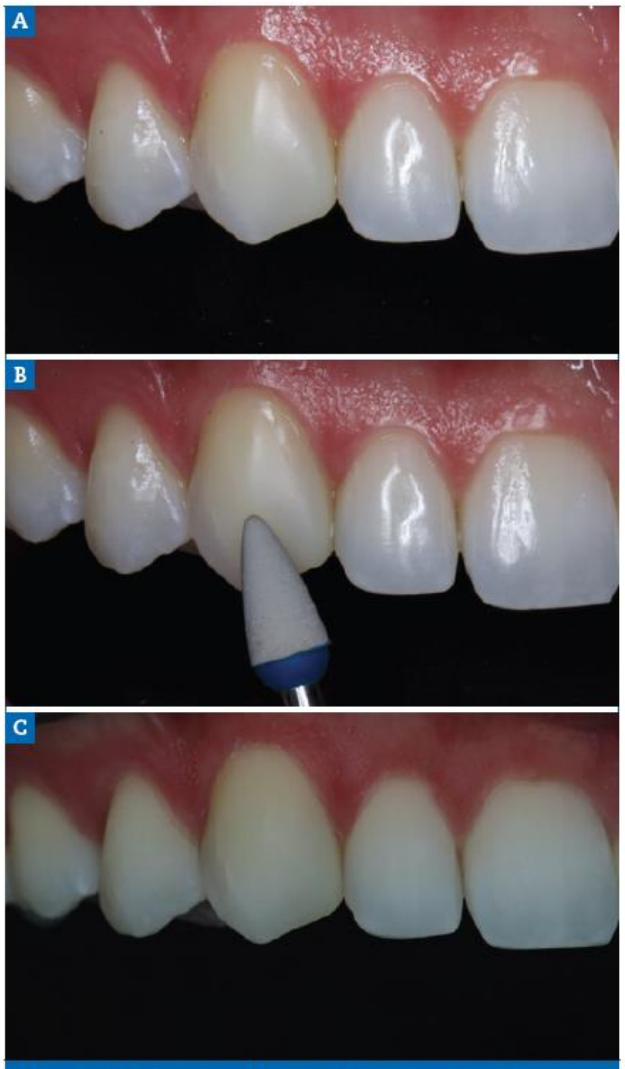

Figure 20. Case 3 lateral view: (A) Postoperative view, (B) Final polishing, (C) Photography with cross-polarization.

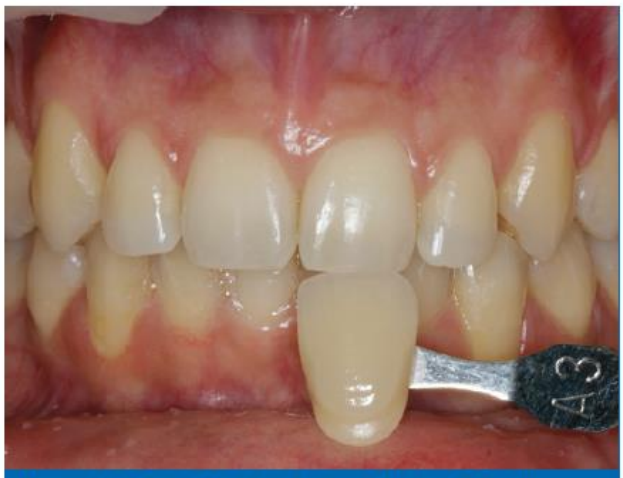

Figure 19. Case 3: Shade selection after orthodontic treatment.

molar typically mimic the gingival architecture associated with a natural smile.[19]

To optimize the harmony of smiles, it is important to evaluate the color before evaluating the morphology and proportion. Canines present a more saturated color than incisors,[20] which is more noticeable in more mesial positions such as in symmetrical bilateral MLIA (Case 1) and asymmetrical unilateral MLIA (Case 2). Following enameloplasty, color correction should be considered to brighten the teeth using bleaching techniques.[21-23] Moreover, the restorative procedure has to be completed at least 3 or 4 weeks after dental bleaching to prevent any negative effects of bleaching on bond strength and stabilize the color.[24]

Dental morphology and ideal tooth proportion were also important in the treatment plans for the three present cases. According to Levin, the golden proportion (i.e., a constant ratio of 1.618:1) can be a guideline for determining the MLI width and, thus, was used in these cases.[25] However, this guideline was more relevant in Case 2, an asymmetric case involving

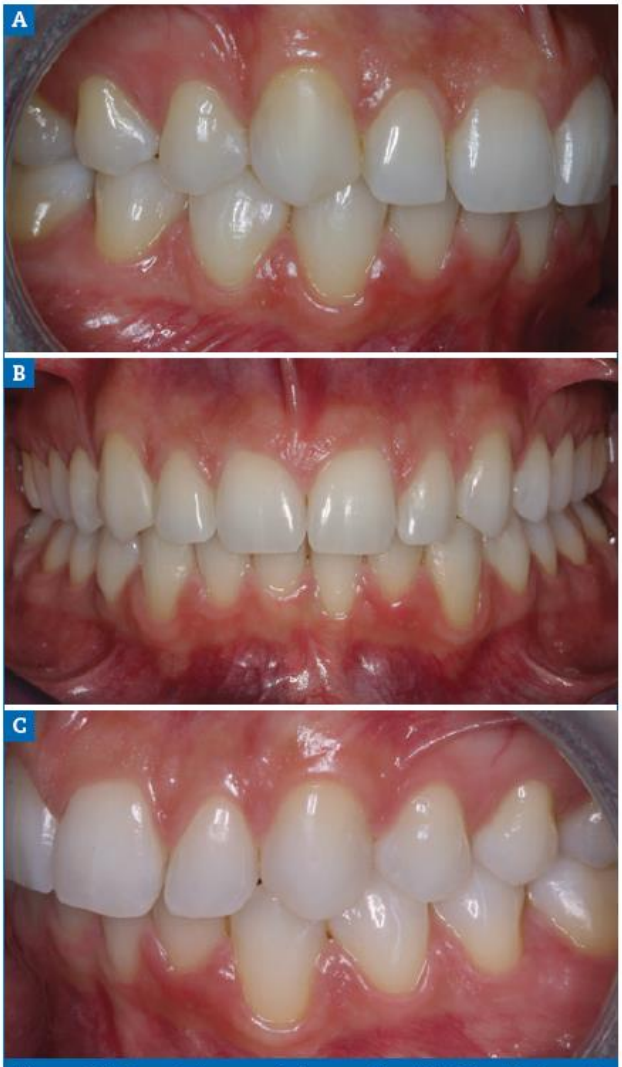

Figure 21. Case 3: Intraoral views of the (A) right, (B) frontal, and (C) left aspects after composite build-ups (three-years recall, 2020).

unilateral agenesis and microdontia on a contralateral tooth.[25,26] Even so, it was somewhat inconsistent in terms of relative tooth width because the golden proportion results from perceived tooth size observed from a frontal aspect and thus had no relation with the teeth' actual width. Another way to determine the amount of space to create for an MLI is the Bolton analysis,[27] which we also used in all three cases. It is widely accepted that a diagnostic wax-up and a smile simulation provide the most effective and esthetic outcomes for patients, as performed on the asymmetric unilateral MLIA in Case 2.

The approach to space closure is improved when the canines have similar dimensions and both lateral incisors are missing, as in Case 1.[18] In turn, in cases of unilateral MLI, like Case 2, the discrepancy in the canine's shape, size, and color compared with the lateral incisor can make it more challenging to achieve an esthetic and, as much as possible, symmetrical result.

In Case 2, the restoration of the upper left canine to mimic the lateral incisor should be more incisal because it looks shorter than the upper right lateral incisor. In addition, the premolars are not totally in a Class II relationship on the left side to compensate for the excess Bolton anterior tooth discrepancy in the maxilla. This situation happens not only because of the permanent mandibular central incisors' agenesis but also due to the presence of their respective deciduous teeth, which presented a reduction in mesiodistal size. To reduce the Bolton discrepancy, the premolars should be more mesialized and the left canine's width reduced. Also, the space in the lower arch (central incisors' area) should be increased to rehabilitate in the future. However, our goal was to leave space to rehabilitate one of the lower central incisors, considering the hypodivergent biotype, agenesis, and the consequent loss of bone structure. For economic reasons, we opted to keep the deciduous tooth. However, the ideal procedure would be a rehabilitation with implants if the bone volume is favorable, or a Maryland.

According to some authors, a canine should usually be 1 mm wider than the lateral incisor that it will replace, which requires mesial and distal reduction and recontouring by enameloplasty to obtain the desired width.[28] In Case 2, the canine eminence of the labial surface was reduced. On the other hand, in Case 1, with bilateral canine mesialization, only the additional approach with composite was needed to perform coronoplasty.

Zachrisson demonstrated that if all of those reductions are executed using diamond burs with water spray cooling, the long-term implications for tooth sensitivity will be minimal.[29] However, if dentine is exposed due to enameloplasty and/or recontouring, restoration may be necessary.[29,30] In Case 1, the direct addition of resin to square off the mesial and distal incisal corners was more relevant to the final symmetrical result. Furthermore, in Case 2, the left side of the first premolar was restored to appear like the maxillary canine.

In Case 3, the patient presented with microdontia, which may represent a different expression of MLIA with a symmetrical outcome. A fundamental part of the overall treatment plan was orthodontic treatment to correct the deep overbite and traumatic occlusion and the Bolton discrepancy due to microdontic mesiodistal maxillary lateral incisors. After the orthodontic treatment, the restorative phase was essential to correct that discrepancy and consequently achieve more esthetic goals.[31]

In the present cases, direct restoration with composite was chosen over indirect restoration primarily because the treatment used did not involve any injury to dental tissue and was more affordable.[32-34] The reversible nature of adding composite is a technique that allows for other treatment approaches in the future. An important benefit of that procedure over other restorative materials is that repair may be possible intraorally without risking the modification of esthetics or mechanical performance.[35]

It is clear from the available independent in vitro and clinical data that simplified adhesives are subpar compared to adhesives that include an extra hydrophobic resin, i.e., two-step self-etch and three-step etch & rinse adhesives.[36-38] However, when bonding to enamel, universal adhesives have good results and a much higher success rate as long as enamel is etched with phosphoric acid, which is the better way to improve the durability and stability of this adhesive system.[36-38]

There is no clinical evidence that nanofilled composite resin results in better overall clinical performance than hybrid

composite resin.[39-41] According to different studies,[42,43] nanofiller resin seems to be the most prone to staining, perhaps due to a sub-ideal dub integration of the nano-aggregated particles. In addition, hybrid composite resins result in a light propagation pattern similar to the natural tooth structure and better than nanofilled composite resin.[44] Thus, one of the advantages of current hybrid composite resins over nanofilled composite resin is their excellent optical properties.[45] Moreover, the nanohybrid ORMOCER-based composite is a BPA-free material. BPA monomer is an endocrine disruptor potentially linked to hormone-related problems like obesity and breast cancer, which should be considered when treating young patients.[46] The decision to apply a single shade in these cases was highly favorable given the good optical color integration of that type of composite resulting from a chameleon-like effect and good polish retention.[45] Photographs taken with a cross-polarizing filter allow clinicians to check the color integration of restorations because it eliminates specular reflections from the surface of teeth.[47-49]

Our results are similar to those reported by other authors who showed how anterior composite restorations demonstrated good clinical performance in the long term, with annual failure rates ranging from 0.0% to 4.1%.[30,50] However, patients should be informed that restorations require periodic maintenance because the texture and shade of the material will change over time.

Patients with MLIA may require complex multidisciplinary care from a young age. Although harmonious outcomes are most challenging to achieve in cases of unilateral agenesis with microdontia, a carefully planned, methodically executed orthodontic-restorative approach can produce satisfactory results with a natural, definitive appearance that improves the patient's self-esteem.

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Conflict of interest

The authors have no conflicts of interest to declare.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed their work center protocols on access to patient data and for its publication.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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Clinical Technique/Case Report

A Multidisciplinary Approach to Maxillary Lateral Incisor Agenesis (MLIA): A Case Report

L Lopes-Rocha • D Rocha • T Pinho

Clinical Relevance

To determine the ideal time for insertion of an implant, the state of skeletal growth and emotional state of a young patient must be taken into consideration. However, with the procedure we described, we can achieve a satisfactory conservative solution with a good esthetic outcome and long-term stability.

SUMMARY

Maxillary lateral incisor agenesis (MLIA) is a condition that significantly compromises smile esthetics and is a particular concern in younger patients. The treatment options include orthodontics for space opening with rehabilitation or space closure with canine camouflage. Currently, there is some controversy regarding the most appropriate treatment. In this case report, we propose a multidisciplinary approach through the combination of orthodontic treatment, frenectomy, and a restorative finishing stage with composite resin and dental implants. More specifically, this treatment was planned to orthodontically close the anterior space by opening the premolar area for subsequent placement of implants and enameloplasty with a composite resin.

The replacement of a missing lateral incisor by an implant is a predictable treatment approach, but it might best be deferred until dental maturity and then accurately placed in a well-developed site through a multidisciplinary approach. Precluding the closure of the anterior spaces and the opening of the posterior zone for implant placement, allows for a more stable and appealing esthetic and functional rehabilitation for young patients, in whom esthetic appearance and self-esteem play a primary role.

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INTRODUCTION

The agenesis of one or both maxillary lateral incisors is a frequent clinical condition, affecting approximately 2% of the population.[1,2] Patients with maxillary lateral incisor agenesis (MLIA) are commonly challenged with functional and esthetic problems at a young age, which may affect their confidence and social relationships.[3-5] Restoring an unbalanced dentition is a challenging process, demanding a multidisciplinary approach that should focus on minimally invasive options to satisfy the expected functional and esthetic objectives.[6-9]

Orthodontically, there are two primary treatment options to be considered: 1) space closure with canine camouflage; or 2) space opening with a tooth-supported, resin-bonded fixed dental prosthesis (RBFDP) or dental implant.[10] In cases where the occlusion and anatomy/dimension of the canine in the lateral incisor position are acceptable for camouflage, orthodontic space closure with canine mesialization provides a satisfactory long-term result both functionally and esthetically.[11] A replacement by implant is also a possible solution. The main advantages of this approach are the possibility of obtaining an ideal occlusion, the maintenance of the canine in its natural position, and the clear benefit of avoiding any damage to the adjacent teeth.[12,13] The patient's age is an inexact predictor of dental maturity because young patients develop at different rates.[14] Nonetheless, the placement of implants in the anterior area presents some visible disadvantages, such as bone resorption, infra-occlusion of the implant, gingival retraction, recession of the interdental papillae, gingival changes (including blue staining of the gingiva), and exposure of the abutments.[12,15-18]

In order to avoid such disadvantages, it is possible to open the spaces in posterior sextants, namely in the premolar area. The closure of the anterior space associated with the re-anatomization of the canine into lateral incisor and the first premolar into canine, with the subsequent placement of implants corresponding to a third premolar, is an achievable solution with outcomes that can be as good or superior to those obtained with implants in the anterior sextants.[19]

The objective of this clinical case report is to illustrate the rehabilitation of a patient affected by bilateral MLIA who received an orthodontic treatment comprising anterior space closure and space opening between the premolars. Dental bleaching and rehabilitation of the canines with a direct restorative procedure and dental implants in the premolar area were also performed.

CLINICAL CASE REPORT

A 14-year-old female patient presented with bilateral MLIA associated with bone loss between central incisors and canines. The patient had many concerns about the esthetics of her appearance and was psychologically affected by her smile disharmony (Figure 1). The patient's medical history did not reveal any systemic diseases, and an intraoral examination showed healthy dentition and no symptoms or signs of periodontal disease.

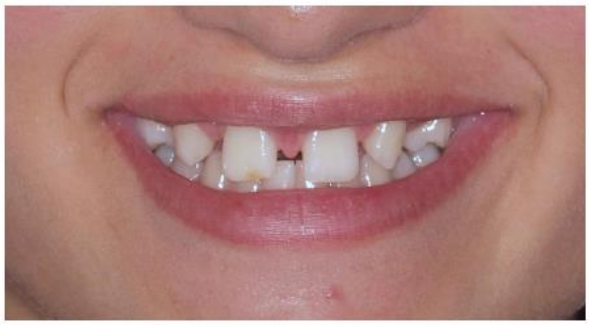

Figure 1. *Smile close-up view before orthodontic treatment.*

In terms of esthetics, the clinical examination of the patient revealed a low smile (considering the patient's young age), competent lips, and a straight profile. Regarding dentition, the examination showed right and left molar Class I and bilateral canine Class II, normal overjet and overbite, first upper premolars in scissor bite relation (Figure 2 A-D); a large maxillary interincisive

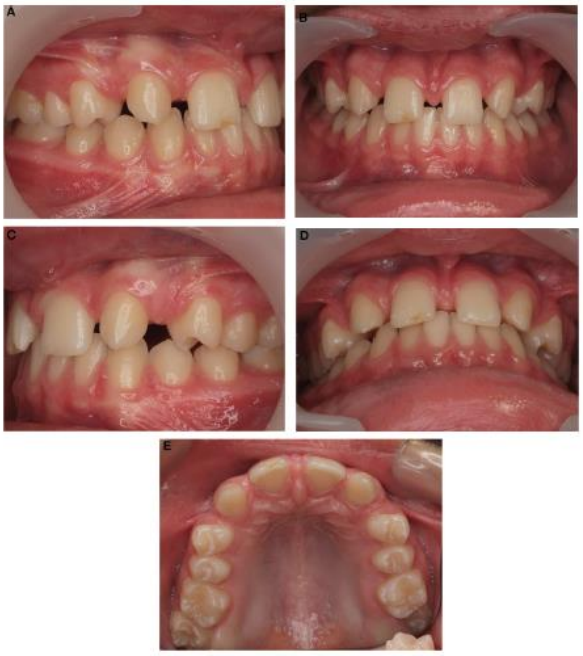

Figure 2. *Preoperative imaging before orthodontic treatment—maxillary lateral incisor agenesis (MLIA).*

true diastema associated with interradicular bone loss and significant atrophy, and a large frenum strongly inserted in the lip and palate (Figure 2E). In addition, the examination revealed a severe hypodivergent biotype, a maxillary dental midline shifted 2 mm to the right in relation to the facial midline, and a slightly negative lower dentomaxillary discrepancy (DDM). The panoramic radiograph showed a congenital absence of maxillary right and left lateral incisors (Figure 3).

First, orthodontic treatment was performed with self-ligated brackets to close the maxillary interincisive diastema and bilaterally close the lateral incisor space with mesialization of the canines. A frenectomy, including the lip and palatal side, was performed (Figure 4). At the end of orthodontic treatment, enameloplasty was performed with dental composite. This treatment allowed for space opening in a more posterior location (between the premolars) for subsequent rehabilitation with implants.

During the orthodontic correction, bite ramps on posterior teeth were necessary for relieving the occlusion, moving the teeth, and correcting dental intercuspation (Figure 5A). Great cooperation was needed from the patient, who was required to use intermaxillary elastics (Figure 5B) throughout the correction. Buttons on lingual surfaces of the first lower premolars with crossed elastics were necessary to correct the scissor bite relation. Coil springs were used between the premolars bilaterally, and they were activated during the correction to enhance mesialization (Figure 5).

During the steel arch wire process (0.19" x 0.25"), some steps were done in three dimensions, with extrusion of the maxillary canines and intrusion of the maxillary first permanent premolars contemplated to improve the gingival architecture and canines' facial-lingual root position (Figure 5B).

The treatment goals of the orthodontic correction were successfully achieved, with anterior space closure and opening of enough space for future placement of implants. At that time, the patient was 17 years old, still too young to place implants between the premolars.

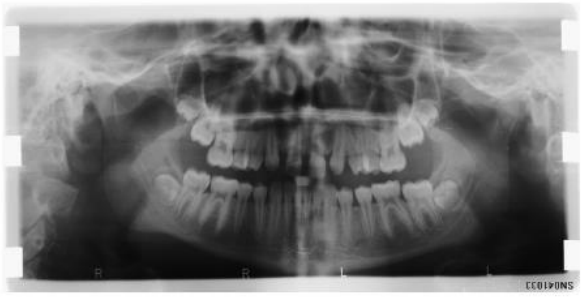

Figure 3. *Initial panoramic radiograph, MLIA.*

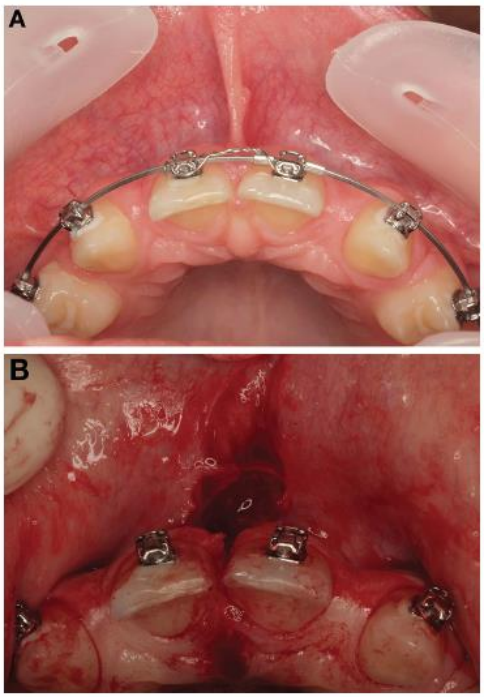

Figure 4. *Preoperative photograph showing high frenal attachment during orthodontic treatment (A) and frenum excised (B).*

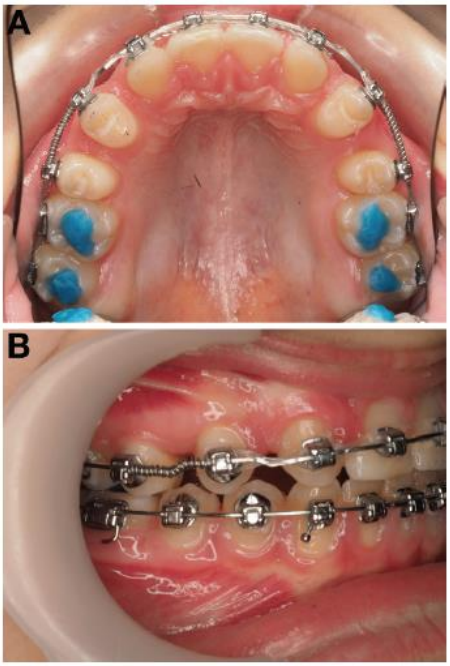

Figure 5. *Orthodontic treatment: (A) Self-ligated brackets with elastic chain and coil spring to close the anterior maxillary spaces; (B) One year later, with wire steps to compensate gingival margins.*

Despite this, the patient was very satisfied with the result and psychologically more confident, as the anterior spaces were closed even though the canines had not yet been remodeled.

To give the patient more occlusal stability and confidence, a removable acrylic maxillary prosthesis with two premolars was provided until the patient had completed skeletal and dental growth and it was possible to place the implants (Figure 6A). In addition, the patient used maxillary and mandibular removable retention, as well as fixed mandibular retention (Figure 6B). At this point, it could be observed that the patient had gingival inflammation (Figure 7), which was controlled mainly through improved oral hygiene habits, an important condition to establish before the dental restorations. However, associated with this inflammation, there was a hypertrophy of the gums that required a gingivectomy prior to the rehabilitation stage.

When the patient was 19 years old, the implants were placed (Figure 8). Considering the clinical observation of the mandibular excursive movements, enameloplasty was attempted on the palatal faces of the mesialized

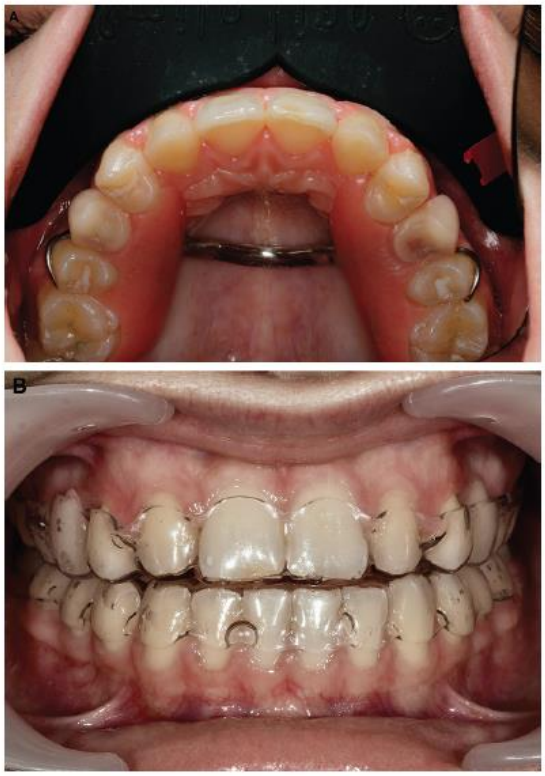

Figure 6. *(A) Placement of removable acrylic maxillary prosthesis for esthetic improvement as well as space retention; (B) Maxillary and mandibular removable retention was used to stabilize tooth positions.*

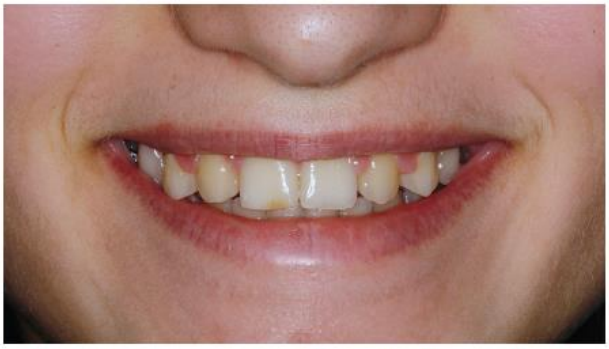

Figure 7. *Smile close-up view after orthodontic treatment with gingival inflammation.*

canines and the palatal cusp of the first premolars. Also, the right central incisor, maxillary canines, and first premolars would be additively remodeled to the shape of lateral incisors and canines, respectively, using direct composite resin. At-home dental bleaching was also included prior to the final restorative phase. This treatment consisted of a two-hour daily regimen of 16% carbamide peroxide (Vivastyle 16%, Ivoclar Vivadent AG, Schaan, Liechtenstein). Four weeks after bleaching, monochromatic restorations were placed on the anterior sextant.

After composite shade selection with the hydrated tooth, rubber dam isolation was used to provide an optimally clean and dry working field. In order to predictably obtain a favorable esthetic outcome with the addition of composite resin, a dimensional guide was fabricated by taking an impression of the palatal surfaces extending over the incisal edges of the maxillary anterior teeth, using an addition silicone putty (Aquasil Soft Putty, Dentsply DeTrey, Konstanz, Germany) from mock-up. The enamel surface was etched with 35% phosphoric acid (Vococid, Voco GmbH, Cuxhaven, Germany) for 30 seconds and rinsed for 20 seconds. Then, a universal adhesive bonding agent was applied (Futurabond U, Voco),

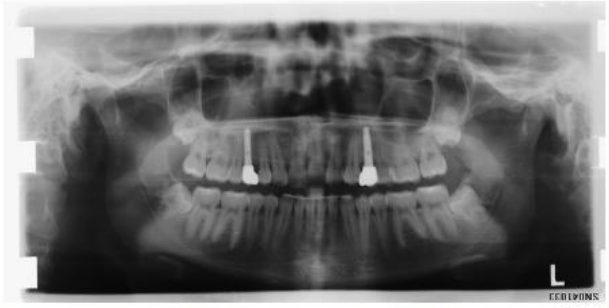

Figure 8. *Radiological investigation after orthodontic treatment and implant placement— panoramic radiograph.*

dried, and polymerized for 20 seconds. The material used for the build-up was a hybrid composite (A1/B1 maxillary central incisors and A2/B2 maxillary canines and first premolars; BRILLIANT EverGlow, Coltène-Whaledent, Altstätten, Switzerland). To prevent the formation of an oxygen-inhibition layer, a glycerin gel was placed on the restoration and polymerized. Any excess material at the margins was removed with a #12 surgical scalpel blade (SwannMorton, Sheffield, England). Finishing and polishing were carried out using fine and extra-fine diamond finishing burs, abrasive disks, and finishing strips (Swiss Flex, Coltene, Cuyahoga Falls, OH/USA). Abrasive silicone points were also utilized (Diamanto, Voco) to obtain better color stability over time and greater wear resistance. Finally, occlusal adjustment was performed.

In the eight-month follow-up, the rehabilitation showed good soft tissue adaptation and excellent esthetic maintenance (Figures 9 and 10).

Figure 9. *Intra-oral record after oral rehabilitation with implants and composite buildups (eight- month recall).*

DISCUSSION

The decision to open space in the posterior area was supported by factors such as the patient's young age, the hypodivergent biotype, the presence of interincisor diastemas with bone defects, the anteroinferior crowding, and the Class I molar relationship.

In cases like the present one, in which esthetics was a major concern, it was important to consider not only the position of the teeth but also the gingival architecture. Orthodontic therapy should involve movement of the teeth in three dimensions; this is more specifically the case in the situation of MLIA, where extrusion of the maxillary canine and intrusion of the first permanent premolar will mimic the gingival architecture of a natural smile.[20]In the present case, in spite of the spaces created by the agenesis, the midline diastema was highly associated with a hypertrophic maxillary interincisive frenum.[21] This was surgically removed after the closure of the diastema because it is believed that heavy orthodontic forces deprive the transseptal fibers of sufficient blood supply. In addition, the maintenance of the outcome probably was due to the newly developed tissue, contributing to the good results achieved.[22,23]

Furthermore, orthodontic mesialization allowed bone recovery at the area affected by the agenesis and, with diastema closure, at the interincisive area. This bone recovery minimized the problems associated with subsequent placement of implants at this area, which had had a bone defect. It has been reported that, when compared with natural contralateral teeth, implant-

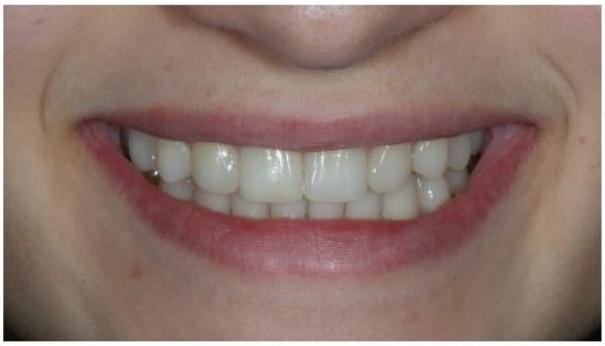

Figure 10. *Smile close-up view after oral rehabilitation with implants and composite buildups (eight- month recall).*

supported crowns replacing upper lateral incisors have shown increased gingival inflammation, increased probing depths, bleeding on probing, and accumulation of plaque.[12,13] Whenever possible and indicated, treatment using anterior space closure is preferable to anterior implants in regard to periodontal health.[12]

The mesial migration of the canine helps to develop the alveolar bone at the place of the congenitally absent lateral incisor, as the bone around the canine will form in the position of the lateral incisor.[24-26] This was taken into consideration during the planning of the orthodontic treatment for this case, and the movements were made in the direction of the bone defects in the interest of bone formation/apposition. Furthermore, it is important that the treatment end during adolescence, to positively impact the individual's self-esteem and social acceptance. The decision to close the anterior spaces was also reinforced by this factor and fortunately did not require a provisional prosthesis in the anterior region before the patient reached the required age to place implants.[27] The authors encourage the use of an maxillary acrylic prosthesis and removable retainers for use at night before placing implants because, as in this case, these measures help to retain tooth position (Figure 6) and provide functional information for the subsequent implant treatment plan.[28,29] Although it is a controversial issue, according to Dietschi and Schatz,[30] implant placement in children younger than 16 to 18 years should be avoided, to prevent infraocclusion due to adjacent alveolar bone growth. Bohner[29] stated that implants must be placed when growth is almost complete. For this reason, the implant surgery on the present case was postponed until the patient reached the age of 19.

In this case, the positioning of the premolars in the place previously occupied by the canines may result in heavy occlusal forces. Occlusion protected by the canine is not possible and this situation can lead to abfraction cervical lesions in the premolars,[12] which must also be re-anatomized for better esthetics and to provide a harmonious smile (Figure 9). However, this substitution is functionally acceptable, giving priority to the occlusion, and consequently distributing the occlusal load between as many posterior teeth as possible.[12] Thus, the opening of the posterior space for implant placement in that region provides an ideal axial load.[19] In this case, the decision to close the space in the anterior area and open the posterior area was taken to eliminate any possibility of bone defect inherent to MLIA and thus eliminate negative esthetic effects in the short and long term.

To optimize smile harmony, before assessing the morphology and proportion, it was important to evaluate the tooth color. Due to their size, canines present a more saturated color when compared with incisors.[31] Hence, following enameloplasty, color correction should be considered to make the teeth brighter, using one of the many available bleaching techniques for vital teeth.[32,33] The restorative procedure was completed four weeks after dental bleaching in order to avoid any possible negative effects of bleaching on bond strength and to allow color stabilization.[34]

The choice of restorative treatment should be based on certain factors that must be well defined, such as preservation of tooth vitality, minimal or no reduction of the dental structure, minimal or no invasion of the gingival area, the esthetic expectations of the patient, cost estimate, and duration of the treatment.[35] In this case, the treatment option chosen was supported by the fact that the canines had a shape and color favorable to space closure, ie, they were small canines with a smaller mesiodistal diameter, and by the fact that a slightly pronounced cusp fits better esthetically and functionally in the position of the lateral incisor. In this way, we were able to solve the problem of the lack of bone in the agenesis area and address the esthetic problem by narrowing spaces in the anterior area during the opening of the space.

The choice of direct restorations with a resin composite over an indirect restoration approach was made mainly due to the fact that the treatment was less expensive and did not involve any injury to the dental tissues.[35-37] Furthermore, the reversible nature of the resin composite technique allows for other treatment approaches in the future. An important benefit of this procedure over others is that the repair may be possible intraorally without the risk of modifying esthetics or mechanical performance.[38] The clinical outcome of anterior resin composite restorations is directly related to the use of a very precise technique, and clinical studies have shown good outcomes without major complications.[36] However, the patient should be mindful that restorations require periodic maintenance because the texture and shade of the material will change over time.[39]

This clinical case demonstrates that a multidisciplinary approach—the combination of initial orthodontic treatment with a restorative finishing stage with composite resin and dental implants, can provide satisfactory esthetic and functional long-term results in a young patient with missing bilateral maxillary lateral incisors.

CONCLUSIONS

In cases of agenesis of the upper lateral incisors, it becomes evident after analyzing the treatment

possibilities that multidisciplinary approaches must be prioritized, linking orthodontics with implantology, prosthodontics, direct restorative dentistry, periodontology, and occlusion. It is important to realize that each patient is unique and needs an appropriate, individualized treatment plan.

Although the usual treatment approach would be the opening of space in the agenesis area, the esthetic limitations in this case resulted in an alternative treatment—the closing of the anterior spaces and opening of the posterior spaces. This solution proved to be viable, showing good results and eliminating any disadvantage of placing implants in the anterior area.

Acknowledgments

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Conflict of Interest

The authors have no financial interest in any of the companies or products mentioned in this article.

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Chapter III

STUDY OF BISPHENOL A RELEASE

1. Toxicity of Bisphenol A (BPA) released from resin composites used in dentistry.

Lopes-Rocha L, Ribeiro-Gonçalves L, Henriques B, Özcan M, Tiritan ME, Souza JCM. An integrative review on the toxicity of Bisphenol A (BPA) released from resin composites used in dentistry. *J Biomed Mater Res B Appl Biomater.* 2021; 109(11):1942-1952. doi:10.1002/jbm.b.34843

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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.

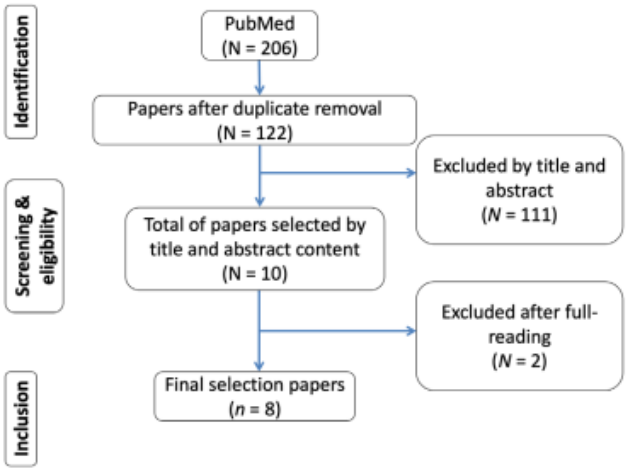

FIGURE 1 Flow diagram of the search strategy used in this study

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 the resin-matrix composite used	-*In vivo*, 22 human participants: 10 male patients (range, 13–25 years) and 12 female patients (range, 13–32 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 increase in BPA compared with	The amount of BPA leaching from Bis-GMA–based composite used for bonding orthodontic lingual retainers was detected at low

(Continues)

TABLE 1 (Continued)

Author (year)	Purpose	Study design	Chemical composition	BPA release (µg/ml)	Biological response
	in a lingual orthodontic retainer during the first month of placement.	- Liquid chromatography/mass spectrometry (LC/MS)	Hybrid resin composite containing Bis-GMA, UDMA and Bis-DEMA (Filtek Z250, 3M ESPE, USA).	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.	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

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

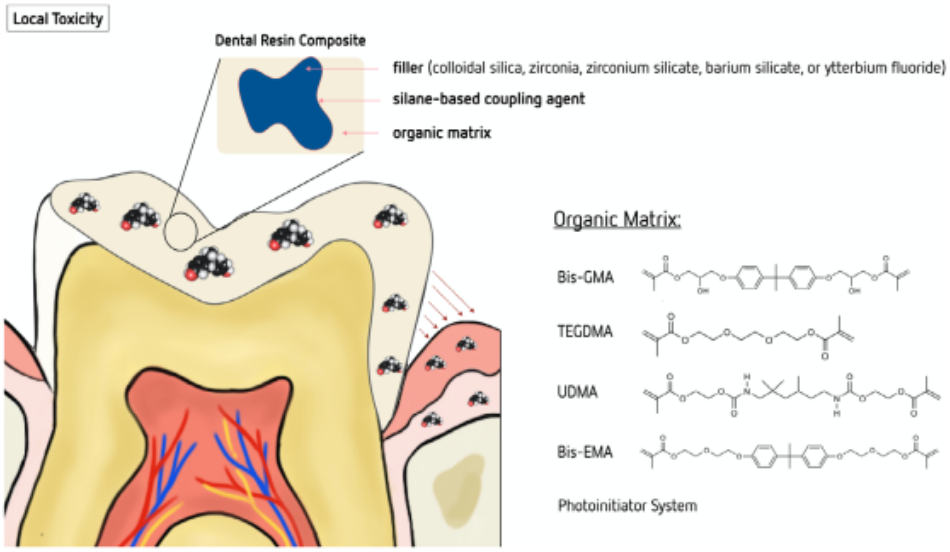

FIGURE 2 Schematics on the chemical composition of the resin-matrix composites used in dental restorations

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,

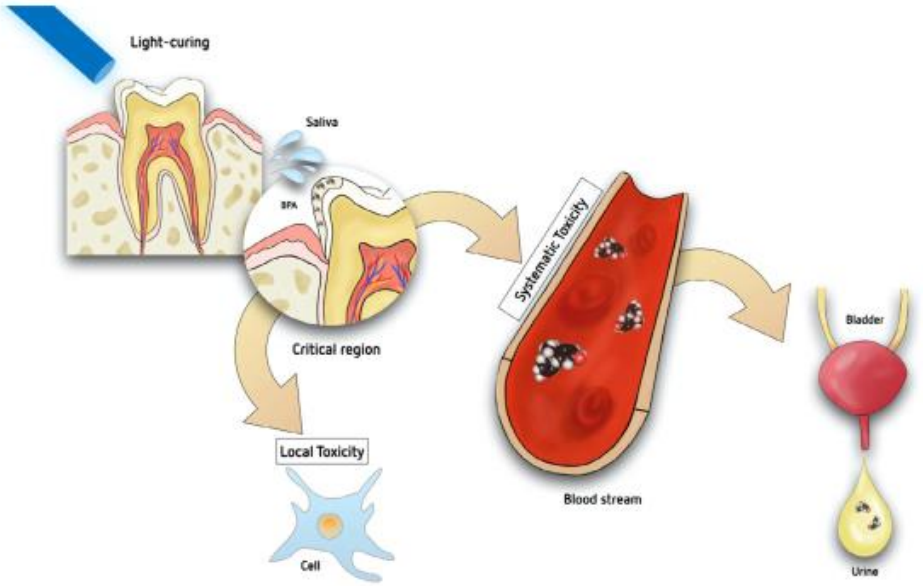

FIGURE 3 Schematics of the localized and systemic toxicity pathways

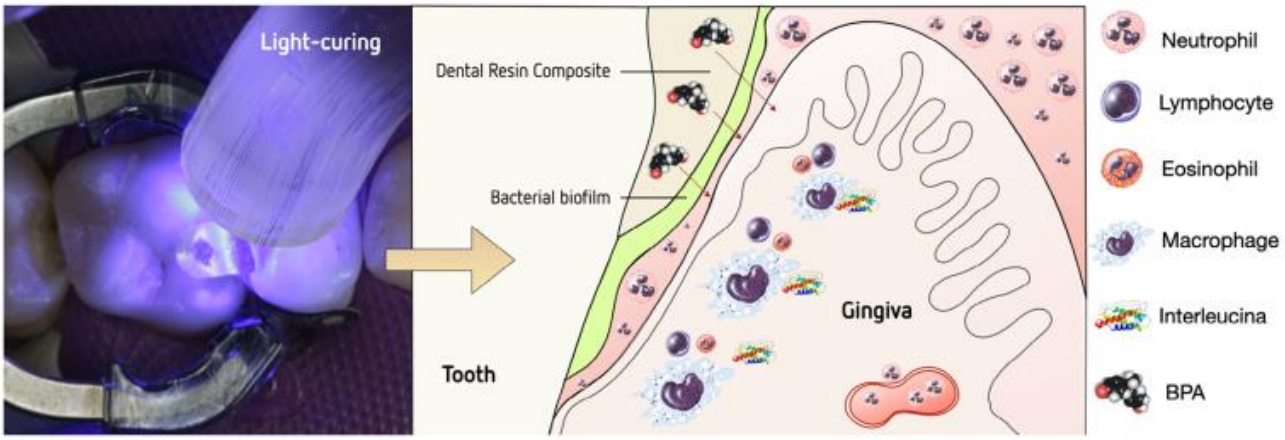

FIGURE 4 Schematics on the release of BPA and its derivatives from resin-matrix composites to the surrounding soft tissues and chemical interaction with cells

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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Analytical Methods for Determination of BPA Released from Dental Resin Composites and Related Materials: A Systematic Review

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ABSTRACT

Knowing the impacts of bisphenol A (BPA) on human health, this systematic review aimed to gather the analytical methods for the quantification of BPA release of BPA in dental materials in *in vitro* and *in vivo* (biological fluids) studies. A brief critical discussion of the impacts of BPA on human health and the possible association with BPA in dental materials was also presented. The research was carried out by three independent researchers, (according to PRISMA guidelines) in PUBMED and SCOPUS databases, by searching for specific keywords and articles published between January 2011 and February 2022. Seventeen articles met the eligibility criteria and were included in this systematic review: 10 *in vitro* and 7 *in vivo*. In *in vitro* studies, the highest amounts of BPA released were from flowable to conventional resins, followed by resin-modified glass ionomer. In contrast, the smallest amount was released from "BPA-free" composites and CAD-CAM blocks. Regarding *in vivo* studies, a higher concentration of BPA were found in saliva than urine or blood. The best analytical method for trace quantifying BPA is LC-MS/MS (Liquid Chromatography with Tandem Mass Spectrometry) due to its selectivity, low quantification limits, and the unequivocal identification. However, further studies are required to develop faster and more sensitive methods, in order to obtain more reliable results.

KEYWORDS

Bisphenol A; dental composite; LC-MS/MS; elution

Introduction

From the 1960s until today, resins have been used as filling and restorative material in dentistry, aiming to improve the mechanical and esthetic properties of dental restorations, having become less harmful biologically and environmentally.[1] Dental resin systems have gained a higher market share after the encouragement of the World Health Organization (WHO) for a global phase-down of dental amalgam. The resin has shown very satisfactory results and has made it possible to replace the amalgam for all cases of restorations on teeth that have suffered from trauma or caries.[2] Resin-based dental materials are composed by an organic and an inorganic phase. In order to obtain the best mechanical and esthetic properties of this material, many monomers of different sizes and fillers have been developed, such as bisphenol A-glycidyl methacrylate (Bis-GMA), Bisphenol A dimethacrylate (Bis-DMA), bisphenol A diglycidyl methacrylate ethoxylated (Bis-EMA), 2,2-bis-(4-(3-methacryloxypropoxy) phenyl) propane (Bis-PMA), bisphenol A diglycidylether (BADGE), triethylene glycol dimethacrylate (TEGDMA), urethane-dimethacrylate (UDMA) (Figure 1).

Bisphenol A (BPA) is an organic compound used in polycarbonates and epoxy resin industrial production.[3] In dentistry, monomers with a BPA-core are commonly used in resin-based materials such as composites, sealants, adhesives, and root canal sealers.[4,5] BPA can become part of these materials in two ways: a product of degradation of other ingredients, or trace material derived from the manufacture of other ingredients. As a natural ingredient, BPA is rarely used as a formula ingredient in composites and sealants.[6–8] The monomer Bis-GMA is the most commonly used BPA derivative in resin-based dental composites.[9] It is a monomer formed by the linkage of a methyl methacrylate groups to each hydroxyl groups of BPA. Bis-GMA has some advantages, including low polymerization shrinkage, suitable mechanical properties, relatively high refractive index, and excellent adhesion to enamel.[10,11] However, the major disadvantage of Bis-GMA is its high viscosity,[10] which prevents the addition of high fillers. Another BPA derivative commonly used in dental materials, predominantly in sealants, is Bis-DMA, which could be degraded in BPA in the

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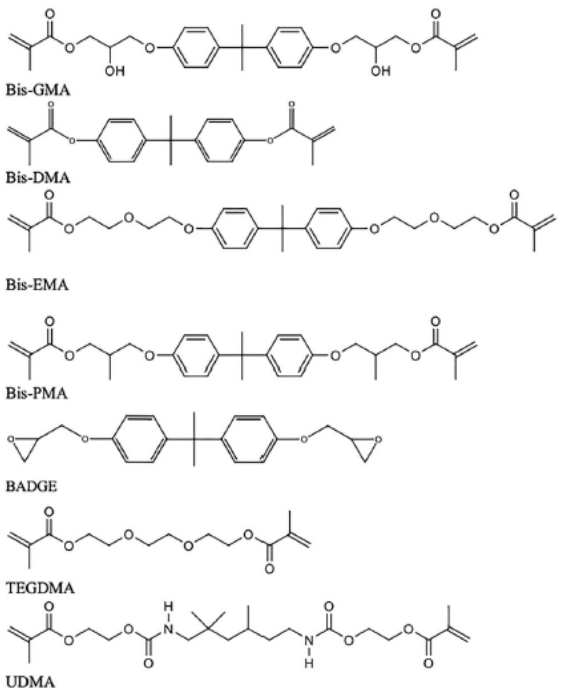

Figure 1. Chemical structures of monomers used in dental materials based on photopolymerization mechanism.

oral cavity by enzymatic hydrolysis through salivary esterase. Bis-EMA, BADGE, and Bis-PMA are also derivatives of BPA.[12] Bis-EMA has a similar molecular structure to Bis-GMA, with a higher molecular weight, but without the two hydroxyl groups. The main advantages of Bis-EMA are its low viscosity, and low polymerization shrinkage.[13,14] TEGDMA is usually added to Bis-GMA to reach feasible viscosity ranges, as the latter monomer has a very high viscosity due to intermolecular hydrogen bonding.[15] However, there is a linear chain between the two methacrylate groups, which has a lower viscosity than Bis-GMA. Because of the lower viscosity, it is used as a diluent for Bis-GMA, enhancing the manipulation of the composite resin and incorporating a higher filler load.[16] However, there are significant disadvantages of adding TEGDMA to the resin-matrix are high water sorption, reduced mechanical properties, and low color stability. Due to the significantly lower viscosity (100 times lower than Bis-GMA) and thus higher mobility of UDMA, Bis-GMA has been partially or wholly replaced by this monomer in many commercially available dental resin materials.[17]

There is a different susceptibility of BPA derivatives to hydrolysis by esterases in saliva. The chemical structure of Bis-GMA prevents hydrolysis, and BPA may be present as an impurity when manufacturing Bis-GMA if polymerization is not complete.[18,19] On the other hand, Bis-DMA can undergo hydrolysis and leaches out BPA as a product of its degradation.[20,21] As a result, more significant amounts of BPA are released from Bis-DMA degradation than from Bis-GMA degradation.[9] Factors such as the number and volume of sealants, physicochemical conditions, and material

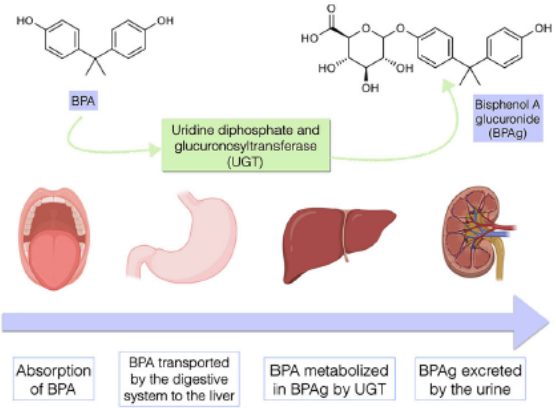

Figure 2. General metabolization scheme of BPA.

properties are essential to BPA release, but BPA exposure due to polymeric dental fillings is considered low versus overall BPA exposure.[22]

Although resins in dentistry are booming, the issue of BPA and its derivatives release into the oral cavity caught the attention of researchers, clinicians, and patients.[2] In the last decades, numerous *in vitro* studies on cell culture has proved that BPA is an endocrine disruptor.[23–25] Additionally, many other deleterious effects have been associated with BPA exposure such as contribution to neurobehavioral effect in children[26] and cancer.[27] Various human health problems have also been associated with urinary BPA levels.[5] The reactive oxygen species generated by bisphenols are involved in chronic diseases like cancer, cardiovascular diseases, diabetes or neurodegenerative diseases, and endocrine disruption impacting fertility because of the disrupt of various signaling pathways.[28] Additionally, the endocrine effects may be cytotoxic, genotoxic, mutagenic, and toxic for the reproductive system.[2,27] Moreover, researchers have developed and tested BPA-free materials to overcome all these potential health risks.[29–31]

In humans, BPA absorption occurs broadly (>99%) in the gastrointestinal tract after the oral exposure, where it suffers a significant first-pass metabolism (>99%), and excreted by urine (Figure 2).[5,32] The information about absorption, distribution, metabolism and, elimination of BPA in humans and non-human primates yields an adequate understanding of the relationship between levels of oral exposure, urinary excretion of BPA, and the resulting serum concentrations are described by Bagley et al.[23]

The type of monomers and the concentration of filler used in the resin-matrix affect the amount of monomers released.[33–35] And, consequently, the diffusion of these by the body after restorative treatment.[33,34] Recent research on this monomer indicates that the health effects of BPA and the associated risks are still debated and uncertain, however it is important to take into account that assessments carried out in several countries have concluded that exposure to BPA that comes from all sources was below the reference values designated as the current toxicity limit.[36–38] Despite this, it has been reported that the amounts of BPA

detected were increased in saliva and urine after dental restoration, sealant, or orthodontic placement.[23]

Therefore, this review gathered the information on BPA release from different dental materials reported in *in vitro* studies and in biological fluids, namely saliva, blood, and urine from different dental materials.

Quantification of BPA can be performed by Gas Chromatography coupled with Mass Spectrometry (GC-MS) or liquid chromatography (LC) with mass spectrometry (MS), ultraviolet (UV) or fluorescence (FDL) detection. However due to its low volatility, BPA needs previous derivatization for GC-MS analysis. LC is the most applied methodology to analyze BPA, FDL and MS detection are much more selective and sensitive than UV detection, but in complex matrices only MS allows unequivocal identification. It is necessary to note that the composites and sealants produced in EU (European Union), Japan, and the USA (United States of America) must meet strict standards determining the maximum amount of BPA released under oral cavity conditions. Furthermore, these standards are based on toxicological aspects and not on estrogenic aspects, which require lower doses to act. From our point of view, the problem has, therefore, to be focused on three questions, to which answers will be given at the end of the review of the literature:

- Is BPA released from resin-matrix composites, adhesives, and fissure sealants?
- If yes, how much of it is released?
- Which is the best analytical method to evaluate BPA release from dental materials?

Materials and methods

Search strategy

The literature search since 2011 was systematically and independently reviewed by three reviewers and restricted to the period between 1st January 20111 and 2nd February 2022. It was conducted in MEDLINE (PubMed (via National Library of Medicine)) and SCOPUS considering that such database includes significant articles in the field of dentistry and biomaterials. The keywords related to the search were "BPA" OR "Bisphenol A" AND "dental resin" OR "dental composite" AND "HPLC" OR "GC-MS" OR "LC-MS/MS"; "dental resin" OR "dental composite" AND "BPA" OR "bisphenol A" AND "chromatography"; and "BPA" AND "Bisphenol A" AND "release" AND "dental materials" AND "dental composite" AND "dental materials" AND "elution." Also, some studies were searched manually by the title in the lists of the relevant primary studies.

Data collection and extraction

The PRISMA guidelines were followed. The flowchart (Figure 3) was applied on the reference lists of all primary sources and eligible studies. Briefly, the titles and abstracts of all the findings were independently evaluated by three

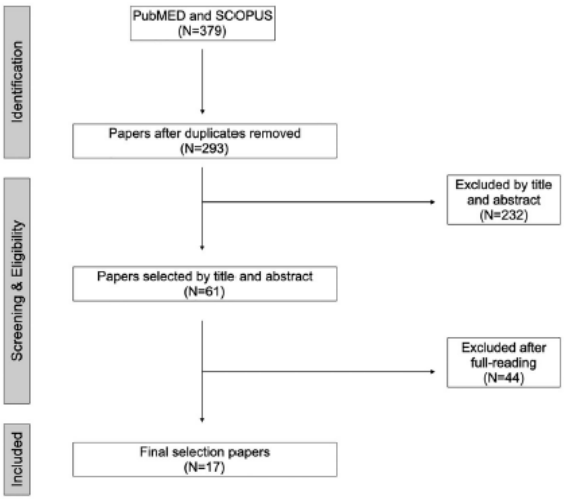

Figure 3. PRISMA flowchart of the article selection.

reviewers. Two reviewers identified relevant articles by screening the titles and abstracts. Full-text versions of the identified papers were retrieved and screened again by the two independent readers for the inclusion criteria. After that, three reviewers were independently reviewed all included articles for methodological features and results. Any discrepancies were resolved through discussion. The inclusion criteria involved articles regarding analytical methods for determining BPA released from resin-matrix composites, fissure sealants and adhesives used in dentistry. The eligibility inclusion criteria used for article searches also involved: *in vitro* studies, *in vivo* studies, case-control, cross-sectional, prospective clinical trials, randomized clinical trials, cohort, etc. It was exclusion criteria were case report with a short follow-up period; BPA release from temporary crown and bridge materials and resin-matrix materials used in other fields. Despite the lack of standardization of measurement intervals, researchers still measured their lease after a time interval. For those papers in which not all necessary information was available, in the paper, attempts were made to obtain the necessary information by contacting (email, letter) the corresponding author or coauthors. Papers with missing information that could not be recovered in this way were not included.

Also, a PRISMA flowchart was performed on the reference lists of all primary sources and eligible studies of this systematic search for additional relevant publications.

Study selection and data collection process

The studies that satisfied the inclusion criteria were handled to extract data. First of all, after identifying the articles in the database, the articles were exported into Mendeley reference manager software version 1.19.8 (Mendeley Ltd.) to remove duplicate articles. Secondly, after reading the abstracts of the included studies, these were thoroughly read and analyzed to verify the hypotheses and data relevant to our study. Finally, the data extraction was carried out using

Table 1. Quantification of BPA in different dental materials from different manufacturers (*in vitro* studies).

Brand	Resin type	Solvent	Analytical method	Detection limit (DL)/ quantification limit (QL)	Quantified monomers (±SD)		Ref.
Tetric Flow® (Ivoclar Vivadent, Ellwangen, Germany)	Nanohybrid composite	Methanol	GC-MS	–	24h: 3.37 (±0.18) mmol/L 7d: 3.36 (±0.26) mmol/L	BPA	Durner et al.[39]
Filtek™ Supreme XT (3M ESPE, Seefeld; Germany)	Nanohybrid composite	Deuterated methanol (CD_3OD)	GC/MS	DL: 0.00008 mmol/L	180d: 0.598 mmol/L	BPA	Sevkusic et al.[40]
Tetric Ceram® (Ivoclar Vivadent, Ellwangen, Germany)					180d: 0.005 mmol/L		
Grandio® (VOCO GmbH, Cuxhaven, Germany)					180d: 0.022 mmol/L 90d: 1.469 mmol/L		
Grandio® Flow (VOCO GmbH, Cuwhaven, Germany)	Nanofillled composite				180 d: 0.598 mmol/L		
Filtek™ Supreme XT (3M ESPE, Seefeld; Germany)	Nanohybrid composite	Deuterated water (D_2O)			0.000 mmol/L		
Filtek™ Supreme XT Flow (3M ESPE, Seefeld; Germany)	Nanofilled composite				120 d: 0.001 mmol/L		
Tetric Ceram® (Ivoclar Vivadent, Ellwangen, Germany)	Nanohybrid composite				150 d: 0.001 mmol/L		
Grandio® (VOCO GmbH, Cuxhaven, Germany)	Nanohybrid composite				180 d: 0.002 mmol/L 180 d: 0.007 mmol/L		
Grandio® Flow (VOCO GmbH, Cuwhaven, Germany)	Nanofillled composite				180 d: 0.001 mmol/L		
Ceram.X (Dentsply DeTrey GmbH; Konstanz, Germany)	ORMOCER composite	Deionized water	UPLC-MS/MS	DL: 0.04 ng/mL QL: 0.1 ng/mL	24 h: 0.36 (±0.06) ng/mL	BPA	Becher et al.[42]
Grandio® Flow (VOCO GmbH, Cuwhaven, Germany)	Nanohybrid composite				24 h: 0.08 (±0.01) ng/mL		
Filtek™ Supreme XTE (3M ESPE, St. Paul, MN, USA)	Nanofilled composite				24 h: 0.6 (±0.1) ng/mL		
Tetric EvoFlow (Ivoclar Vivadent, Schaan, Lichtenstein)	Nanoparticles composite				24 h: 6.5 (±0.4) ng/mL		
Clinpro™ Sealant (3M ESPE, St Paul, MN, USA)	Fissure sealant				24 h: 0.17 (±0.09) ng/mL		
DELTON® (Dentsply DeTrey GmbH; Konstanz, Germany)	Fissure sealant				24 h: 9.6 (±2.2) ng/mL		
Helioseal F (Ivoclar Vivadent AG, Amherst, NY, USA)	Fissure sealant				24 h: 0.04 (±0.04) ng/mL		
Filtek™ Supreme XTE (3M ESPE, Seefeld, Germany)	Nanofilled composite	Salivary , Bacterial *S. mutans*	UPLC-MS/MS	DL= 73 pg BPA QL= 1926 pg BPA	24 h: < QL	BPA	Nys et al.[44]
Solitaire 2 (Heraeus Kulzer, Dormagen, Germany)	Microhybrid composite						
G-aenial Posterior (GC Europe, Leuven, Belgium)	Hybrid composite						
Ceram.x Spectra ST HV (Dentsply Sirona; Konstanz, Germany)	Nano-ceramic composite						
Fissurit FX (VOCO GmbH, Cuxhaven, Lower Saxony, Germany)	Fissure sealant						
Scotchbond™ Universal (St. Paul, MN, USA)	Adhesive						
Clearfil SE Bond2 (Kuraray Co. Ltd., Osaka, Japão)	Adhesive						
AH Plus Jet (Dentsply Sirona, Konstanz, Germany)	Root canal sealer						
	Hybrid composite	Water	UPLC-MS/MS		1 y: 89.4 (±5.1) pmol/mL	BPA	Nys et al.[43]

(*continued*)

Table 1. Continued.

Brand	Resin type	Solvent	Analytical method	Detection limit (DL)/ quantification limit (QL)	Quantified monomers (±SD)		Ref.
G-aenial Posterior (GC Europe, Leuven, Belgium)				DL= 0.20 pmol BPA			
Venus (Kulzer, Hanau, Germany)	Hybrid composite				1 y: 62.6 (±16.3) pmol/mL		
Ceram.X mono (Dentsply Sirona, Konstanz, Germany)	Nanohybrid composite				1 y: 45.7 (±2.7) pmol/mL		
Filtek™ Ultimate Universal Restorative (3M ESPE, St. Paul, MN, USA)	Nanofilled composite				1 y: 249.5 (±23.8) pmol/mL		
G-aenial Posterior (GC Europe, Leuven, Belgium)	Hybrid composite	Ethanol			1 y: 582.4 (±12.4) pmol/mL		
Venus (Kulzer, Hanau, Germany)	Hybrid composite				1 y: 258.2 (±8.8) pmol/mL		
Ceram.X mono (Dentsply Sirona, Konstanz, Germany)	Nanohybrid composite				1 y: 250.7 (±5.4) pmol/mL		
Filtek™ Ultimate Universal Restorative (3M ESPE, St. Paul, MN, USA)	Nanofilled composite				1 y: 195.7 (±28.4) pmol/mL		
Charisma Classic (Kulzer, Hanau, Germany)	Microhybrid composite	Methanol	LC-MS/MS	QL: 0.042 ng/mL	24 h: 0.66 (±0.09) ng/mL 4 d: 0.54 (±0.07) ng/mL 9 d: 0.58 (±0.07) ng/mL 16 d: 0.99 (±0.16) ng/mL 35 d: 2.17 (±0.22) ng/mL 65 d: 2.26 (±0.26) ng/mL 130 d: 3.37 (±0.49) ng/mL	BPA	Šimková et al.[41]
Filtek™ Ultimate Universal Restorative (3M ESPE, St. Paul; MN, USA)	Nanocomposite composite				24 h: 1.01 (±0.06) ng/mL 4 d: 0.27 (±0.14) ng/mL 9 d: 0.43 (±0.08) ng/mL 16 d: 0.57 (±0.09) ng/mL 35 d: 1.50 (±0.33) ng/mL 65 d: 3.59 (±0.16) ng/mL 130 d: 4.64 (±0.30) ng/mL		
Charisma Diamond (Kulzer, Hanau, Germany)	Nanohybrid Composite				24 h: 0.11 (±0.05) ng/mL 4 d: 0.08 (±0.02) ng/mL 9 d: 0.10 (±0.06) ng/mL 16 d: 0.05 (±0.01) ng/mL 35 d: 0.08 (±0.05) ng/mL 65 d: 0.07 (±0.02) ng/mL 130 d: 0.16 (±0.03) ng/mL		
Admira Fusion (VOCO GmbH, Cuxhaven, Germany)	ORMOCER composite				24 h: 0.15 (±0.01) ng/mL 4 d: 0.08 (±0.08) ng/mL 9 d: 0.03 (±0.02) ng/mL 16 d: 0.04 (±0.01) ng/mL 35 d: 0.03 (±0.01) ng/mL 65 d: 0.02 (±0.01) ng/mL 130 d: 0.03 (±0.01) ng/mL		
Charisma Classic (Kulzer, Hanau, Germany)	Microhybrid composite	Artificial saliva	LC-MS/MS	QL: 0.042 ng/mL	Cumulative values over 260d: 20 s, 1300 mW/cm^2: 143.7 (±6.0) ng/g 5 s, 3000 mW/cm^2: 126.4 (±24.7) ng/g	BPA	Tichy et al.[35]
Filtek™ Ultimate Universal Restorative (3M ESPE, St. Paul, MN, USA)	Nanofilled composite				Cumulative values over 260d: 20 s, 1300 mW/cm^2: 180.4 (±39.1) ng/g 5 s, 3000 mW/cm^2: 154.1 (±23.1) ng/g		
Charisma Diamond (Kulzer, Hanau, Germany)	Nanohybrid composite				Cumulative values over 260d: 20 s, 1300 mW/cm^2: 2.09 (±0.48) ng/g 5 s, 3000 mW/cm^2: 3.34 (±0.42) ng/g		

(*continued*)

Table 1. Continued.

Brand	Resin type	Solvent	Analytical method	Detection limit (DL)/ quantification limit (QL)	Quantified monomers (±SD)		Ref.
Admira Fusion (VOCO GmbH, Cuxhaven, Germany)	ORMOCER composite				Cumulative values over 260d: 20 s, 1300 mW/cm^2: 2.54 (±0.95) ng/g 5 s, 3000 mW/cm^2: 3.39 (±0.59) ng/g		
Photac Fil Quick (3M ESPE, St. Paul, MN, USA)	Resin modified glass ionomer				Cumulative values over 260d: 20 s, 1300 mW/cm^2: 14.1 (±1.7) ng/g 5 s, 3000 mW/cm^2: 27.6 (±1.1) ng/g		
GC Fuji II LC Capsule (GC, Tokyo, Japan)	Resin modified glass ionomer				Cumulative values over 260d: 20 s, 1300 mW/cm^2: 8.84 (±1.28) ng/g 5 s, 3000 mW/cm^2: 12.9 (±1.2) ng/g		
Charisma Classic (Kulzer, Hanau, Germany)	Microhybrid composite	Methanol			Cumulative values over 260d: 20 s, 1300 mW/cm^2: 213.1 (±4.90) ng/g 5 s, 3000 mW/cm^2: 170.8 (±19.0) ng/g		
Filtek™ Ultimate Universal Restorative (3M ESPE, St. Paul, MN, USA)	Nanofilled composite				Cumulative values over 260d: 20 s, 1300 mW/cm^2: 299.0 (±18.2) ng/g 5 s, 3000 mW/cm^2: 358.0 (±16.3) ng/g		
Charisma Diamond (Kulzer, Hanau, Germany)	Nanohybrid composite				Cumulative values over 260d: 20 s, 1300 mW/cm^2: 7.98 (±0.46) ng/g 5 s, 3000 mW/cm^2: 8.53 (±0.82) ng/g		
Admira Fusion (VOCO GmbH, Cuxhaven, Germany)	ORMOCER composite				Cumulative values over 260d: 20 s, 1300 mW/cm^2: 5.11 (±1.65) ng/g 5 s, 3000 mW/cm^2: 4.49 (±1.03) ng/g		
Photac Fil Quick (3M ESPE, St. Paul, MN, USA)	Resin modified glass ionomer				Cumulative values over 260d: 20 s, 1300 mW/cm^2: 206.1 (±17.0) ng/g 5 s, 3000 mW/cm^2: 4.40 (±1.03) ng/g		
GC Fuji II LC Capsule (GC, Tokyo, Japan)	Resin modified glass ionomer				Cumulative values over 260d: 20 s, 1300 mW/cm^2: 22.7 (±3.0) ng/g 5 s, 3000 mW/cm^2: 25.4 (±2.2) ng/g		
Ceram X® (Dentsply Sirona, Bensheim, Germany)	ORMOCER composite	Wastewater	HPLC-FLD	DL: 0.001 µg/mL	6 m: 0.082 (±0.062) µg/mL	BPA	Polydorou et al.[45]
Filtek™ Supreme XTE (3M ESPE, Seefeld, Germany)	Nanofilled composite			QL: 0.005 µg/mL	6 m: 0.082 (±0.064) µg/mL		
Core-X® flow (Dentsply Sirona, Bensheim, Germany)	Dual-cure core build-up				6 m: 0.022 (±0.018) µg/mL		
Vita Enamic (VITA Zahnfabrik, H. Rauter, GmbH & Co KG)	CAD-CAM Blocks:polymer-infiltrated ceramic network materia	Distilled water	HPLC-UV	DL = 0.083 ng/µL (TEGDMA and BPA) DL = 0.166 ng/µL (UDMA and Bis-GMA)	1 d: n.d.	BPA	Mourouzis et al.[46]
				DL = 0.333 ng/µL (Bis-EMA)	7 d: n.d.		
				QL = 0.25 ng/µL (TEGDMA and BPA) QL= 0.5 ng/µL (UDMA and Bis-GMA)	30 d: n.d.		
				QL = 1 ng/µL (Bis-EMA)	60 d: n.d.		
Cerasmart (GC Corporation, Tokyo, Japan)	CAD-CAM Blocks: Nanoceramic				1 d: n.d.		

(*continued*)

Table 1. Continued.

Brand	Resin type	Solvent	Analytical method	Detection limit (DL)/ quantification limit (QL)	Quantified monomers (±SD)		Ref.
					7 d: n.d. 30 d: n.d. 60 d: n.d.		
KATANA AVENCIA (Kuraray, Noritake Dental Inc)	CAD-CAM blocks: Nanoceramic				1 d: n.d. 7 d: n.d. 30 d: n.d. 60 d: n.d.		
BRILLIANT Crios (Coltène/ Whaledent AG, Switzerland)	CAD-CAM Blocks: Reinforced resin composite				1 d: n.d. 7 d: n.d. 30 d: n.d. 60 d: n.d.		
Shofu HC (SHOFU Dental GmbH)	CAD-CAM Blocks: Nanoceramic				1 d: n.d. 7 d: n.d. 30 d: n.d. 60 d: n.d.		
Vita Enamic (VITA Zahnfabrik, H. Rauter, GmbH & Co KG)	CAD-CAM Blocks:polymer-infiltrated ceramic network materia	75% ethanol /water			1 d: n.d. 7 d: n.d. 30 d: n.d. 60 d: n.d.		
Cerasmart (GC Corporation, Tokyo, Japan)	CAD-CAM Blocks: Nanoceramic				1 d: n.d. 7 d: n.d. 30 d: n.d. 60 d: n.d.		
KATANA AVENCIA (Kuraray, Noritake Dental Inc)	CAD-CAM blocks: Nanoceramic				1 d: n.d. 7 d: n.d. 30 d: n.d. 60 d: n.d.		
BRILLIANT Crios (Coltène/ Whaledent AG, Switzerland)	CAD-CAM Blocks: Reinforced resin composite				1 d: n.d. 7 d: n.d. 30 d: n.d. 60 d: n.d.		
Shofu HC (SHOFU Dental GmbH)	CAD-CAM Blocks: Nanoceramic				1 d: n.d. 7 d: n.d. 30 d: n.d. 60 d: n.d.		
Clinpro™ Sealant (3M ESPE, St Paul, MN, USA)	Fissure sealant	Acetonitrile	HPLC - UV	DL: 1.6 µg/mL QL: 5 µg/mL	0.1 mg: 15.82 (±0.37) µg/mL 0.2 mg: 22.70 (±0.74) µg/mL 0.3 mg: 22.70 (±0.46) µg/mL 0.4 mg: 34.77 (±0.29) µg/mL	BPA	Al-Tannak et al.[47]
Filtek™ One Bulk Fill (3M ESPE GmbH, Seefeld, Germany)	Bulk fill composite				0.1 mg: 12.88 (±0.60) µg/mL 0.2 mg: 21.48 (±0.96) µg/mL 0.3 mg: 32.70 (±1.80) µg/mL 0.4 mg: 44.62 (±0.45) µg/mL		
Filtek™ Universal Restorative (3M, St. Paul, MN, USA)	Microhybrid composite				0.1 mg: 5.87 (±0.10) µg/mL 0.2 mg: 9.21 (±0.90) µg/mL 0.3 mg: 13.65 (±0.59) µg/mL		

(*continued*)

Table 1. Continued.

Brand	Resin type	Solvent	Analytical method	Detection limit (DL)/ quantification limit (QL)	Quantified monomers (±SD)	Ref.
					0.4 mg: 14.63 (±0.97) µg/mL	
Filtek™ Supreme XT Flow (3M ESPE, Seefeld, Germany)	Nanofilled composite				0.1 mg: 8.0 (±2.61) µg/mL	
					0.2 mg: 11.53 (±0.95) µg/mL	
					0.3 mg: 12.15 (±0.39) µg/mL	
					0.4 mg: 28.29 (±0.42) µg/mL	
Filtek™ Z350 XT (3M ESPE, Seefeld, Germany)	Nanofilled composite				<QL	

Abbreviations: Analytical method: GC-MS, gas chromatography coupled with mass spectrometry; UPLC-MS/MS, ultra-performance liquid chromatography–tandem mass spectrometry; LC-MS/MS, liquid chromatography with tandem mass spectrometry; HPLC-FDL, high performance liquid chromatography with fluorescence detection; and HPLC-UV, high performance liquid chromatography with ultra violet. Time: y, years; m, months; d, days; h, hours; and s, seconds. n.d., not detectable.

a standard and classified form (Tables 1 and 2), following predefined specific terms. The data were as follows: reference of the dental material; type of the dental material; the solvent used in release studies, and the biological matrix in which BPA was analyzed *in vitro* (Table 1) and *in vivo* (Table 2) studies, respectively; analytical method, detection and quantification limits, quantified BPA, general results, references.

Results

A total of 379 references were searched from the database. Figure 3 depicts the results of the pieces of research carried out on PubMed and SCOPUS. Indeed, this has allowed us to find 293 studies; among them, 86 duplicates were removed. As shown on the diagram, after reading the titles and abstract, 232 articles were eliminated, because they did not match the target subject. In addition, 61 papers were left to be read in full; 44 of them were not included because they used experimental composite resins or used unpolymerized samples. Finally, 17 papers seemed to constitute the data required for the systematic review: ten articles *in vitro* and seven articles *in vivo*.

The sample size of the *in vivo* studies fluctuates from 4 to 1001 individuals, on average 176.9 individuals. The individuals in these studies have a different range in age, from 3 years to approximately more than 50 years old. All the studies were done on males and females.

Concerning the techniques used for the quantification of BPA, in the *in vitro* studies, five methodologies were used: GC-MS,[39,40] LC-MS/MS (Liquid Chromatography with Tandem Mass Spectrometry),[35,41] UPLC-MS/MS (Ultra-Performance Liquid Chromatography–tandem Mass Spectrometry),[42–44] HPLC-FDL (High Performance Liquid Chromatography with Fluorescence Detection),[45] and HPLC-UV (High Performance Liquid Chromatography with Ultra Violet).[46,47] BPA release were evaluated in eight different solvents: methanol,[35,39–41] deionized water,[40,42] saliva,[35,44] ethanol,[43] wastewater,[45] distilled water,[46] 75% ethanol/water,[46] and acetonitrile.[47]

In the *in vivo* studies, six different methodologies were used: HPLC-MS,[37,48] LC-MS/MS,[49] LC-MS,[50,51] GC-MS,[52] Flow cytometry,[53] ELISA (Enzyme-Linked Immunosorbent Assay).[36] In addition, three biological fluids were analyzed: saliva,[36,48,50,51] urine,[37,48–51,53] and blood.[53]

Regarding the *in vitro* studies, 5 of them have only one value measured for each dental material.[40,42–45] In contrast, five studies for each dental material tested has several values measured at different times: one between 1 day to 7 days;[39] one between 1 to 60 days;[46] one between 1 day to 130 days.[41] Only one study aimed to investigate the level of BPA leakage from four selected weights (0.1, 0.2, 0.3, 0.4 mg).[47]

All *in vivo* studies have measured BPA released between different dental material tested, before and at least one time after exposure. Four studies compare BPA concentrations in two biological fluids.[48,50,51,53] Six studies compare BPA concentration in urine,[37,48–51,53] while five compare BPA in saliva[36,48,50–52] and one study compare BPA concentration in blood.[53]

However, the most relevant were the studies in which there was observed a significant change in the quantity of BPA detected at different times in monitoring studies.

By bringing together all the data from the studies, 33 different dental materials, from twelve different manufacturers were tested, allocated in different categories: dental resin composite, adhesive, fissure sealant, CAD-CAM blocks, glass ionomer, root canal sealer, compomer and dual-cure build-up. Ten dental resin composites were analyzed *in vitro*, and eleven were analyzed *in vivo*; only four dental resin composite types were analyzed in both environments (microhybrid, nanohybrid, nanofilled and ORMOCER).

The major findings are shown in Tables 1 and 2 (*in vitro* and *in vivo* studies, respectively) and drawn as follow: In *in vitro* studies fast polymerization increased the initial release of BPA.[35] The *in vitro* studies showed that the highest amounts of BPA were released from conventional composites, followed by resin-modified glass ionomer, while the smallest amount released was observed from "BPA-free" composites[35,41] and CAD-CAM blocks.[46] On *in vivo* assessment, placement of resin-matrix composite was associated with

Table 2. Quantification of BPA in different dental materials from different manufacturers (*in vivo* studies).

Brand	Resin type	Biological matrix	Analytical method	Detection limits (DL)/ quantification limit (QL)	Quantified monomers (±SD)		Ref.
Filtek™ Z250 Universal Restorative (3M ESPE, St Paul, MN, USA)	Microhybrid composite	Saliva	LC-MS	–	No rubber dam placed:	BPA	Kingman et al.[50]
					After: / Before:		
					0 to <1 h: / 0 to <1 h:		
					0.71 ng/mL / 0.46 ng/mL		
					1 to 8 h: / 1 to 8 h:		
					0.39 ng/mL / 0.41 ng/mL		
					9 to 30 h: / 9 to 30 h:		
					0.41 ng/mL / 0.48 ng/mL		
					With rubber dam placed:		
					After: / Before:		
					0 to <1 h: / 0 to <1 h:		
					0.60 ng/mL / 0.41 ng/mL		
					1 to 8 h: / 1 to 8 h:		
					0.38 ng/mL / 0.38 ng/mL		
					9 to 30 h: / 9 to 30 h:		
					0.41 ng/mL / 0.42 ng/mL		
Heliomolar or Heliomolar HB (HH, Ivoclar Vivadent, Schaan, Liechtenstein)	Microfilled composite	Urine			No rubber dam placed:		
					After: / Before:		
					0 to <1 h: / 0 to <1 h:		
					0.99 ng/mL / 1.70 ng/mL		
					1 to 8 h: / 1 to 8 h:		
					1.82 ng/mL / 1.94 ng/mL		
					9 to 30 h: / 9 to 30 h:		
					2.35 ng/mL / 1.63 ng/mL		
					With rubber dam placed:		
					After: / Before:		
					0 to <1 h: / 0 to <1 h:		
					1.10 ng/mL / 1.80 ng/mL		
					1 to 8 h: / 1 to 8 h:		
					2.51 ng/mL / 1.98 ng/mL		
					9 to 30 h: / 9 to 30 h:		
					2.41 ng/mL / 1.70 ng/mL		
Filtek™ Flow (3M Unitek, Monrovia, CA, USA)	Nanohybrid composite	Saliva	LC-MS	LD: 0.5 ng/mL	T0: 0.00 ng/mL	BPA	Kang et al.[51]
					30 min: 2.32 (±2.20) ng/mL		
					1 d: 0.55 (±1.56) ng/mL		
					1 w: 0.09 (±0.27) ng/mL		
					1 m: 0.00 ng/mL		
		Urine			T0: 0.79 (±1.65) ng/mL		
					30 min: n.d.		
					1 d: 0.58 (±1.14) ng/mL		
					1 w: 0.69 (±1.02) ng/mL		
					1 m: 2.81 (±4.01) ng/mL		
Filtek™ Z250 Universal Restorative (3M ESPE, St Paul, MN, USA)		Saliva			T0: 0.83 (±2.26) ng/mL		
					30 min: 7.26 (±6.81) ng/mL		
					1 d: 0.36 (±1.22) ng/mL		
					1 w: 0.85 (±2.32) ng/mL		
					1 m: 0.00 ng/mL		
		Urine			T0: 0.32 (±0.71) ng/mL		
					30 min: n.d.		
					1 d: 4.11 (±6.31) ng/mL		
					1 w: 3.33 (±6.27) ng/mL		
					1 m: 0.80 (±1.56) ng/mL		
Filtek™ Z100 Restorative (3M ESPE, St. Paul, MN)	Microhybrid composite	Urine	HPLC-MS	LD: 0.1 ng/mL	T0: 3.33 (±3.84) ng/mL	BPA	Maserejian et al.[37]
					24 h: 5.04 (±10.11) ng/mL		
					14 d: 3.45 (±3.97) ng/mL		
OptiBond Solo Plus (Kerr, Orange, CA)	Adhesive				6 m: 3.07 (±3.01) ng/mL		
Teethmate-F-1 2.0 (Kuraray, Tokyo, Japan)	Fissure sealant	Urine	HPLC-MS	–	T0: 1.41 (±1.06) ng/mL	BPA	Kim and Choi[48]

(*continued*)

Table 2. Continued.

Brand	Resin type	Biological matrix	Analytical method	Detection limits (DL)/ quantification limit (QL)	Quantified monomers (±SD)		Ref.
		Saliva			24 h: 2.43 (±1.54) ng/mL T0: 2.84 (±2.84) ng/mL 24 h: 3.69 (±2.70) ng/mL		
Filtek™ Supreme Ultra (3M Oral Care; St Paul, MN, USA)	Nanofilled composite	Urine	LC-MS/MS	–	T0: 0.51 ng/mL	BPA	McKinney et al.[49]
Ultraseal XT Plus (Ultradent, South Jordan, UT, USA)	Fissure sealant				Treatment: 0.57 ng/mL 2 d: 1.00 ng/mL 1 w: 0.69 ng/mL 4 w: 0.55 ng/mL 16 w: 0.57 ng/mL		
Ultraseal XT Plus (Ultradent, South Jordan, UT, USA)	Fissure sealant	Urine Blood	Flow cytometry (renal function): Gamma-glutamyl transpeptidase (gamma-GT); Albumin; N-acetyl-β-D-glucosaminidase (NAG)	LD = 0.1 l/L	Primary teeth	BPA	Trachtenberg et al.[53]
Revolution (Kerr; Orange, CA, USA)	Microhybrid composite				5 y: 39.9 (±21.2) surface-years Permanent teeth		
Filtek™ Z100 composite (3M ESPE, St. Paul, MN, USA)					5 y: 10.4 (±17.0) surface-years		
Dyract AP (Dentsply, DeTrey, Konstanz, Germany)	Polyacid-modified composite				Primary teeth		
Filtek™ Z250 Universal Restorative (3M ESPE, St Paul, MN, USA)	Nanofilled composite	Saliva	Ecologiena supersensitive BPA ELISA kit	Quantitative analysis ranges from 0.05 to 10 ug/L (ppb)	5 y: 11.8 (±18.1) surface-years T0: 0.15 (±0.42) µg/L 5 min: 3.64 (±2.32) µg/L 7 d: 0.59 (±1.27) µg/L	BPA	Lee et al.[36]

Abbreviations: Analytical method: GC-MS, gas chromatography coupled with mass spectrometry; LC-MS, liquid chromatography–mass spectrometry; HPLC-MS, high performance liquid chromatography–mass spectrometry. Time: m, months; w, weeks; d, days; h, hours; min, minutes; and s, seconds. n.d., not detectable.

an increase in BPA (and other compounds) detection in saliva within one hour after restoration placement[36,50,51] and an increasing concentration of BPA in urine until 24 hours after restoration.[37,48–50] An *in vivo* study[50] showed that rubber dam use did not reduce BPA absorption (measured as BPA level in urine). On *in vivo* assessment, the maximum concentration (24 hours) of BPA in saliva was around 0.55 ng/mL from placing lingual bonded retainer (nanohybrid composite).[51] The maximum concentration (24 hours) of BPA in urine was around 5.04 ng/mL from the placement of microhybrid resin-matrix composite.[37] However, all released amounts of BPA (*in vivo* and *in vitro* studies) were significantly lower than the current tolerable daily intake (4 µg/kg body weight/day), even in methanol, the solvent that is prone to increase the release of BPA.[35,41]

Discussion

A dental restoration is a clinical treatment that replaces natural structures with long-lasting materials such as metal, ceramic, and composite. Research and development of dental resin composites have been gradually enlarged for direct restoration in the anterior and posterior tooth due to their excellent optical and mechanical properties.[11,54,55] Dental composites have another advantage; there are sculptable, which allows the clinician to recreate the complex structure of the damaged tooth by designing the natural and correct anatomy.[35] Nowadays, this is also increasingly a requirement of patients.[56] Due to the current and growing requirement for a low-cost material, non-toxic[57] and with greater longevity, significant investment have been done by companies in betting on new resin-matrix materials.

Dental resins are constituted by an organic resin-matrix and an inorganic filler. The inorganic content may be up to 90 wt% resin-matrix composite containing one or two types of silanized ceramic or glass-ceramic fillers, such as colloidal silica and zirconium silicate, barium silicate, or ytterbium fluoride.[1,58,59] Coupling agents are used to building the covalent interaction between organic matrix and inorganic fillers. Among these coupling agents, the most commonly used is silane, which enhances bonding or adhesion between

the filler particles and matrix and helps transfer load and stresses.[60]

The proportion of organic matrix highly influences the properties of the resin-matrix composite.[61] Indeed, affecting the thermic expansion coefficient, shrinkage, water absorption, and solubility. The optical and mechanical properties of the resin-matrix composite before and after polymerization are dependent on the type of monomers and the ratio of the monomer fraction. The resin-matrix is chemically active, contains monomers that make it compatible with other resin matrices and adhesives. These monomers come from organic polymer matrices, mainly Bis-GMA, Bis-EMA, UDMA and TEGDMA.[60] The organic matrix based on Bis-GMA has many drawbacks due to the viscosity.[62] In order to counterweigh the high viscosity of Bis-GMA, lower viscosity monomers, like TEGDMA, are combined to attain a more flowable composite. It allows the addition of filler particles.[63] The resin-matrix also contains photo-initiators, mainly camphorquinone, stimulated by visible light at a wavelength of around 470 nm.[64] Moreover, co-initiators (e.g., tertiary amines) activate photopolymerization to reduce the negative points of monomers release.[65,66] The degree of conversion during polymerization of the monomers varies between 50 and 70%,[67,68] although the maximum degree of conversion is only reached during 24 h from photopolymerization. As shown in Tables 2, for the higher values of monomers concentration in the first hour, the degree of conversion is deficient (40%), which indicates the instability of the polymeric bonds leading to susceptibility to degradation in the oral cavity.[69,70] In addition, an improper composite polymerization or fast polymerization significantly increased the initial release of BPA, from dental composites.[35] On the other hand, Sonkaya et al.[34] showed that polymerization time did not affect the monomer release when the initiators and activators of the tested materials were similar. However, the type of the monomers and concentration of the filler used in the composite affected the amount of monomers released.[34] The decrease of free radicals induces polymerization reaction, and this depletion is not absolute, which means that these are remaining unreacted monomers residues of methacrylate in the restoration matrix.[11,71] Then, BPA can be leached from the organic matrix of the resin-matrix into the oral environment (saliva and oral tissues).[70,72] The toxicity of BPA and its derivatives comes from leaching of the organic matrix and the degradation of BPA-based monomers. Thus, continuous monitoring may be fruitful to improve the quality of these resin composites and to accelerate, even more, the investigation on BPA-free composites used in dental treatments.[71]

Analytical methods for monitoring BPA

In vitro studies

In vitro studies are essential to analyze substances released in the human body because they can reproduce better or worse conditions while being standardized.[73] The most used solvent in selected studies was methanol.[35,39–42] Notably, according to specific authors, methanol should be the better solvent because of the recommendations of the US Food and Drug Administration.[41,42,45] On the other hand, methanol would be the worst condition because, besides its toxicity, it enhances the maximum elution of monomers. Methanol can be replaced by an environmental friendly mixture of 75% ethanol and 25% water.[74,75]

Natural saliva is a mixture of fluids secreted by parotid, submaxillary and sublingual glands. Thus, being a complex system with many components and varying according to time of day, exact replication is not possible.[76] Many *in vitro* studies require relatively large amounts of saliva. Although natural saliva would be the material of choice,[77] it is difficult to obtain in adequate quantities and varies in composition. Furthermore, human saliva in experimental studies can lead to ethical concerns with reproducibility and standardization of experiments due to human variation.[78] For this reason, some studies use artificial saliva,[35] which includes only inorganic components and does not include human salivary proteins. Furthermore, other authors have preferred to use only water.[37,51]

Analytical methods used for the quantification/detection in those studies were based on GC-MS,[39,40] LC-MS/MS,[35,41] UPLC-MS/MS,[42–44] HPLC-FDL,[45] and HPLC-UV.[46,47] For best results with GC-MS a derivatization procedure is needed to analyze BPA, while the LC-MS methods can analyze BPA and derivatives straightforward.[48] Most studies used LC and only two studies used GC-MS for quantification of BPA. The most applied detection method for LC was MS, two studies used UV and one FL detection. Beyond the separation methods (GC or LC), the use of a high selective and sensitive detection method is mandatory for analysis of complex matrices such as dental resin composites. The MS detection combined with both GC and LC increases the sensitivity and selectivity of the technique. However, samples are heated at high temperatures in GC technique and can degraded other monomers that can overestimate the amount of BPA. Deviot et al.[] evaluated the *in vitro* release of BPA from an orthodontic resin composite by GC/MS and LC/MS as well as the *in vitro* Bis-GMA release by LC/MS. The possible influence of the heat used in GC/MS on the measure of BPA release from resin composite and the thermal stability of Bis-GMA were investigated. In the results obtained by GC/MS, BPA was detected in saliva and in acetonitrile in high quantity while by results obtained by LC/MS, BPA was detected from samples immersed in saliva samples and only traces in acetonitrile samples. This study pointed that the heat applied in GC/MS may overestimate BPA release from resin composite.

Concerning the *in vitro* studies (Table 1), the follow-ups of the release were from 1 hour to 180 days. In addition, some studies[35,41,79] examined the kinetics of BPA release. The average daily release of BPA decreased after the first day and steadily decreased. However, the long-term release was examined only by Polydorou et al.[80] who did not detect any BPA after 1-year storage in 75% ethanol, probably due to the insufficient quantification limit (QL = 0.5 µg/mL).

Computer-aided design and manufacturing (CAD/CAM) in dentistry for indirect dental restorations has developed remarkably during the past decade. Dental restorations produced by CAD/CAM can comply with standardized manufacturing processes, offer satisfactory reproducibility, and reduce manufacturing costs. In addition, CAD/CAM technology can process a diversity of restorative materials such as glass/ceramic, composites, and acrylic resins due to new scanning technologies and tools.[81,82] Regarding the type of resin linked with the quantity of monomers released, the CAD-CAM blocks release less monomer when immersed in aqueous solutions in the short-term and long-term periods than conventional resin composite materials. The amount of monomer release declined until no monomer could be detected. The difference could be because those materials have been previously polymerized into ready-to-mill blocks and thus possess better chemical properties.[46]

Another relevant piece of information was the positive level of BPA and its derivatives detected for two dental composites[35,41] presented as "BPA-free," Charisma Diamond (CD; Kulzer, Hanau, Germany) and Admira Fusion (AF; VOCO, Cuxhaven, Germany), although in significantly lower amounts than from Bis-GMA-containing composites. This could be explained by a false positive, an error occurred during the preparation of the sample relative to the technique of polymerization, the time of polymerization, or even a failure in the protocol of polishing. Also, it could be linked with an exaggerated time of storage or with an inadequate container which must be in glass or a plastic "BPA free." Furthermore, what manufacturers report about the composition of products or when they declare something as BPA-free does not always imply that such a statement is true. Even if it were, contamination could also occur at the factory by mixing with the material or being stored in plastic tubes/cartridges/syringes.

Comparing *in vitro* studies, the detection of BPA revealed significant concentrations in three mediums (water, methanol, or 75% ethanol/water). Most studies monitored only BPA, but other the monomers were also quantified in few works, the Bis-GMA was found up to 42.31 µg/mL with 72 h release in 75% ethanol/water.[74] The remarkable value of 0.000 mmol/L (Filtek Supreme XT; 3M ESPE; Seefeld; Germany)[51] should be linked to the quantification limit.

Dental grinding procedures present an additional source of BPA released in wastewater.[45] The wastewater remains in the environment for a long time (e.g., in rivers), resulting in a long period of contact of the resin composite powder with the water. BPA concentrations in effluents after wastewater treatments are reported to be in concentrations of 100–300 ng/L.[83,84] The contamination of wastewater with endocrine disruptor micropollutants can, unfortunately, impact the wildlife of the receiving waters. For example, the feminization of fish and mussels and reproductive disturbance in fish has been observed in several rivers by wastewater treatment discharges, which is thought to be related to the release of endocrine disruptors in wastewater.[85–87] According to Polydorou et al.,[45] dental grinding procedures present an additional proven source of BPA released in wastewater, requiring accurate and effective wastewater treatment even for small amounts or traces. This release appears to be material dependent. Of the assessed filtration methods, catalytic carbon seems to be an accurate method for removing BPA in water, providing a promising treatment for wastewater accumulated from dental treatments.[45]

In vivo studies

In vivo studies (Table 2), some studies (33%) have a sample of participants under 25 individuals, which is inconsistent for epidemiologic studies and possible further consequent association.[48,51,52] Concerning the analytical methods, different methodologies were used: HPLC-MS,[37,48] LC-MS/MS,[49] LC-MS,[50,51] Flow cytometry,[53] ELISA (Enzyme-Linked Immunosorbent Assay).[36] The biological fluids used in *in vivo* studies are complex matrices that demand high selective and sensitive methods, thus MS detection is mandatory for unequivocal identification and low detection limits.

According to the population in each study both sexes were represented in equivalent proportion. Nevertheless, BPA and its derivatives are endocrine disruptors,[53] particularly for children and women.[88] Regarding the age of the participants, in some studies the groups were very heterogeny,[36,51] while others were based on a younger population with children or teenagers.[37,48–50,53]

The *in vivo* studies are more complex than *in vitro* studies because of the bias and the complexity of the biological fluids matrices. For example, the received dental composite placement, which could be a source of BPA, Bis-GMA, or other monomer, however, there are many other environmental sources of contamination of these monomers, for example, canned food, plastic bottle of water, etc. Also, by analyzing biological fluids, participants have many variations because of their bodyweight and/or their metabolization.[89]

As shown in Figure 2, saliva is the first element in direct contact with the composite and BPA and its derivatives are at a higher level than urine or blood, right after the placement and for a short time.[50] The studies analyzing saliva with follow-ups indicate an increase of BPA or Bis-GMA between the moment just after the dental composite placement in the oral cavity and 24 hours after.[40,50,75,90] Using a rubber dam to isolate the tooth during the dental composite placement did not reduce significantly the level of BPA detected in urine.[50] Then, after the oral ingestion, metabolization by the uridine diphosphate and glucuronosyltransferase (UGT) and the passage by the gastrointestinal tract and the liver, BPA and its derivatives suffer another transformation by the sulfotransferase.[89] Finally, the next step is the clearance of the blood by excretion of metabolized monomers in the urine,[32] in which the augmentation occurs a particular time after, the higher level detected in urine was 24 hours after the placement of the dental composite.[40,91] Due to the problematic collection of blood, only 22% of the studies monitored blood or plasma,[39] a decrease of Bis-GMA at 6 months and 1 year after placement was observed. Then, at 5 years post-placement, the concentration of BPA and derivatives detected was null.[36,91] Most studies

monitored only BPA, which was quantified up to 5.04 ng/mL in saliva (24 h),[37] and Bis-GMA was also quantified up to 458.60 ng/mL in saliva (1 h).[50] Flow cytometry was also reported with the objective to analyze the immune function.[53,90]

Comparison between in vivo/in vitro

Biological fluids from humans as saliva, urine, and blood are complex and requires the participants' agreement in each study. However, *in vitro* studies must be ethical but do not need consent. Another advantage of the *in vitro* studies is that all the bias and mediums can be controlled much better than in the *in vivo* studies. Also, the number of samples can be lower than in the *in vivo* studies because of the randomization of the conditions. Another inconvenience of the *in vivo* study is the collection of the samples (saliva, urine, and blood) which require more organization and the collaboration of the participants.

Based on new data and methodologies, EFSA (European Food Safety Authority) has rated a low level of safety, known as the Tolerable Daily Intake (TDI) of BPA, for 4 µg/kg of body weight per day. However, concerning the units, there is significant variability in the presentation of the results, making comparisons between different studies difficult. Therefore, the unit ng/mL was the most used in the studies selected for this review.[37,41,42,49–51] For example, in *in vitro* studies, expressing the results may vary between µg/mL,[45,47] ng/mL,[41,42] mmol/L,[39,40] pmol/mL.[43] But it is easier to convert to compare the outcomes. On the other hand, in *in vivo* studies, few articles mention the units of µg/kg of body weight to verify whether it is above or below the TDI. Also, there are studies that it is impossible to compare because they do not present data such as the weight of the child/adult. Additionally, most studies are concerned about the quantification of BPA, but the original monomers can also be released and give contribute to endocrine disruptions as recently reported by Boonen et al.,[92] which agonistic estrogenic activity was found for the monomer Bis-DMA, the photostabilizer 2-hydroxy-4-methoxy-benzophenome (HMBP) and photoinitiator 2,2-dimethoxy-2-phenylacetophenone (DMPA).

Regarding the quantification limit, it is easier to achieve acceptable values in the *in vitro* studies than in the *in vivo* studies due to the lower matrix effects and the more controlled and standardized type of matrices. The bias of the study can be, for example, the materials of the containers for the collection of the samples the composition of the medium in which the sample is eluted.

A common denominator about the studies with follow-ups was that after a specific time of immersion, the level of BPA detected tends to stabilize[53] or decrease.[36,40–42,45,50]

Conclusion

The exposure of humans to BPA is constant and must be reduced as possible because of its toxicity, mainly concerning the endocrine system. Dental composites are direct sources of contamination, as shown in the previous studies.

At first, we concluded that equally *in vitro* and *in vivo* studies demonstrated that flowable composites and sealants released a higher amount of BPA than conventional ones.

The majority of the *in vivo* studies analyzed urine or saliva, and not blood because the difficulty in collection. The level of BPA was higher in saliva than in urine and blood because of its proximity with a dental composite.

GC/MS should be avoided to measure BPA release from resin composite, because of the high risk of contaminations due to the potential cleavage of BPA derivatives (such as Bis-GMA) in BPA. Therefore LC-MS/MS is the most suitable method for trace quantification of BPA in *in vitro* and *in vivo* studies.

The use of LC-MS/MS allows to obtain a better selectivity, low quantification limit, and unambiguous identification of the target compounds.

Nevertheless, innovation in sample preparation to attain a faster and more efficient method to increase the monitoring reports is still needed.

Declaration of interest statement

The authors report there are no competing interests to declare.

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3. Evaluation of BPA and Bis-GMA release from recent dental composite materials by LC-MS/MS

Lopes-Rocha L, Gonçalves VMF, Cunha S, Garcez J, Pinho T, Tiritan ME. Evaluation of BPA and Bis-GMA release from recent dental composite materials by LC-MS/MS. *Int J Paediatr Dent* .

(Submitted)

3.1. Introduction

Resin-matrix composites are standardly used in various areas of dentistry. [1] Primarily, they are the most conservative approach in esthetic and functional rehabilitation in young patients. [2], such as in maxillary lateral incisor agenesis. [3] In addition, they have an ample range of utilizations, such as pit and fissure sealant, luting cement or temporary material, and adhesive for brackets and splints. [1]

Resin-matrix composites contain an organic portion consisting of a polymer matrix and inorganic filler particles attached to the resin matrix through a silane coupling agent. [4] In recent resin-matrix composites, the sources of Bisphenol-A (BPA) that leach from dental materials include trace levels of BPA from impurities of BPA-derivatives such as bisphenol A-Diglycidyl Methacrylate (Bis-GMA); Ethoxylated Bisphenol A Glycol Methacrylate (Bis-EMA), dimethacrylate (Bis-DMA) and 2,2-bis-(4-(3-methacryloxypropoxy) phenyl) propane (Bis-PMA). In addition, the presence of BPA due to degradation/ hydrolysis of Bis-DMA has also been reported. [4,5] Bis-GMA (**Figure** 1) is the predominant base monomer used in the resin-matrix composite, and its chemical structure is supposed to prevent hydrolysis. Still BPA may be found as an impurity in Bis-GMA manufacture if polymerisation still needs to be completed. [5] While the acceptance of resin-matrix composites has grown in the last decade, the release of endocrine-disrupting chemicals (EDCs), such as BPA and its derivatives, has gained the attention of scientists, clinicians and patients. [6,7] BPA is a chemical intermediate producing polycarbonate-, epoxy-, and methacrylate-matrix materials used in several industrial and health fields. [6,8] BPA is an organic compound having two phenolic functional groups. (**Figure 1**), and was categorised by the European Chemicals

Agency (ECHA) as a 'substance of very high concern' since it was classified as an EDC with threats to human health (toxic for human reproduction) and the environment, as determined in Regulation (EC) No 1907/2006. [6] Furthermore, researchers found that BPA leached into the saliva and urine of treated patients and was responsible for the estrogenicity of some commercial composites and sealants used in Dentistry. [9] There are children with a positive association between composite restorations (with BPA) and attention deficit hyperactivity disorder. [10]

As resin-matrix materials are predicted to have a shelf life of several years in the mouth, long storage periods are required for *in vitro* studies to study the long-term release of numerous composite components. For example, it has been demonstrated *in vitro* that monomers (BPA-based) can elute from resin-matrix composites for up to one year after a week-to-week extraction solution update protocol (i.e. ethanol, water, and artificial saliva).[11]

Bis-GMA is one of the primary sources of BPA release from dental resin materials. Therefore, several research studies proposed a new formulation (using non-BPA dimethacrylates) as a substitution for Bis-GMA monomer in resin formulations to reduce human exposure to BPA derivatives. [4] To formulate dental materials without Bis-GMA, urethane-dimethacrylate (UDMA), another typical dimethacrylate monomer applied in odontology, was envisaged to substitute Bis-GMA as the base resin for dental materials. [12] The UDMA-based resin, unfortunately, had a critical flaw in its higher volumetric shrinkage. [13] Therefore, alternative monomer compositions of Bis-GMA were introduced to address the limitations of this product in terms of durability and toxicity. One alternative to the methacrylate-based resin-matrix composites is a hybrid organoceramic, ORganically MOdified CERamic (ORMOCER®, VOCO, Germany), which is an ORMOSIL (Organically Modified SILicate). One of the most

exciting ORMOCER® characteristics is combining polysiloxane groups with light-curing methacrylate groups covalently bonded to silica fillers. [14] ORMOCER® provides high biocompatibility due to the lack of residual monomers, lesser polymerisation shrinkage, high wear resistance, increased opacity, and improved handling characteristics. [14,15]

Due to the constantly increasing use of resin-matrix composites, consideration should be given to the biocompatibility of such dental restorative fillings. There is an increasing need to bridge the gap between the alarming literature regarding hazardous effects and the limited clinical investigations, which can be only accomplished by acquiring expertise on the leaching of compounds from resin-based dental materials.

This work aims to evaluate the BPA and Bis-GMA (**Figure 1**) monomers released from six resin–matrix composites using a sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) quantification method.

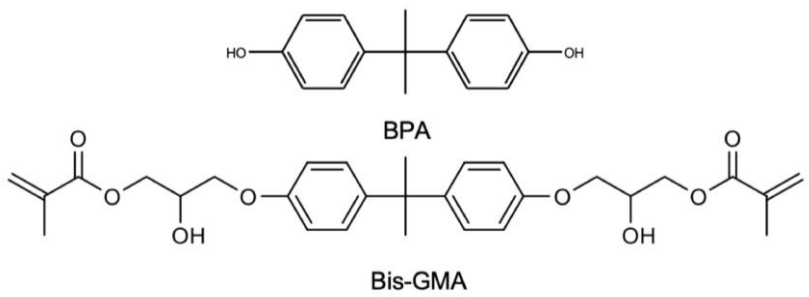

Figure 1 Molecular structure of BPA and Bis-GMA

3.2. Materials and Methods

3.2.1. Resin composites used

Six resin composites were tested: five commercial resins and one experimental resin, from different commercial brands. The manufacturer specifications and composition of the six resin-matrix composites are presented in **Table 1.**

Table 1. Composite materials specifications.

Resin	Brand	Manufacturer	Composition	Filler by weight (%)	Filler dimension (μm)	Water Sorption (μg/mm³)	Water Solubility (μg/mm³)
AF	Admira® Fusion	VOCO, Cuxhaven, Germany	ORMOCER® resin, SiO_2 Ba-Al-B-Si-glass fillers	84	2.5 to 3.0	13.4	≤ 0.1
BF	Enamel Plus HRI BIO Function	Micerium SpA, Avegno, Italy	UDMA, TCDDMA, no co-monomers, and no Bis-GMA glass filler, high dispersion silicon dioxide, fluorine	74	0.2 to 3.0	15.27	0.31
NC	Experimental resin	Coltène-Whaledent, Altäsatten, Switzerland	n.a.	n.a.	n.a.	n.a.	n.a.
BE	BRILLIANT EverGlow™	Coltène-Whaledent, Altäsatten, Switzerland	Bis-GMA*, TEGDMA, Bis-EMA*, ZnO, Amorphous silica fillers	79	0.4 to 0.7	15.1	< 0.1
ED	IPS Empress Direct	Ivoclar Vivadent,Schaan Liechtenstein	Bis-GMA*, UDMA, TCDD, Ba-Al-Si-glass, YbF3, SiO_2/ZrO_2, MO, Nanomodifier	78	0.1 to 0.3	19.6	< 0.1
FS	Filtek™ Supreme XTE	3M ESPE, MN, USA	Bis-GMA*, UDMA, TEGDMA, Bis-EMA*, ZrO_2/SiO_2 cluster SiO_2 nano-scale fillers	72.5	0.6 to 20	n.a.	n.a.

Asterisk (*) indicates BPA-based monomers.
Abbreviations: n.a.: not available; Bis-GMA: bisphenol A diglycidyl methacrylate; Bis-EMA: ethoxylated bisphenol A dimethacrylate; TEGDMA: triethylene glycol dimethacrylate; UDMA: urethane dimethacrylate; TCDDMA: tricyclodecane dimethanol dimethacrylate; TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin.

3.2.2. Chemicals and reagents used

Standards of Bisphenol A (BPA) (CAS- 80-05-7) and Bisphenol A-glycidyl methacrylate (Bis-GMA) (CAS- 1565-94-2) were from Sigma Aldrich (St Louis, MO, USA), acetonitrile and ethanol HPLC gradient grade were from Fisher Scientific (Loughborough, United Kingdom), formic acid 98-100% from Merck (Darmstadt, Germany) ammonium acetate (97% purity) was purchased from AppliChem Panreac ITW Co. (Barcelona, Spain) and methanol (MeOH, for HPLC LC-MS grade) was purchased from VWR. Ultra-pure water grade was supplied by a SG Water System (Ultra Clear UV model). BPA and Bis-GMA stock solutions were prepared at 1mg/mL in ethanol and stored at -20°C in amber glass flasks.

3.2.3. Preparation of resin composite samples and eluates

Specimen disks (6 mm diameter and 2 mm thickness) were prepared in a stainless-steel mold. A glass plate covered the top and bottom to limit oxygen inhibition, ensure smooth surfaces, and avoid excess material. Samples were polymerized for 20 s by light-curing using a LED light-curing unit (Celalux 3, High-Power LED curing-light; VOCO, Cuxhaven, Germany) at an average of 1300 mW / cm^2, 450/480 nm and, before each polymerization, the power of the equipment was confirmed through the radiometer. Grinding was carried out under standardized conditions to simulate the clinical procedures in the dental practice. Each resin composite sample was polished using a silicon carbide (SiC) abrasive paper of 2400 Mesh. Each disc had its abrasive paper to avoid contamination with BPA-containing particles from previous experiments. An electronic caliper (Mitutoyo, Tokyo, Japan) was used to confirm the precise and equal sizes of the disks (**Figure 2**).

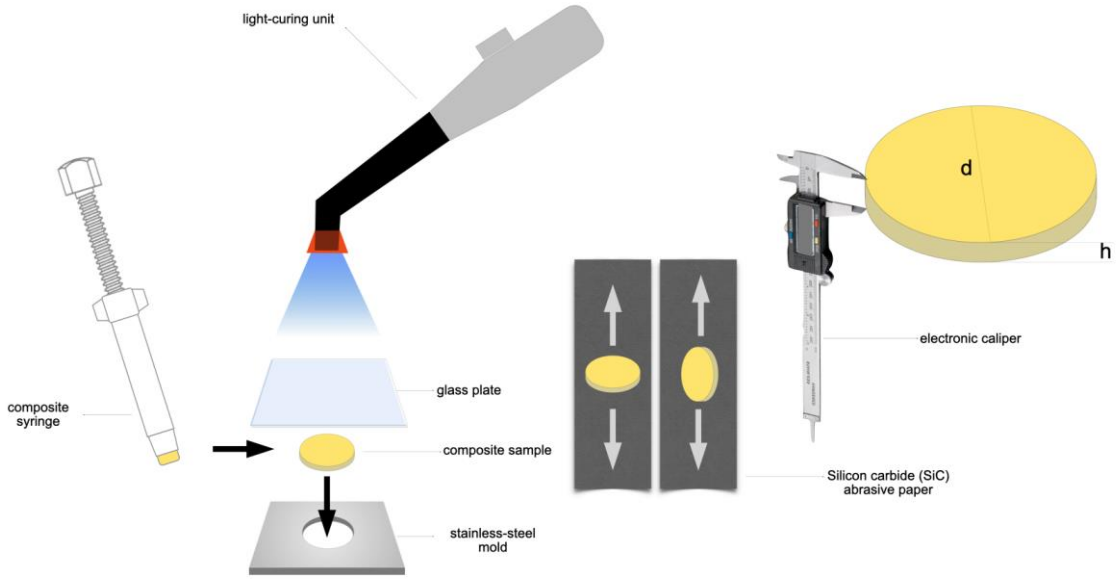

Figure 2. Schematic illustration of composite sample preparation. d- diameter; h-height.

Subsequently, the disks (n =5 for each composite type) were immediately immersed in 1 mL of a 75% ethanol-water solution in a sealed amber glass vial. In order to ensure that the entire surface of the disc was in contact with the solution, the disc was held by a fisherman's string and held in suspension in the solution (**Figure 3**).

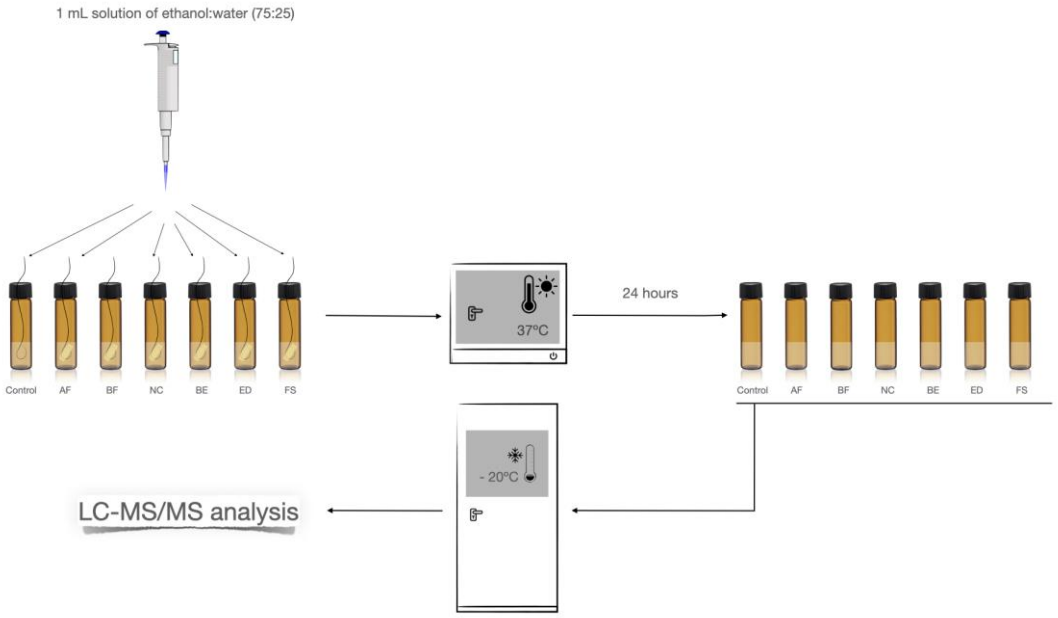

Figure 3. Schematic representation of the release assay.

A control assay was also performed using just de fisherman's string, putting it into a vial with 1mL of 75% ethanol-water solution. All the samples were incubated at 37°C (Optic Ivymen system, COMECTA), and, at each 24h (for seven days), the 75% ethanol-water solution was replaced with a fresh solution. All samples were stored at -20°C until analysis by Liquid Chromatography (LC) analysis.

3.2.4. High Performance Liquid Chromatography with UV and Fluorescence detection (HPLC-UV/RF) analysis

In a first approach, the samples were analyzed by High Performance Liquid Chromatography with UV and Fluorescence detection (HPLC-UV/RF). For that, a Shimadzu Prominence UFLC System (Shimadzu Corporation, Tokyo, Japan) was used. It was composed by a degasser DGU-20A5, two LC-20AD pumps, a SIL-20AC autosampler, a CTO-20AC column oven, a CBM-20A System Controller. A SPD-20A UV detector (set at 230nm) and a RF-10AXL fluorescence detector (set at 225nm and 310nm, for excitation and emission wavelength,

respectively), were coupled to the LC system. All the system was controlled using the LC Solution software, (V. 1.24 SP1, Shimadzu). Chromatographic analysis was performed in a Luna PFP2 (150x4.6mm, 3µm) column from Phenomenex (Torrance, CA, USA) operating at 40°C in a gradient mode at a flow rate of 0.8mL/min. The mobile phase consisted in A) 0.1%Formic acid (in water) and B) Acetonitrile (with 0.1% formic acid). The gradient was as follow: 0-6min 55%A:45%B; at 10 min 20%(A):80%(B) and remain at this condition until 25min; at 26min the pump return to the initial condition (55%A:45%B) and remain at this condition during 10min to stabilize the column for the next injection. Injection volume was 20µL.

3.2.5. LC-MS/MS analysis

Sample analysis was performed by a HPLC system Waters Alliance 2695 (Waters, Milford) interfaced to a Quattro Micro triple quadrupole mass spectrometer (Waters, Manchester, UK). The chromatographic separation was achieved using a Kinetex C18 2.6 µm particle size analytical column (150 × 4.6 mm) with a Phenomenex pre-column (Tecnocroma, Portugal) at a flow-rate of 200 µL/min. The column was kept at 30 °C, and the autosampler was maintained at room temperature (±25 °C). The mobile phase consisted of 90% MeOH and 10% aqueous solution of 5 mM ammonium acetate (pH 5), in isocratic mode. The total run time was 15 min. The sample injection volume was 10 µL. The MS/MS acquisition was operated in negative-ion mode with multiple reaction monitoring (MRM); the collision gas was argon 99.995% (Gasin, Portugal) with a pressure of 2.9×10^{-3} mbar in the collision cell. Capillary voltages of 3.0 KV were used in the negative ionization mode (**Table 2**). Nitrogen was used as desolvation gas, and cone gas at the flow of 350 and 60 L/h, respectively. The desolvation temperature was set to 350 °C and the source temperature to 150 °C. Dwell times of 0.1 s/scan were selected. The data were collected using the software MassLynx4.1.

Table 2. Selected reaction monitoring (SRM) parameters for tandem mass spectrometry analysis of target analytes.

Compound	MRM Transition (m/z)	Cone voltage (V)	Colision energy (eV)
BPA	227>133	35	25
	222>211	40	30
Bis-GMA	513>277	30	11
	513>427	30	11
	513>496	30	11

For sample quantification, individual standard calibration curves were performed for the monomers of BPA and Bis-GMA at the concentrations of 0.2, 0.4, 0.8, 3.2, and 6.4 ng/mL and 0.4, 0.8, 1.6, 12.5 and 25,0 ng/mL, respectively. A quality control was also used for both monomers, at 1.6 ng/mL for BPA and 6.4 ng/mL for Bis-GMA, to evaluate method accuracy and precision. Limit of detection (LOD) and limit of quantification (LOQ) were determined by the signal to noise ratio (S/N=3 LOD and S/N=10 LOQ). For sample quantification, individual standard calibration curves were performed for the monomers of BPA and Bis-GMA at the concentrations of 0.2, 0.4, 0.8, 3.2, and 6.4 ng/mL and 0.4, 0.8, 1.6, 12.5 and 25,0 ng/mL, respectively. A quality control was also used for both monomers, at 1.6 ng/mL for BPA and 6.4 ng/mL for Bis-GMA, to evaluate method accuracy and precision. Limit of detection (LOD) and limit of quantification (LOQ) were determined by the signal to noise ratio (S/N=3 LOD and S/N=10 LOQ).

3.3. Results

3.3.1. HPLC-UV/RF analysis

In a first approach, sample release was analyzed by LC with UV and RF detections. The chromatographic analysis clearly demonstrated the different chemical patterns between the resins (**Figure 4 and 5**). BPA was not detected in none of the six composite materials, but the chromatograms indicated the occurrence of Bis-GM. Due to the poor selectivity and sensibility of the LC-UV/RF method for quantification of BPA and Bis-GMA monomers, a LC-MS/MS method was established for monitoring the release studies.

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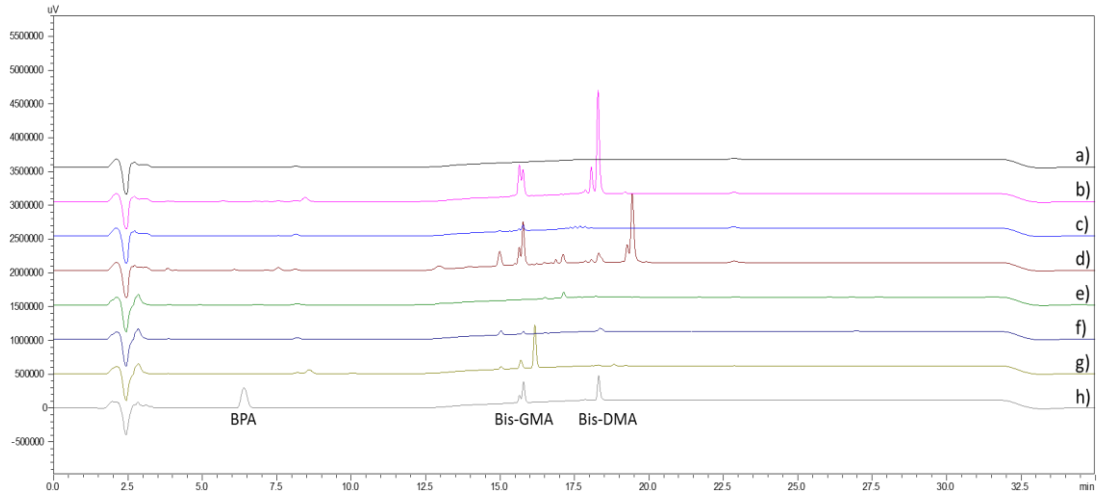

Figure 4. Chromatograms profile of BPA and Bis-GMA release from the resin samples after 24h immersed in 1 mL of a 75% ethanol-water solution: a) Control; b) BE; c) FS d) ED; e) AF; f) BF; g) NC; h) standard mixture at 50 μg/mL; obtained by UV detection at 230nm.

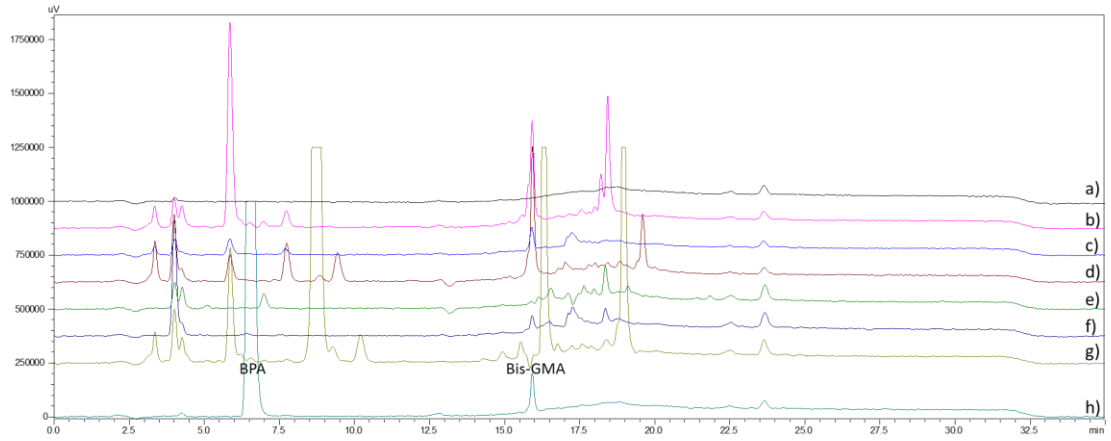

Figure 5. Chromatograms profile of BPA and Bis-GMA release from the resin samples after 24h immersed in 1 mL of a 75% ethanol-water solution: a) Control; b) BE; c) FS d) ED; e) AF; f) BF; g) NC; h) standard mixture at 50 μg/mL; obtained by RF detection at ex225/em310nm.

The quantification of the target monomers was performed by LC-MS/MS through a validated method considering the following parameters: selectivity, linearity and range, LOD, LOQ, accuracy, recovery, and precision. The injection of the reconstituted extracts gave correlation coefficients between 0.9914 and 0.9986 for BPA and Bis-GMA (**Table 3**). No carryover was observed. The LOD was 0.06 ng/mL and 0.03 ng/mL for BPA and Bis-GMA, respectively, while the LOQ was 0.2 ng/mL and 0.1 ng/mL, respectively. The method presented accuracy close to 100%.

Table 3. Method validation parameters.

	LOD (ng/mL)	LOQ (ng/mL)	Range	Linear Regression	r^2	Quality Control (ng/mL)	Accuracy (%)
BPA	0.06	0.2	0.2 – 6.4	y = 1804.1x – 314.55	0.9914	1.6	[92,52 – 105,9]
Bis-GMA	0.03	0.1	0.4 – 25	y = 2539.2x – 852.62	0.9986	6.4	[82,60 – 122,7]

Abbreviations: LOD: Limit of detection; LOQ: Limit of quantification.

3.3.2. BPA and Bis-GMA leaching experiment

The concentration of eluted monomers (BPA and Bis-GMA) from the six resin-based dental materials quantified by the validated method LC-MS/MS is shown in **Figure 6** and **Table 4**.

BPA was not detected in any sample, even in the Bis-GMA resin-based samples (**Figure 6** and **Table 4**).

As shown in **Figure 6** and **Table 4**, only on day one, an important amount of Bis-GMA was released from ED> BE> FS resins. The concentration, however, decreased significantly over the following days, but the release persisted during the seven days. The ED and BE had a similar behavior during the following days, but the FS presented the lowest and constant of Bis-GMA release.

Regarding **Figure 6** and **Table 4**, for the AF, NC and BF resins, samples analysis was only performed for the first five days because the results remained practically null and constant regarding the release of BPA and Bis-GMA since day 3.

To the best of our knowledge, no release studies were performed on the composite NC, which is an experimental brand resin-based material considered as BPA-free. Despite this method was not able to detect any concentration of BPA, a minimal concentration of Bis-GMA, was detected on days 1 and 2, as shown in **Figure 6** and **Table 4**.

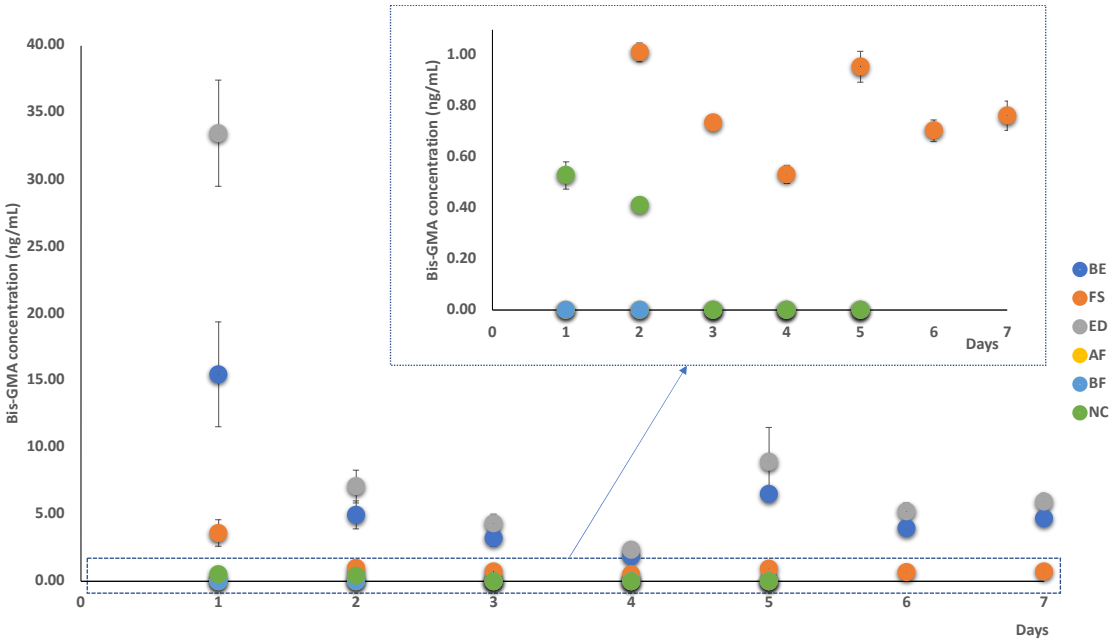

Figure 6. Released concentration of Bis-GMA from resin-based dental materials for 5 days (AF, BF, NC) and for 7 days (BE, ED, FS), expressed in mean ± SD (n=5).

Table 4. Released concentration of BPA and Bis-GMA from resin-based dental materials over a period of 5 and 7 days. Values are expressed as mean ± SD (n=5).

Concentration released by day (ng/mL) (n=5)										
Sample		1	2	3	4	5	6	7	Total concentration released (ng/mL)	Total concentration released (ng/mm³)
AF	BPA	ND	ND	ND	ND	ND	-	-	ND	ND
	Bis-GMA	ND	ND	ND	ND	ND	-	-	ND	ND
BF	BPA	ND	ND	ND	ND	ND	-	-	ND	ND
	Bis-GMA	ND	ND	ND	ND	ND	-	-	ND	ND
NC	BPA	ND	ND	ND	ND	ND	-	-	ND	ND
	Bis-GMA	0.53±0.05	0.41±0.05	<LOQ	<LOQ	<LOQ	-	-	0.94	0.02
BE	BPA	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Bis-GMA	15.46±3.94	4.96±1.04	3.22±0.62	1.88±0.19	6.51±0.53	4.00±0.43	4.71±0.39	40.75	0.72
FS	BPA	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Bis-GMA	3.60±0.983	1.01±0.037	0.74±0.029	0.53±0.035	0.95±0.061	0.71±0.042	0.76±0.057	67.43	1.19
ED	BPA	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Bis-GMA	33.46±3.97	7.09±1.21	4.35±0.66	2.38±0.29	8.95±2.55	5.22±0.64	5.98±0.60	67.43	1.19

3.4. Discussion

The present study evaluated the leaching of BPA and Bis-GMA from six recent resin-based dental materials. For that, a simple analytical method, without pre-concentration and/or derivatisation of BPA, was established for monitoring the release of BPA and Bis-GMA in *in vitro* studies using a non-toxic matrix.

In vitro studies are an essential part of analysing substances released in humans since they can be reproduced under more or less favourable conditions while they are being standardised. [16] Several solutions, such as artificial saliva, distilled water, ethanol, methanol, and acetonitrile, have been used in studies investigating the elution of monomers. [17,18] The outcomes of prior reports have suggested that the type of solvent affects the amount of eluted monomer from a composite resin. [17] The environment in the oral cavity is between water and the most aggressive solvents (ethanol, methanol and acetonitrile). [19] Ethanol and ethanol/water mixtures can penetrate the methacrylate polymer network, resulting in more sorption and swelling. [17] Moreover, a mixture of 75% ethanol and 25% water is not toxic for the operator in the laboratory and is considered environmentally friendly. The United States Federal Drug Administration recommends a 75% ethanol-water solution as a clinically relevant food–oral simulating liquid, and it has been used in several studies. [18] Furthermore, other authors explained that water is more similar to saliva, which would reproduce saliva and dentin fluid, the latter being the path to the dental pulp. [20] Therefore, 75% ethanol-water solution was used in this study.

Most studies monitored BPA release and neglected Bis-GMA, claiming that BPA can be detected in dental materials. However, it occurs as an impurity of the synthesising process and/or possibly as a degradation product of BPA-based monomers since BPA itself is not an intended ingredient. [21] Although leachable, Bis-GMA monomer is considered cytotoxic and inflammatory.[22,23] In this work, the release was monitored by LC with different types of Detection, but only LC-MS/MS allowed the suitable conditions for quantification of BPA and Bis-GMA. Regarding the release from six target resin–matrix composites, the chromatograms illustrated in **Figures 4** and **5** show that the profile differs according to the

resin type. The quantification by the validated LC-MS/MS method demonstrated that BPA release was lower than LOD (0.06 ng/mL) from all the samples over seven days after incubation of the composite samples with 75% ethanol-water solution (**Figure 6 and Table 4**), while Bis-GMA was released in detectable amounts from four of the six analysed samples: ED> BE> FS, including the NC sample, an experimental resin, called BPA-free. Higher concentrations of Bis-GMA were detected in the first few days, followed by a consistently lower release in the following days for ED>BE>FS; however, for the NC sample, the presence of Bis-GMA was detected just in the two first days (**Figure 6 and Table 4**). Bis-GMA was quantified in levels of up to 33.46 ng/mL on the first monitoring day. According to Nys et al [24], one possible explanation for the absence of BPA is the development of a recent new filler technology that alloys more free resin than standard fillers. This could delay the release of monomers and BPA. Thus, a BPA-based monomer in the raw material does not necessarily point to BPA being released in traceable amounts from this specific material after light curing. Furthermore, this may indicate the requirement for even more sensitive detection methods. [24]

The concentration of BPA-based monomers can explain the differences between the composites in the resin and their physicochemical properties (solubility in the 75% ethanol-water solution). Furthermore, the extent and rate of elution of components from composites depend upon the degree of conversion (DC) of monomers, the composition and solubility characteristics of the extraction solvent, and the size and chemical characteristics of the leachable species. [25,26] To minimise sources of error, we standardised the process by, among others, using only one fully charged light-emitting diode (LED) device for each material, which is common in daily dental work and superior to a halogen light-curing unit. [25] In addition, custom-made polymerisation stands were used to maintain the exact distance. It has been reported that the DC of light-polymerised resin materials is 55–80%. [25,27,28] There is an inverse correlation between DC and the amount of eluted monomer. The greater the polymerisation reactions, the fewer residual monomers available to elute. [27] Time also plays a relevant role in the elution of monomers. Several studies have stated that acute monomer release occurs within 24 hours. [28] Though, some current work has demonstrated that the elution of monomers is still ongoing within the first 24 hours and that leaching on particular monomers continues for a longer time. [11]

Finishing and polishing protocol is also crucial to eliminate excess resin-rich external layer that may be the source of eluted unreacted monomers in the oral cavity. [27]

Several studies used analytical methods which may have needed specificity and sensitivity to provide accurate detection and quantification of low levels of BPA, as exemplified by the high Limit of Detection (i.e., 100 ng/mL) reported by Noda et al [29]. Instead, there is insufficient information about the long-term release. Usually, samples are incubated for long periods without renewal of the incubation solution at equal time breaks. [30] In contrast, in a study developed by Putzeys et al [16], the long-term release of (BPA-based) monomers was determined in an equal-range solvent-changing configuration with the release of particular monomers after a 52-week incubation period. The major shortcoming of this non-specific approach is the loss of sensitivity. There is a inconsistency between different reports, which can be explained by BPA concentration being too low for detection. Moreover actual absence of BPA or due to a minute amount of BPA and BPA-based monomers in the resin. In the present study, the sensitivity of LC-MS/MS method analysis is reflected in the low LOD (0.06 ng/mL), which has a reasonable sensitivity compared with other studies. It is also important to consider that quantification by GC/MS may overestimate the BPA release from resin composite due to the heat used in GC/MS and the thermal stability of Bis-GMA. [31] However, making direct comparisons and drawing conclusions is not straightforward because the studies are not standardised. The modifications in the composition of the resin-matrix composite could result in variability in the release of the quantity and type of monomers.

3.5. Conclusions

This study described a simple, sensitive, and accurate method to detect low levels of BPA and Bis-GMA, released from resin-based dental materials, in a 75% ethanol-water solution. The results indicate that, if it is present, BPA release is below the LOD of the method. However, Bis-GMA was quantified in levels of up to 33.46 ng/mL in the first day of monitoring. Bis-GMA was detected even in the NC sample, an experimental resin, called BPA-free. Continual monitoring in *in vitro* and *in vivo* studies may be helpful to improve the quality of the resin composites and to stimulate, even more, the clinical application on BPA

free composites used in dental treatments, especially in young patients with esthetic and functional rehabilitation such as in cases of maxillary lateral incisors agenesis.

BPA-free resins are a good option, and the brands studied and marketed show that they are also free of BPA derivatives such as Bis-GMA. However, further studies are needed regarding other monomers also derived from BPA.

3.6. References

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Chapter IV

CHEMICAL AND MECHANICAL PROPERTIES OF RESIN-MATRIX COMPOSITES

1. Effect of aging on the mechanical properties among dental resin with and without Bisphenol A

Lopes-Rocha L, Carbas RJC, Borges CSP, Gonçalves VMF, Tiritan ME, Medeiros IS, da Silva LFM, Pinho T. Effect of aging on the mechanical properties among dental resin with and without Bisphenol A. *Strain*

(Submitted)

1.1. Introduction

The composition of resin-matrix composites differs according to their clinical purposes and companies. The inorganic content can reach up to 90 wt% of the resin-matrix composite, including one or two types of silanized ceramic or glass-ceramic fillers.[1–3] The balance in the ratio of the organic matrix and inorganic fillers establishes the physicochemical properties of the resin-matrix composites,[2–7] affecting the thermic expansion coefficient, shrinkage, water absorption, and solubility.[8] The organic matrix often involves Bisphenol A (BPA) monomers and derivates such as Bis-GMA (bisphenol A-diglycidyl methacrylate), Bis-DMA (bisphenol A-dimethacrylate), Bis-EMA (ethoxylated bisphenol A methacrylate), BADGE (bisphenol A diglycidil-ether).[9] In addition, TEGDMA (triethylene glycol dimethacrylate), UDMA (urethane dimethacrylate), and photoinitiators can also be found in the organic matrix composition.

Bis-GMA is a monomer widely used in resin-based dental materials. Bis-GMA's dominance is ascribed to its high reactivity, controlled volume shrinkage, good mechanical behavior, tissue diffusibility, and low volatility. [10] However, Bis-GMA has negative aspects like high hydrophilicity and viscosity.[11] In addition, Bisphenol A (BPA) and its derivatives have also been identified as an endocrine disrupter.[12] UDMA, another typical dimethacrylate monomer, was considered to manufacture dental materials without Bis-GMA. [13] Unfortunately, UDMA-based resin had a critical flaw in its greater volumetric shrinkage compared to Bis-GMA-based resin.[14] The greater volumetric shrinkage of UDMA-based resin may result in a more significant marginal gap between teeth and restorations, leading to a higher risk of secondary caries,[15] stainings,[16] and partial loss or fracture the restoration.[17] Therefore, alternative monomer compositions of Bis-GMA were introduced to address the limitations of this product in terms of durability and toxicity.[18] One alternative is a hybrid organoceramic to the methacrylate-based resin-matrix composites, known as ORganically MOdified CERamic (ORMOCER®, VOCO, Germany), which is an Organically Modified SILicate (ORMOSIL). One of the most interesting features of ORMOCER® is the combination of polysiloxane groups with light-curing methacrylate groups covalently linked to silica fillers.[19–21] It results in high biocompatibility because of the absence of residual monomers,

lower polymerization shrinkage, higher opacity, high wear resistance, and enhanced handling characteristics.[22–25]

A biomimetic treatment would promote similar mechanical properties of resin-matrix composites, such as compressive strength, strain-to-failure, and the elastic Young's modulus (*E*). The strength of the enamel–dentin, enamel, and dentin specimens were 126.1 ±54.6, 62.2 ±23.8, and 193.7 ±30.6 MPa. The strain-to-failure of the enamel–dentin, enamel, and dentin specimens was 8.7 ±2.7%, 4.5 ±0.8%, and 11.9 ±0.1%. The Young's modulus of the enamel–dentin, enamel, and dentin specimens were 1628.6 ±482.7 MPa, 1338.2 ±307.9, and 1653.7 ±277.9, respectively.[26]

This study aimed to evaluate the chemical and mechanical properties of six different dental resin-matrix composites (three BPA and three without BPA) having different filler loading, filler types, and organic matrices under the same curing and testing conditions. The null hypothesis showed no significant difference in these properties: (1) water sorption, solubility, and diffusion coefficient, and (2) compressive strength, strain, Young's modulus, and biaxial flexural strength with or without BPA among resin matrix composites without BPA.

1.2. Materials and methods

1.2.1. Preparation of specimens

The six resin-matrix composites tested in this study (**Table 1** and **2**) were as follows: Admira Fusion (VOCO, Cuxhaven, Germany), Enamel Plus HRi Bio Function Enamel (Micerium SpA, Avegno, Italy), N/C (Coltène-Whaledent, Altstätten, Switzerland), Brilliant EverGlow™ (Coltène-Whaledent, Altstätten, Switzerland), IPS Empress Direct (Ivoclar Vivadent, Liechtenstein) and Filtek™ Supreme XTE (3M GmbH, Seefeld, Germany).

While condensating, the unpolymerized composite was placed in a stainless-steel mold, and a mylar matrix strip was applied on the surface to limit oxygen inhibition. Excess material was removed with a sterile scalpel. Polymerization was accomplished using a LED light

source (Celalux, High-Power LED curing-light; VOCO, Cuxhaven, Germany) at an average of 1300 mW/cm^2, 450/480 nm for 20 seconds on each face. Before each polymerization the power of the equipment was confirmed through the radiometer. Finally, finishing and polishing were performed on all samples. Six specimen disks were prepared for each composite. The specimens had been stored in distilled water at 37±1 ˚C in the dark for 24 h, immediately after curing.

Table 1. Materials used in this study and manufacturers' information.

Group	Brand	Code	Lot number	Classification
AF	Admira® Fusion	A2 enamel	19076721	Nanohybrid-ORMOCER
BF	Enamel Plus HRI BIO Function	A2 enamel	2018003080	Nanohybrid
NC	Experimental resin	----	C1971	Nanohybrid
BE	BRILLIANT EverGlow™	A2/B2	J18375	Submicron hybrid
ED	IPS Empress Direct	A2 enamel	Y28474	Nanohybrid
FS	Filtek™ Supreme XTE	CT enamel	NA28205	Nanofilled

Table 2. Materials used in this study, their composition, and manufacturers' data.

	Group	Organic matrix	Filler	Filler Wt%	Filler dimension (µm)	Wsp (µg/mm3)	Wsl (µg/mm3)
Without BPA	AF	ORMOCER® resin	SiO2 Ba-Al-B-Si-glass fillers	84	2.5 to 3.0	13.4	≤ 0.1
	BF	UDMA, TCDDMA, no co monomers, and no Bis-GMA	glass filler, high dispersion silicon dioxide, fluorine	74	0.2 to 3.0	15.27	0.31
	NC	not available	Barium glass submicron	not available	not available	not available	not available
With BPA	BE	Bis-GMA TEGDMA Bis-EMA	ZnO Amorphous silica sillers	79	0.4 to 0.7	15.1	< 0.1
	ED	Bis-GMA UDMA TCDD	Ba-Al-Si-glass YbF3, SiO2/ZrO2, MO, Nanomodifier	78	0.1 to 0.3	19.6	< 0.1
	FS	Bis-GMA UDMA TEGDMA Bis-EMA	ZrO2/SiO2 cluster SiO2 nano-scale fillers	72.5	0.6 to 20	not available	not available

1.2.2. Test of specimens

Different tests were performed to evaluate the stiffness as a function of the side that will be exposed to irradiation, and to occur the reticulation of the resin through Knoop hardness tests and the effect of aging on the compression strength of resins.

1.2.2.1. Knoop hardness and Biaxial strength

Hardness values of brittle materials generally increase as indentation forces decrease, a phenomenon known as the indentation size effect (ISE).[28,29] Because of the ISE, a specified force or functional relationship over a test range is necessary to compare the hardness of brittle dental materials accurately.

The sampler (Styleitaliano™ products) allows making specimens of composite discs with the desired thickness in cylindrical molds: 12±0.2 mm in diameter and 1±0.1 mm in thickness. Visible light was irradiated for 20 s. All specimens were entirely covered with

transparent plastic matrix strips during light irradiation.

The Knoop microhardness of the irradiated side and the bottom side of the hardened resin composite were then measured with Hardness Tester (Model no. HMV-2; Shimadzu, Kyoto, Japan). The specimens were positioned centrally below the diamond indenter to measure the Knoop hardness number (KHN). The hardness of each specimen was measured with a 50 g load, applied for 15 s. The KHN equivalent to each indentation was calculated by computing the indentation dimensions and using the formula $KNH = 14.2\ x\left(\frac{F}{d^2}\right)$, where *F* is the test load and *d* corresponds to the longer diagonal of an indentation (**Fig. 1**).

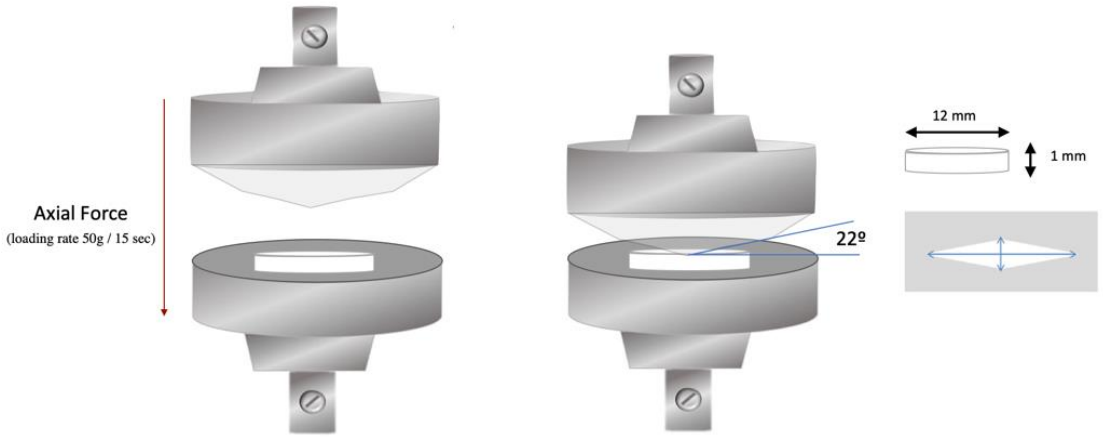

Figure 1. Knoop test. (Adapted from Ilie 2017[117]).

1.2.2.2. Water sorption and solubility

Water sorption and solubility tests were determined according to the specification standard for composite (ISO 4049:2009).[29] Six specimens were manufactured for each testing condition. Specimens with diameter of 12±0.2 mm and thickness of 1±0.1 mm were manufactured using the above-described sampler (**Fig. 2**).

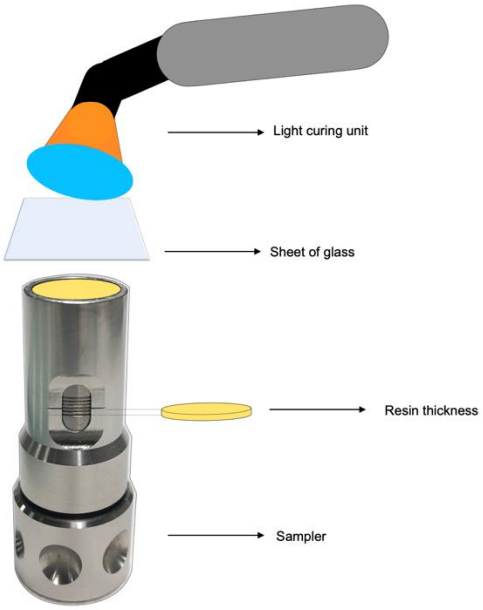

Figure 2. Production process of the discs: 1) sampler resin application, 2) placement of a glass sheet, 3) light-curing, 4) removal from the sampler, and 5) light-curing opposite side.

The thickness and diameter of the disks were measured with a digital electronic caliper (Mitutoyo Corporation, Tokyo, Japan). The specimens were prepared for each composite material and were weighed by an analytical scale accurate up to 0.0001mg (Sarorius, BI210s, Germany). Prior to the immersion in water, the specimens were fully dried. For this, all specimens were kept on a vacuum desiccator at 37°C until a constant weight was obtained. At this point, it was assumed that no water was left on the specimens (M_1). The samples were then stored in 10 mL of distilled water. The mass of the specimens was periodically measured until a stable value was reached. At this point, it was considered that the specimens were saturated. After this storage period, the samples were removed. The excess water at the surface was removed with absorbent paper until the water was no longer visualized to make sure the mass of water measured was inside the specimen and not left on its surface. Then, the weights were re-recorded (M_2). Afterwards, the specimens were subsequently reinserted in the desiccator at 37 °C and weighed daily until a constant

mass was reached (M_3). At this point, it was considered that the specimen was dry again.

Equations 1 and 2 were used to calculate the water sorption (WS) and solubility (SL) values, respectively.

$$W_{sp} = \frac{M_2 - M_3}{V} \quad (1)$$

$$W_{sl} = \frac{M_1 - M_3}{V} \quad (2)$$

M_1 is the initially dry specimen's, constant mass, in micrograms (mg), before water immersion, M_2 is the mass of the specimen (mg) after immersion in water for seven days; M_3 is the mass of the reconditioned specimen (mg), and V is the volume of specimen, in cubic millimeters (mm^3).

A Fick's law of diffusion can be fitted to the sorption and desorption behavior of the specimen. Fick's law is given by equation 3.[30]

$$M_t = \left[1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left(\frac{-D(2n+1)^2\pi^2 t}{4h^2}\right)\right]M_\infty \quad (3)$$

Where t represents the time starting from the immersion and h represents the thickness of the specimen.

The water uptake at the time increment t, M_t, is given by equation 4.

$$M_t = \frac{M}{M0} \quad (4)$$

Where M is the mass of the specimen on the time increment t and M_0 is the reference mass of specimen in the beginning of the process.

A script was run in *MATLAB* to determine the best fit of the coefficient of diffusion, D, and infinite water uptake, M_t, to the experimental results. For the series a sample size of n=50 was used.

1.2.2.3. Compressive tests procedure

Compressive strength is defined as the stress at which it fractured. The tests were conducted using a universal testing machine INSTRON® model 3367 (Norwood, Massachusetts, USA) equipped with a load cell of 30 KN, at a displacement rate of 0.02 mm/min (**Fig. 3**). First, load and strain were recorded electronically using the software TestXpert II (Zwick GmbH & Co. KG, Ulm, Germany). Afterward, the recorded data wasconverted in the mechanical parameters: Young's modulus and compressive strength.

Six samples (n=6) of each investigated material were prepared with 6 mm diameter and 2mm height (**Fig. 2**). First, all specimens were kept on a vacuum desiccator at 37°C to obtain the same condition. Next, the specimens were stored in 10 mL in distilled water for seven days, 36 days, and one year. After this storage period, the samples were removed, and the excess water was removed with absorbent paper until the water was no longer visualized. Finally, the compressive test was done in specimens after preparation (0 days) and after 7, 36 days, and one year of immersion in distilled water.

Compressive strength (CS) was calculated from the formula: $CS = 4F/\pi d^2$ F is the maximum load, and d is the cylindrical specimen diameter.

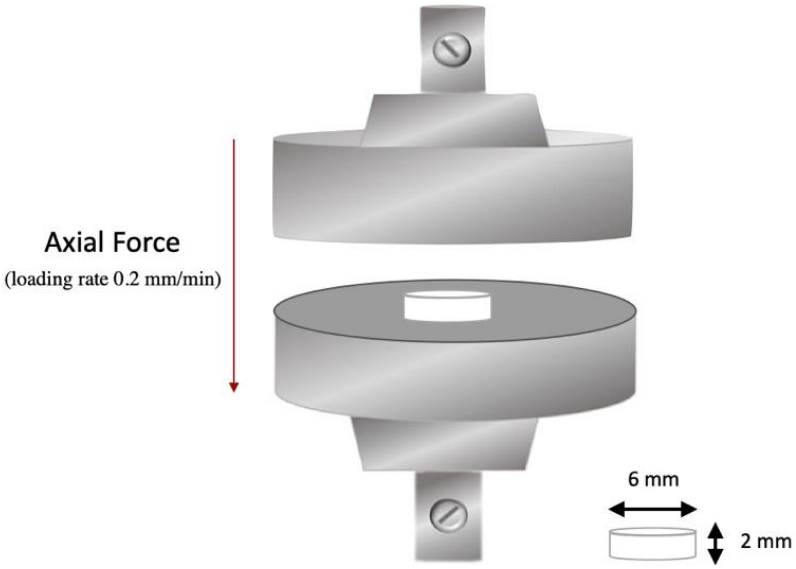

Figure 3. Compressive test.

1.3. Results

1.3.1. Knoop hardness and Biaxial strength

The obtained Knoop hardness values on the irradiated surface were statically superior to those obtained on the opposite surface (**Fig. 4** and **Table 3**). The AF group shows the greater Knoop hardness values (57.31±3.88) and the FS the lowest value (48.19±4.37) (**Table 3**). The Knoop hardness is higher in the resin group without BPA than with BPA (AF>BF>NC>BE>ED>FS).

In terms of biaxial flexural strength, as can be seen in **Fig. 5** and **Table 4**, the results recorded for the BE and ED groups were quite similar (BE: 150.0±15.00 and ED: 146.9±12.90). Moreover, the AF group showed the lowest value (133.7±13.90), and the FS group the highest (178.9±18.50).

Although the AF group showed the highest hardness value, it was the group with the lowest flexural strength. On the other hand, the FS group was the soft material but with the highest flexural strength. Therefore, considering the hardness and flexural strength, the BF and NC group were the ideal materials for the scope of dental resin, because they were hard, but showed moderate flexibility to avoid the material's fracture.

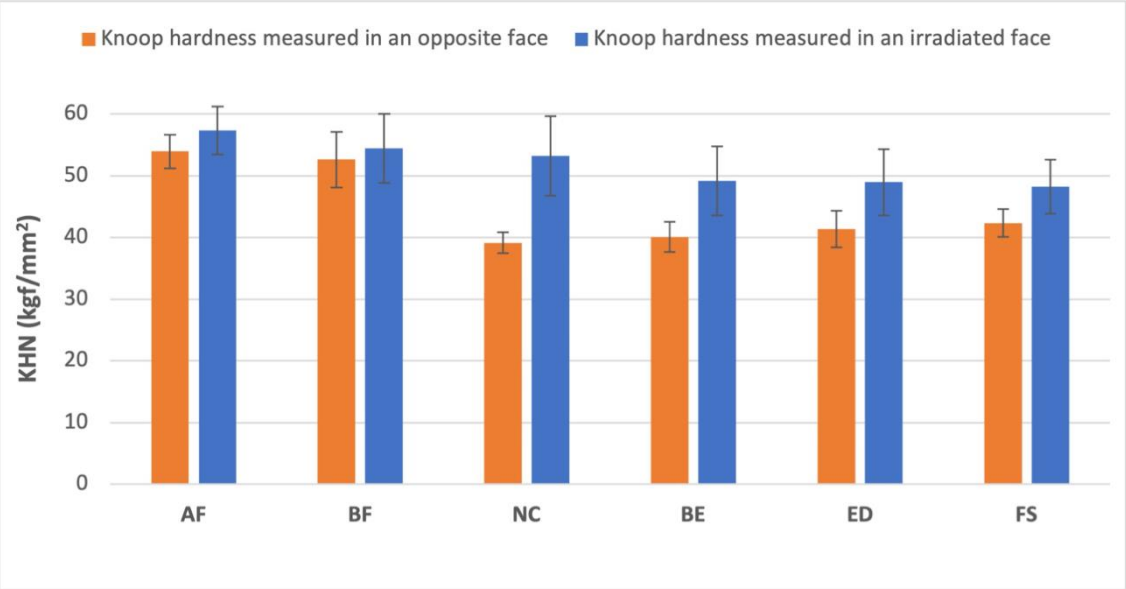

Figure 4. Material properties of the resin composite specimens.

Table 3. Mean, SD values, and coefficient of Knoop hardness for all groups.

MATERIAL	Irradiated face		Opposite face	
	Knoop hardness	Coefficient (%)	Mean±SD	Coefficient (%)
AF	57.31±3.88	7	53.92±2.75	5
BF	54.44±5.62	10	52.60±4.53	9
NC	53.18±6.42	12	39.13±1.70	4
BE	49.18±5.60	11	40.08±2.43	6
ED	48.95±5.36	11	41.36±2.99	7
FS	48.19±4.37	9	42.31±2.26	5

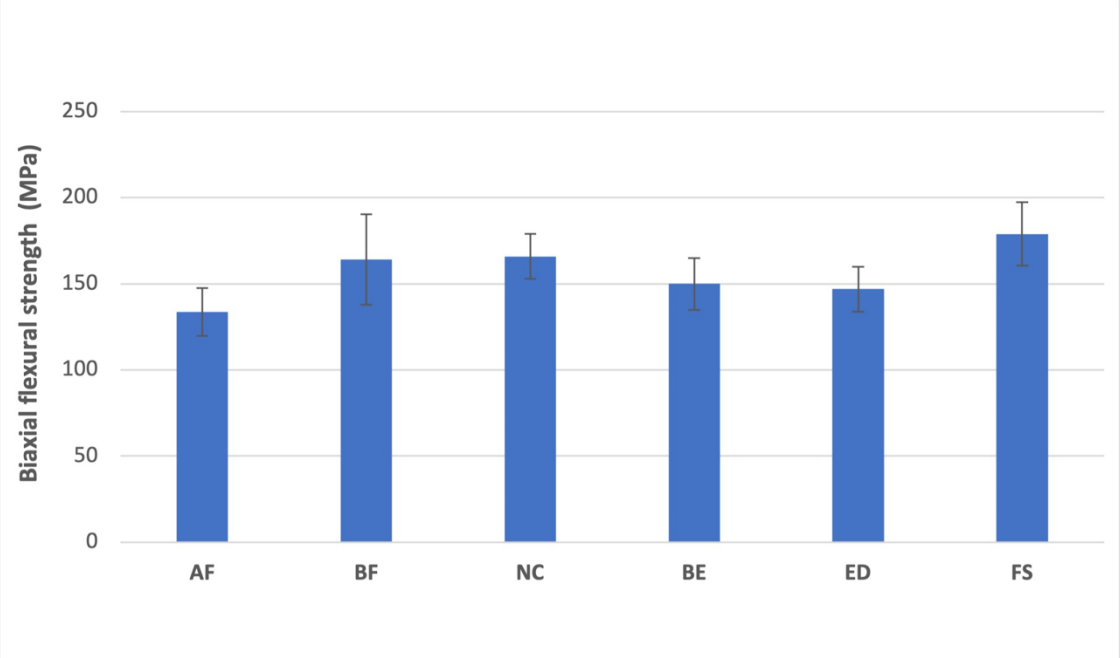

Figure 5. Material properties of the resin composite specimens: Biaxial flexural strength (MPa).

Table 4. Mean, SD values, and coefficient of biaxial flexural strength (MPa) for all groups.

MATERIAL	Flexural strength [MPa]	Coefficient (%)
AF	133.7±13.90	10
BF	164.0±26.30	16
NC	165.9±13.10	8
BE	150.0±15.00	10
ED	146.9±12.90	9
FS	178.9±18.50	10

1.3.2. Water sorption and solubility

The FS group exhibited the most significant water sorption (31.17 μg/mm^3), while the BF showed the lowest (12.23 μg/mm^3), as shown in **Table 5**. The water sorption of the materials showed no significant changes between 7 days and 36 days of immersion, **Table 5**. The AF group maintained the exact value of WS (18 μg/mm^3). After 20 days of storage, FS continued to exhibit the most significant water sorption (33.69 μg/mm^3). The BF group is the most hydrophobic resin, displaying the lowest water sorption (**Table 5**). The water sorption values of different groups are within the range of ISO standard [29] ($W_{sp} \leq 40$ μg/mm^3). Water solubility also showed differences within the groups (**Table 6**). FS was the material with the lowest mean water solubility value, followed by NC. Conversely, ED and AF were the materials that showed the highest solubility values. The water solubility values of different groups are remarkably lower than ISO guidelines [29] ($W_{sl} \leq 7.5$ μg/mm^3).

Table 5. Mean and SD values of water sorption for all groups for a total immersion period of 7 and 36 days.

MATERIAL	Water sorption (μg/mm³)		
	Brand	7 days	36 days
AF	13.4	18.19±0.93	18.16±1.03
BF	15.27	12.23±0.82	14.08±0.75
NC	not available	15.89±0.83	19.16±1.21
BE	15.1	15.80±1.55	18.02±0.76
ED	19.6	19.47±0.82	20.05±0.89
FS	not available	31.17±3.53	33.69±4.23

Table 6. Mean and SD values of water solubility for all groups.

MATERIAL	Water solubility (μg/mm³)	
	Brand	Study
AF	≤0.1	2.29±0.53
BF	0.31	1.55±1.10
NC	not available	1.28±0.53
BE	<0.1	1.48±0.75
ED	<0.1	3.48±1.88
FS	not available	1.11±0.40

The results obtained by Fick's law of diffusion (**Fig. 6** and **7**) show that the FS group (nanofilled resin) absorbs more water (1.6%) in the first seven days than the other groups (nanohybrid resins). However, after seven days, and for all groups, the water uptake rate is slower until it reaches a constant value. The amount of water that resin-matrix composite can absorb depends on the hydrophilicity of the polymeric matrix and the filler composition. It may be due to the decrease in sites where the water molecules can form hydrogen bonds with the material since they are formed between matrix and water. Additionally, the material has lower porosity due to the portion of the material occupied by inorganic filler, decreasing the water absorbed in the free volume of the polymeric chain of the resin matrix. In terms of diffusion coefficient (*D*), the group AF is the fastest in absorbing water and, the group NC is the slowest (**Table 7**).

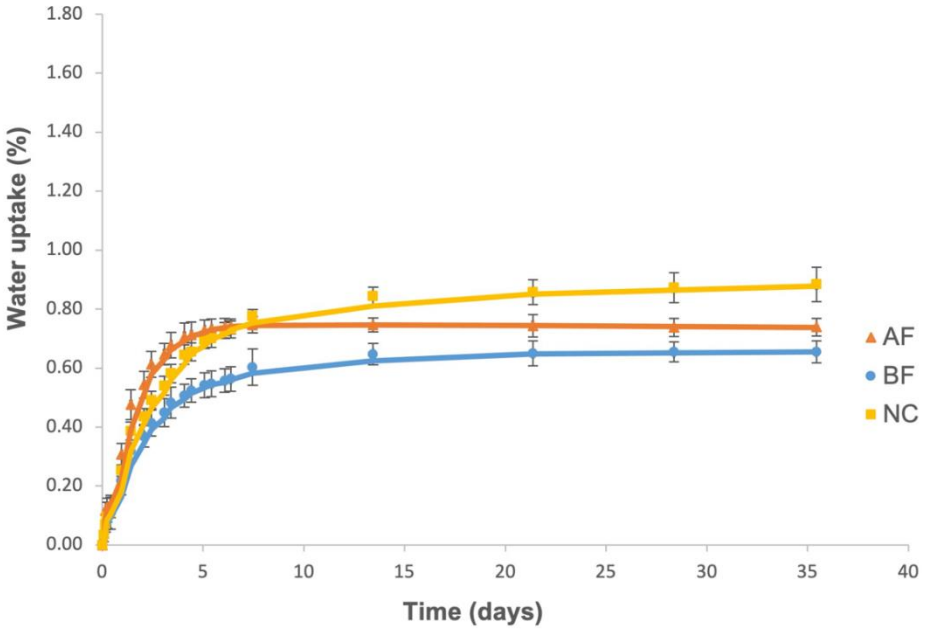

Figure 6. Experimental results and analytical model of the gravimetric test of resin groups without BPA (AF, BF, NC).

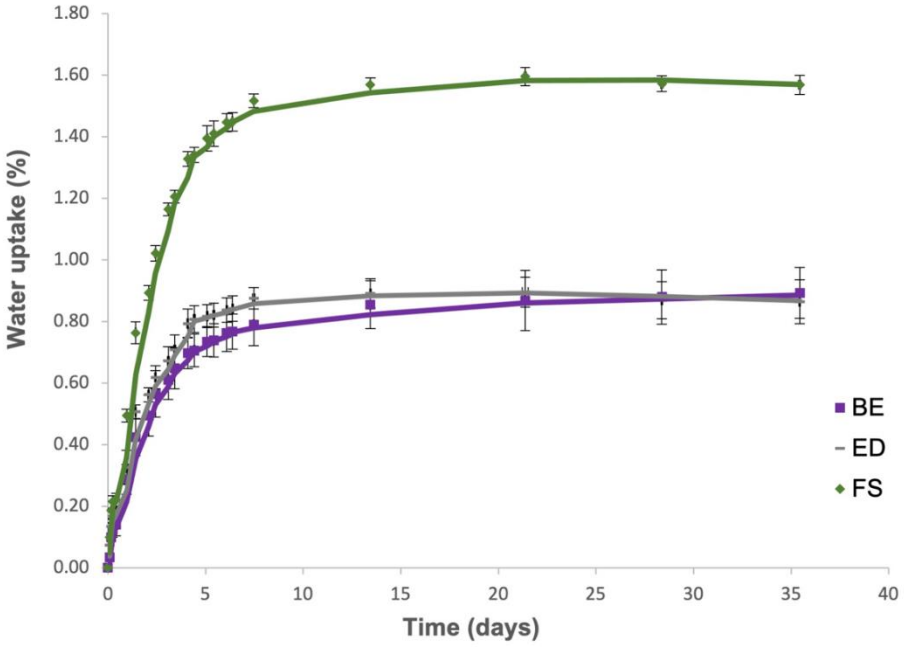

Figure 7. Experimental results and analytical model of the gravimetric test of resin groups with BPA (BE, ED, FS).

Table 7. Water uptake properties of the materials analyzed.

MATERIAL	D (m^2 /s)	M∞ (%)
AF	9.8E-13	0.63
BF	7.5E-13	0.57
NC	3.8E-13	0.84
BE	8.4E-13	0.59
ED	9.3E-13	0.83
FS	5.2E-13	0.12

1.3.3. Compressive tests

Mechanical properties results assessed compressive test are shown in **Fig. 8 to 10** and **Tables 8 to 10**. Regarding flexural strength (FS), after 7 days of wet storage, all groups were statically similar except AF and BF, which decreased their strength, and FS, which increased its strength (**Table 8**). After one year hydrated, the materials have maintained the same behavior they presented before water immersion (0 days). According to the tests performed, the effect of diffusion on the behavior of materials in compressive tests is practically negligible.

Results of statistical analysis of biaxial flexural strength and compressive strength are shown in **Fig. 5** and **8**. In both test methods, the resistance to flexural loading of the AF group was significantly lower than all other resin composites evaluated. Furthermore, the biaxial test method arrived at the same conclusions (FS>BF>NC>BE>ED>AF) except for BE group that had a lower result compared with the compressive test (BE>FS>NC>BF>ED>AF).

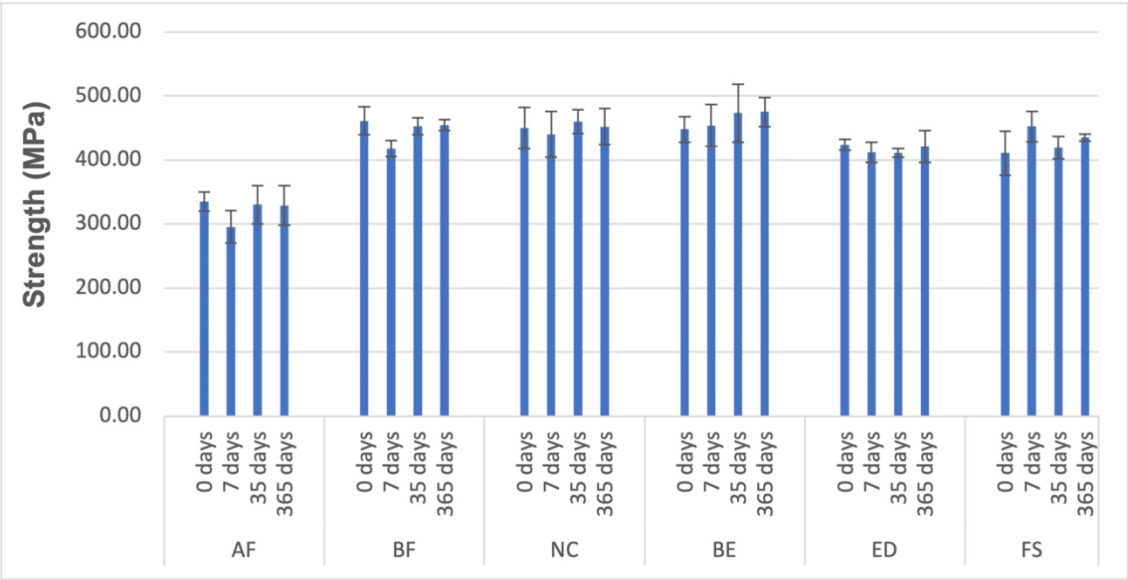

Figure 8. Material properties of the resin composite specimens: Strength (MPa).

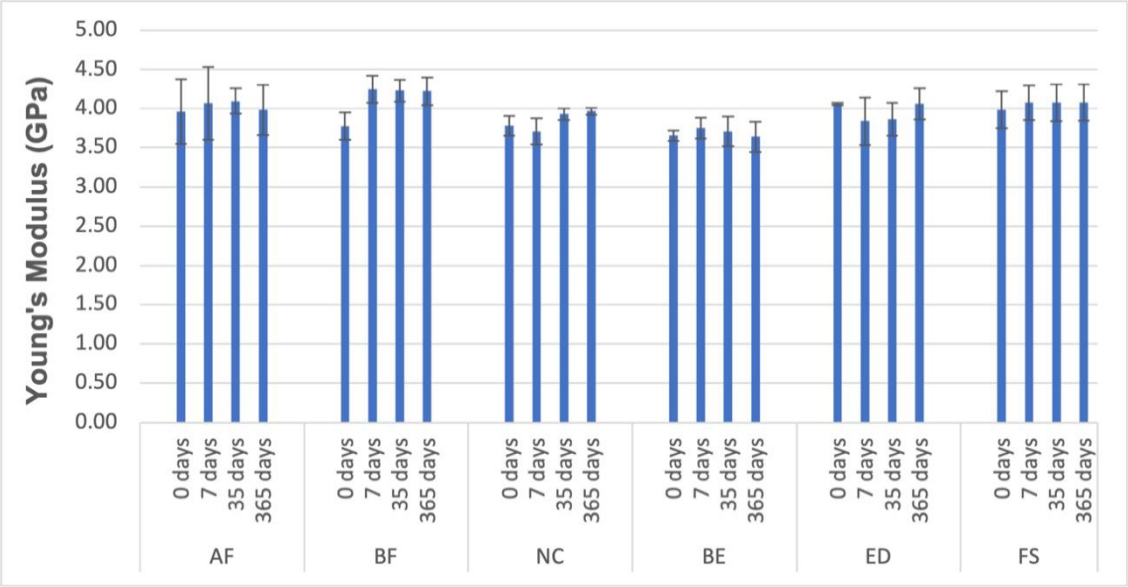

Figure 9. Material properties of the resin composite specimens: Young's Modulus (GPa).

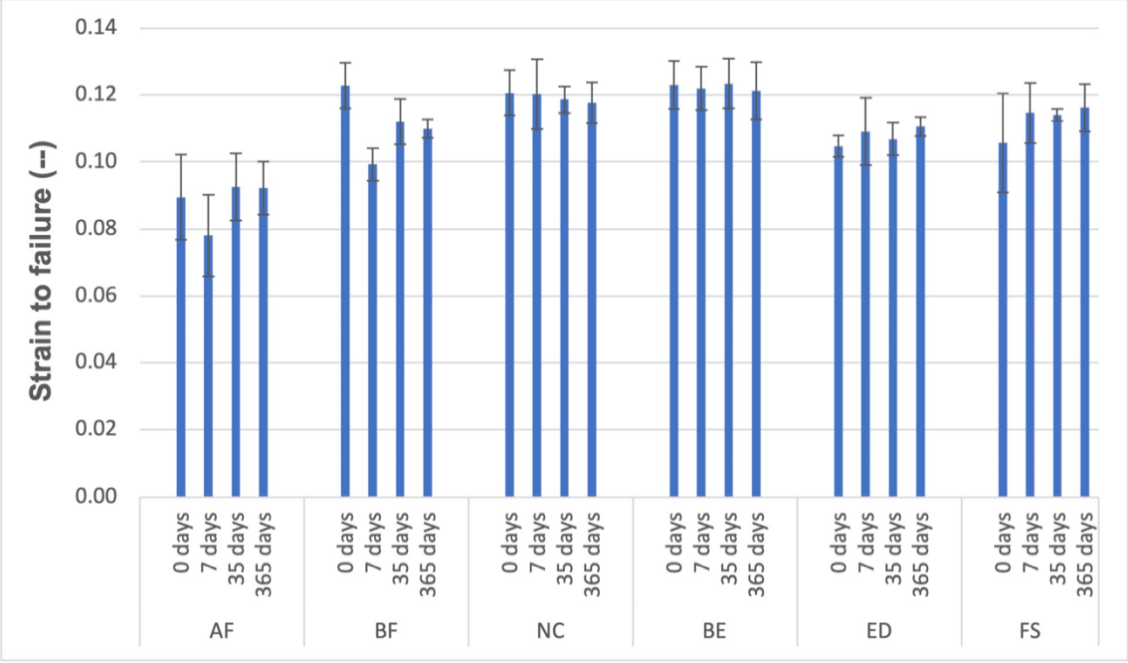

Figure 10. Material properties of the resin composite specimens: Strain to failure (--).

Table 8. Mean, and SD values of strength, young's modulus, and strain fail for all groups for a total immersion period of 7 days.

MATERIAL	Strength (MPa)	Young's Modulus (GPa)	Strain to Failure (--)
AF	295.55±25.45	4.07±0.47	0.08±0.01
BF	417.73±12.81	4.25±0.18	0.10±0.00
NC	440.52±35.82	3.71±0.16	0.12±0.01
BE	453.89±32.73	3.75±0.13	0.12±0.01
ED	411.87±16.04	3.84±0.31	0.11±0.01
FS	452.59±23.41	4.07±0.22	0.11±0.01

Table 9. Mean, and SD values of strength, young's modulus, and strain fail for all groups for a total immersion period of 36 days.

MATERIAL	Strength (MPa)	Young's Modulus (GPa)	Strain to Failure (--)
AF	330.11±29.58	4.10±0.17	0.09±0.01
BF	452.98±13.37	4.23±0.14	0.11±0.01
NC	460.05±18.91	3.93±0.08	0.12±0.00
BE	473.33±45.24	3.70±0.19	0.12±0.01
ED	411.10±6.70	3.86±0.21	0.11±0.00
FS	419.10±17.84	4.07±0.24	0.11±0.00

Table 10. Mean, and SD values of strength, young's modulus, and strain fail for all groups for a total immersion period of 1 year.

MATERIAL	Strength (MPa)	Young's Modulus (GPa)	Strain to Failure (-)
AF	328.93±30.65	3.98±0.32	0.09±0.01
BF	454.85±8.73	4.22±0.18	0.11±0.00
NC	452.32±28.46	3.96±0.04	0.12±0.01
BE	475.23±22.82	3.64±0.19	0.12±0.01
ED	421.25±24.70	4.06±0.20	0.11±0.00
FS	435.30±5.41	4.07±0.23	0.12±0.01

1.4. Discussion

Surface hardness has been applied as an indicator for the conversion of monomers.[21,31–34] A low value of hardness of a dental composite means a weak chemical/physical bond between the matrix and the filler interface.[32,35,36] The hardness of composites has been described with a correlation between how the organic matrix is obtained [37] and filler weight % [35,36,38,39] (**Table 1**), as we can see in this study (AF>BF>BE=ED>FS). For this reason, we choose to evaluate the compression testes by the irradiated face of resin discs.

As expected, materials made from hydrophilic monomers, such as resin-matrix composites, have high-water sorption. Dental materials' water sorption (WS) affects the longevity of dental materials applications in an aqueous environment. Because water intrusion can cause various adverse consequences, such as hydrolysis of the polymeric network,[40] reduced thermal stability,[41] impaired mechanical properties,[42] and elution of non-reacted monomers.[43] In addition, water solubility (WSL) indicates the quantity of non-reacted monomers released from the polymeric networks. The leaching of monomers is the primary source of cytotoxicity and tissue inflammation.[44] For these reasons, evaluating WS and WSL

of the resin-matrix composites under in vitro conditions with distilled water is essential. The results will be more effective than in saliva, where we could have other substances that could serve as a barrier to water absorption by the material. The FS group exhibited the most significant water sorption (31.17 μg/mm^3), while the BF showed the lowest (12.23 μg/mm^3), as shown in **Table 4**. These results are consistent with these reports by Kumar and Sangi,[45] which showed that nanofilled composite (FS group) compared with nanohybrid resin-like (AF, BF, NC, BE, and ED groups) has higher water sorption and solubility. It can be explained by the pores of the nanoclusters, which are prone to ion leaching and hydrolysis of the silane coupling agent, resulting in detachment and loss of the filler particles. Dental composites absorb water, slowly accumulating between the matrix and inorganic fillers. It may require up to 2 months until equilibrium is reached.[46,47] However, the water sorption of the materials showed no significant changes between 7 days and 36 days of immersion. Water uptake of fluorinated resins was much lower than that of Bis-GMA-based resin.[48] Therefore, the lower WS and water uptake of the BF group (**Figure 1** and **Table 5**) might be due to the fluorinated structure in their filler's composition (**Table 1**). Due to its lower WS, BF resin may have better water resistance than Bis-GMA-based resin.

Dental composites can be subjected to biting force and undesired loadings (dental injuries due to car accidents or sports-related injuries). The stress-strain curves of the studied materials are determined according to the results obtained, and the curves are used to determine Young's modulus. One reason for enhanced compressive strength in the FS (resin nanofiller) group could be the reduced inter-particle distance between the fillers, which decreases the tendency to form and propagate cracks. Furthermore, the smooth, rounded edges of the spherical nanoparticles lead to a more uniform distribution of stress across the composite resin, which is identical to the study reported by De Moraes *et al.*.[49]

According to Hooke's law, the elastic modulus (*E*), or Young's modulus, is the stress to strain ratio within the material's elastic range. Reveals the material's ability to store elastic energy related to recoverable elastic deformation. A stiffer material has a higher Young's modulus, i.e., the less elastic strain caused by the applied stress. High values were reported in the nanohybrid BF group, and the lowest was reported in the submicron hybrid BE group (**Figure 2**). BF proved to be considered the stiffest composite tested. The

significance of the elastic modulus is mainly concerned with selecting the appropriate composite for a specific clinical situation. An example, flowable composites are usually applied in class V restorations because their higher elasticity can absorb, at best, the mastication force in this particular tooth area. Hydrolytic degradation is at a constant rate. This diffusion effect process is determined by the type of polymer and filler particles and their surface treatment. In the present study, water aging did not cause a significant reduction in strength after immersion in water. When the resin matrix becomes saturated with water, the structure stabilizes, and no further decrease in properties is observed within the studied period. Carreiro et al. [50] also conducted a similar trial. They soaked different resin composites in distilled water for 180 days and tested their compressive strengths. They verified that the compressive performance of the same composite material did not change significantly. The difference in compressive strength among different types of composite materials could be due to the filler volume and the formulation of the resin. AF and ED resins, which had barium glass in their composition (**Table 1**), showed decreased flexural strength (**Figure 2**). Barium silicate glass may be related to the decrease in flexural strength as it was observed when encountered with water, the barium was exchanged with the hydrogen ion of the water, making the barium silicate glass sensitive to it.[51]

To summarize, according to all the parameters studied, we verified that the BF group is the one that presents the best overall chemical and mechanical behavior. It is a nanohybrid resin-matrix composite without BPA, which absorbs less water, presents a high strength, and with some flexural resistance compared to the other groups.

1.5. Conclusions

Within the limitations of this study, the following conclusions were drawn:

1- There are no significant differences between the groups of resins containing BPA in their composition and those free of this monomer and its derivatives. It can be stated that the fact that resins are free of BPA may not influence the chemical-mechanical performance of composite resins.
2- The AF group is a promising Bis-GMA-free and Ormocer®- based material, but it does not show comparable mechanical performance to conventional Bis-GMA-

containing resin-matrix composites. In addition, it appears that AF shows more susceptibility to mechanical degradation effects induced by water, especially at the early stages of diffusion. The inorganic matrix may have more influence than the organic matrix in the parameters studied, where BPA and its derivatives are found in its composition.

In the future, the ideal would be to develop a study in which the same methodology is applied, but with two groups of resins with the same composition in which the only difference would be the absence of BPA.

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Chapter V

FINAL REMARKS

1. Discussion

Patients with maxillary lateral incisor agenesis (MLIA) are commonly challenged with functional and esthetic problems at a young age, affecting their confidence and social relationships. Our clinical cases of MLIA (**Paper 1 and 2**-Chapter II) with orthodontic treatment (space closure with canine camouflage) required a complex multidisciplinary team. It is essential to realize that each patient is unique and needs an appropriate treatment plan. The approach to treating cases with lateral incisor agenesis is still, at present, the subject of debate. According to Kokich and Kinzer,[27] the treatment of choice should be the least invasive option that satisfies the expected esthetic and functional goals. However, the lack of scientific evidence to support the different treatment options [118] makes decision-making difficult. One of the latest published systematic reviews [40] suggests that the space closure option may be better than the opening option. However, it only addresses the specific limitations of each of the two treatment options, without considering other factors for opting for one or the other. There are a number of facial and dental criteria that should be evaluated when selecting a treatment option, such as malocclusion and amount of crowding; facial profile; shape and color of the canine; labial level; age of the patient; opinion of the parents or the patient himself; and the economic situation, among others. According to a retrospective study,[36] that compared the esthetic, functional, and periodontal results of the two types of treatment, it was observed that, while the esthetic objectives were satisfactory for patients in both cases, the periodontal health of patients treated with space closure was better than that of those treated with implants. In contrast, a second study that compared cases treated with space closure and with space opening and implant placement, concluded that the same quality and stability of esthetic and periodontal objectives can be achieved up to 5 years post-treatment.[119] In this way, the implants should be placed using 3D technology, particularly in cases where the periodontium is thick, so as to preserve 2 mm of vestibular bone.[120]

In **Paper 2** (Chapter II), the generally attempted treatment would be the opening of space in the agenesis area. However, the decision to open space in the posterior area was supported by factors such as the patient's young age, the hypodivergent biotype, the

presence of interincisor diastemas with bone defects, the anteroinferior crowding, and the molar Class I relationship. In cases like the presented one, where there was a high esthetic demand, it was important to consider not only the position of the teeth but also the gingival architecture.[121] In addition, the mesial migration of the canine helps to develop the alveolar bone at the congenitally absent lateral incisor place, as the bone around the canine will form in the position of the lateral incisor.[122–124] In this case (symmetrical bilateral MLIA), it resulted in an alternative treatment – closing of the anterior space and opening of the posterior spaces for an implant. Although it is a controversial issue, according to Dietschi and Schatz,[125] implant placement in children younger than 16 to 18 years must be avoided, or they will remain in infraclusion due to adjacent alveolar bone growth. Bohner [126] stated that implants must be placed when growth is almost complete. For this reason, the implant surgery on the present case was only performed at the age of 19.

Dental morphology and ideal tooth proportion were also important in the treatment plans (**Paper 1 and 2**, Chapter II). In cases of symmetrical bilateral MLIA (**Paper 1**-case 1; **and Paper 2**), the treatment option was supported by the fact that the canines had a shape and color favorable to space closure, *i.e.* , they were small canines with a smaller mesiodistal diameter, and by the fact that a slightly pronounced cusp fits better esthetically and functionally in the position of the lateral incisor. In this way, we were able to solve the lack of bone in the agenesis area and the esthetic problem. According to Levin, the golden proportion (*i.e.*, constant ratio of 1.618:1) can be a guideline for determining the width of the maxillary lateral incisor (MLI) and it was thus used in these cases.[127] However, the guideline was more relevant in **Paper 1**-case 2 as it was an asymmetric case involving unilateral agenesis and microdontia on a contralateral tooth.[127,128] Even so, it is somewhat inconsistent in terms of relative tooth width, because the golden proportion results from perceived tooth size observed from a frontal aspect, and thus had no relation with the teeth's real width. Another way to determine the amount of space to create for a MLI is Bolton analysis,[129] which we also used in all four cases (**Paper 1 and 2**). It is widely accepted that a diagnostic wax-up and a smile simulation provide the most effective and esthetic outcomes for patients, as performed on the asymmetric unilateral MLIA in **Paper 1**-case 2.

In **Paper 1**, case 2 (unilateral asymmetrical MLIA), the left upper canine restoration should

be more incisive to mimic the lateral incisor because it seems shorter than the right upper lateral incisor. In addition, it was necessary to compensate for the excess of Bolton's anterior dental discrepancy in the maxilla because the deciduous mandibular central incisors had a reduced mesiodistal dimension, and she had agenesis of the respective permanent teeth. For this reason, the premolars are not entirely in a Class II relationship (left side). To reduce the Bolton discrepancy, the premolars should be more mesialized and reduce the width of the left canine. Also, there should be increased space in the lower arch (central incisors' area) to rehabilitate in the future. However, our goal was to leave space to rehabilitate one of the lower central incisors, considering the hypodivergent biotype, agenesis, and the consequent loss of bone structure. For economic reasons, we opted to keep the deciduous tooth. However, the ideal would be a rehabilitation with implants if the bone volume is favorable, or a resin-bonded fixed partial denture (commonly referred to as a Maryland bridge).

In **Paper 1**-case 3, the patient presented with microdontia, which can represent a different expression of MLIA with a symmetrical outcome. A fundamental part of the overall treatment plan was the orthodontic treatment to correct not only the deep overbite and traumatic occlusion, but also the Bolton discrepancy due to microdontic mesiodistal maxillary lateral incisors. At the end of orthodontic treatment, the restorative phase was essential to correct that discrepancy and consequently achieve better esthetics.[130]

After orthodontic treatment, the patients were rehabilitated with a dental composite (**Paper 1 and 2**). Considering that these were young patients, direct restorations with a resin-matrix composite were chosen over an indirect restoration approach, because the treatment used was less expensive and did not involve any injury to the dental tissues.[43,51,52] An important benefit of this procedure over others is that the repair may be possible intraorally without the risk of modifying esthetics or mechanical performance.[57] Furthermore, anterior composite restorations have shown promising long-term clinical performance, with annual failure rates ranging from 0.0% to 4.1%.[131,132]

In **Paper 7** [105] revealed that clinicians should be aware of the type of resin-matrix composites and their exposure performance to dietary and therapeutic solutions. Even through the

limitations of an *in vitro* study, the restorative materials tested (AF, BE and FS) showed clinically noticeable color differences after their exposure to coffee and red wine solutions. Coca-Cola® and Eludril Care® were shown to have a lower influence on the color stability of resin-matrix composites. Moreover, patients must be warned of the chemical interaction between colored solutions and resin-matrix composites. For this reason, patients should be informed that restorations require periodic maintenance because the texture and shade of the material will change over time.

Most dental composites for direct restorations have Bisphenol A (BPA) and derivatives in their organic matrix composition. The results found in several studies expressed concerns on the use of BPA, because human exposure to BPA is widespread. The BPA molecule can be locally absorbed by the surrounding tissues (*e.g.*, gingival margin) and diffused into the bloodstream resulting in local and systemic toxicity. BPA is an endocrine disruptor and an estrogen receptor agonist that can cause toxicity, as validated in previous studies.[133–136] BPA derivatives also induce a similar endocrine disrupter effect. 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, BPA in food contact packaging has been banned in many countries in Europe.[137,138] Our results showed that considering the defined criteria in **Paper 3** (Chapter III), BPA might be released from dental resin-matrix composites at different amounts depending on the chemical composition of the restorative materials and the complexity of the oral environment. Although the popularity of resin-matrix composites has increased in recent years, the concern on the release of toxic substances such as BPA and derivatives has gathered attention by scientists, clinicians, and patients.[67,68,139] In this way, the industry has developed dental products, such as dental resin-matrix composites free of BPA and its derivatives.

It is necessary to note that the composites and sealants produced in UE (United European), Japan, and the USA (United States of America) must meet strict standards determining the maximum amount of BPA released under the conditions of the oral cavity. Few studies reported that the release of BPA and its derivative is hardly measurable.[68,139,140] However, due to the kinetic release of monomers from *in vitro* studies, the solvent's saturation could influence the monomers.[141] In the oral environment, the overall degradation of resin-matrix

composites can be progressive, and the saturation could never be reached due to the continuous removal of the monomers by the human saliva flow.[142] Considering the importance of this issue, our results in **Paper 4** (Chapter III) demonstrated that among the *in vitro* selected studies, just two had used a solution of 75% ethanol-water, half of them had used a methanol solution, another half used water. The United States Federal Drug Administration recommends a 75% ethanol-water solution as a clinically relevant food–oral simulating liquid, and it has been used in several studies.[143–147] In addition, methanol can be replaced with a mixture of 75% ethanol and 25% water to be more environmentally friendly. Another relevant piece of information was the positive level of BPA and its derivatives detected for two dental composites[145,148] presented as "BPA-free", Charisma Diamond (CD; Kulzer, Hanau, Germany) and Admira Fusion (AF; VOCO, Cuxhaven, Germany), although in significantly lower amounts than from Bis-GMA-containing composites. This could be explained by a false positive, an error occurred during the preparation of the sample[145,148] relative to the technique of polymerization, the time of polymerization, or even a failure in the protocol of polishing. Also, it could be linked with an exaggerated time of storage or with an inadequate container which must be in glass or a plastic "BPA free".

The results from review (**Paper 4**, Chapter III) showed the great majority of the *in vivo* studies had analyzed urine or saliva, and others studied blood. The level of BPA detected was higher in saliva than in urine and blood because of its proximity with a resin-matrix composite. The studies analyzing saliva with follow-ups indicate an increase of BPA or Bis-GMA between the moment just after the dental composite placement in the oral cavity and 24 hours after.[143,149–151] The use of a rubber dam to isolate the tooth during the dental composite placement significantly reduces the level of BPA detected in saliva.[149] That is one of the reason we adopt the use of rubber dam in our restorative protocol (**Paper 1 and 2**, Chapter II). Therefore, liquid chromatography with tandem mass spectrometry (LC-MS) as a suitable analytical was mainly used in the studies, others had used gas chromatography coupled with mass spectrometry (GC-MS), and a small portion used flow cytometry or was based on enzyme-linked immunosorbent assay (ELISA). The use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) provides a higher selectivity, low quantification limit, and unambiguous identification of the target compounds.

Based in these parameters we evaluated the elution of BPA and Bis-GMA monomers from six composite (three BPA and three without BPA) resin–matrix composites using sensitive LC–MS/MS quantification method and a 75% ethanol-water solution (**Paper 5**, Chapter III). To our knowledge, this is the first time that the resin groups without BPA (AF, BF and NC) were studied under these conditions. The results of the **Paper 5** indicate that BPA concentrations was null or below the LOQ (0.06 ng/mL) of the method. According to Nys *et al.*,[152] one possible explanation is the recent development of new filler technology that binds more free resin than standard fillers, which could hinder the release of monomers and BPA. Hence, the presence of a BPA-based monomer in the raw material does not necessarily indicate that BPA will be released in detectable amounts from this material after light-curing. In addition, this might point out the need for even more sensitive detection methods.[152] On the other hand, Bis-GMA was released in detectable amounts from all groups with BPA (Table 1, Chapter I) (ED> BE> FS) and from the NC group, an experimental resin BPA-free. As expected, the resins with BPA (G2) higher amounts were detected in the first few days, followed by a consistent lower release in the following days. The concentration of BPA-based monomers can explain the differences between the composites in the resin and their physicochemical properties (solubility in the 75% ethanol-water solution). There is a discrepancy between different studies, which can be explained by BPA concentration being too low for detection, either by actual absence of BPA or due to a minimal amount of BPA and BPA-based monomers in the resin. In our study (**Paper 5**, Chapter III), the sensitivity of our method of LC-MS analysis is reflected in the low LOQ (0.06 ng/mL), which has a higher sensitivity compared with other studies. However, it is not simple to make direct comparisons and draw conclusions because many studies present the results in mass units rather than concentration. Another limitation is that the differences in the composition of the resin-matrix composite could result in variability at the release of amount and type of monomers. These resin-based composites may be considered sources of BPA from degradation of Bis-GMA, which might be relevant in patients with multiple and extensive restorations like in cases reports in **Paper 1 and 2**, in Chapter II. The toxicity is mainly reported by BPA that comes from the degradation of BPA-based monomers. However, although studies focus mainly on BPA, Bis-GMA was quantified in levels of up to 33.46 ng/mL in the first day of monitoring and was detected even in the NC sample, an

experimental resin, called BPA-free (**Paper 5**, Chapter III). Release of Bis-GMA monomer should also systematically monitored due the toxicity associated to this monomer. [153–155] Continual monitoring *in vitro* and *in vivo* studies may be helpful to improve the quality of the resin composites and to stimulate, even more, the clinical application on BPA free composites used in dental treatments, especially in young patients with esthetic and functional rehabilitation, such as in cases maxillary lateral incisors agenesis (MLIA) (**Paper 1 and 2**, Chapter II).

The resin-matrix composites should be BPA-free, but it is equally important to keep their chemical and mechanical properties. It is known that a biomimetic approach would advocate similar mechanical properties of resin-matrix composites, such as the compressive strength, strain-to-failure, and the elastic or Young's modulus (*E*). **Paper 6** (Chapter IV), aimed to evaluate the chemical and mechanical properties of the same dental resin-matrix composites used in **Paper 5** (Chapter III) having different organic matrices, filler loading, and filler types under the same curing and testing conditions. This is the only study (**Paper 6**), as far as we know, that evaluated this resin-matrix without BPA. According to all the parameters studied, there are no significant differences between the groups of resins containing BPA in their composition and those free of this monomer and its derivatives. Therefore, it can be hypothesized that the fact that resins are free of BPA may not influence the chemical-mechanical performance of composite resins. The AF resin (G1) is a promising Bis-GMA-free and Ormocer®-based material, however AF resin shows more susceptibility to mechanical degradation effects induced by water, especially at the early stages of diffusion. In resin-matrix composite the inorganic matrix may have more influence than the organic matrix in the parameters studied, where BPA and its derivatives are found in its composition. In line with all the parameters studied, we verified that the BF resin is the one that presents the best chemical and mechanical behavior. It is a nanohybrid resin-matrix composite (without BPA), which absorbs less water, presents a high strength, and with some flexural resistance compared to the other groups. Unfortunately, we did not use it in our clinical cases because this resin was not being marketed in Portugal when we started.

Concerning the limitations of the studies performed, some of the tasks were adapted. At the beginning of the project, the goal was to use the same resins in the *in vivo* and *in vitro*

studies. However, when we initiated the clinical cases back in 2015, the only BPA-free resin-matrix composite being marketed in Portugal was AF resin. Over the final years of this thesis, new brands and BPA-free resins have emerged, and so, we decided to incorporate those into the project for the *in vitro* assays. Therefore, we ended up with different sets of resins for the *in vivo* and *in vitro* studies, but we feel the addition of new resins for the *in vitro* assays, really improved our work.

Thus, according to the work developed and described in the literature[27,40,118], we can state that in the case of young patients with MLIA, the orthodontic treatment option with space closure rehabilitated with resin composite may be the most indicated. Also, it is the most minimally invasive option compared with the orthodontic treatment option with space opening rehabilitation. However, in the case where the option is to space open, as in the clinical report of **Paper 1** (Chapter II), we can do it posteriorly, minimizing the impact of the problems of placing implants in the anterior sector[3-5]. This way, we eliminate adverse aesthetic effects in the short and long term.

2. Conclusions

In the context of a minimally invasive approach, this thesis aimed to recover esthetic disharmony due to MLIA after orthodontic space closure in young patients. Furthermore, we have studied, *in vivo,* the clinical performance, and *in vitro*, the chemical and mechanical properties of contemporary dental resin composites with and without BPA.

The main outcome of this work can be summarized as follows:

- Beginning at a young age, patients with MLIA can require complex multidisciplinary care. It is important to realize that each patient is unique and needs an appropriate treatment plan. This option proved to be viable, showing good results and eliminating any disadvantage of placing implants in the anterior area.
- 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.
- Clinicians should pay attention to the proper use of resin-matrix composite considering light-curing parameters and equipment for the polymerization and decrease of residual toxic monomers.
- It can be stated that the fact that resins are free of BPA may not influence the chemical-mechanical performance of composite resins. There are no significant differences between the groups of resins containing BPA in their composition and those free of this monomer and its derivatives.
- The inorganic matrix may have more influence than the organic matrix in the chemical and mechanical parameters studied, where BPA and its derivatives are found in its composition.
- According to all the parameters studied, we verified that the BF group is the one that presents the best chemical mechanical behavior overall. It is a nanohybrid resin-matrix composite (without BPA and derivates), which absorbs less water, presents a high strength, and with some flexural resistance compared to the other groups tested.

- A simple, sensitive, and accurate method to detect low levels of BPA and Bis-GMA, released from resin-based dental materials, in a 75% ethanol-water solution was developed. The results indicate that BPA release was below the LOD (0.06 ng/mL) of the method. However, Bis-GMA (LOD: 0.03 ng/mL) was quantified in levels of Bis-GMA up to 33.46 ng/mL (ED resin) in the first day of monitoring. Bis-GMA was detected even in the NC resin (total concentration released: 0.94 ng/mL), an experimental resin, called BPA free.

This thesis provides a thorough document that can be used by dentists as a way of differentiating different types of materials and learning about their chemical and mechanical properties. This renders this thesis as an important tool that will help any dentist in their choice of material to rehabilitate young patients with MLIA after orthodontic treatment with space closure.

3. Future perspectives

The presented work may be improved upon by studying additional topics, such as:

- Develop a new and more simple analytical method to facilitate the quantification of BPA and derivatives by resin-matrix composites in biological fluids (saliva and urine).
- Further *in vivo* studies are required to validate the localized and systemic toxicity of BPA and its derivatives released from resin-matrix composites on biological fluids.
- Develop a study in which the same methodology should be applied, but with two groups of resins with the same composition in which the only difference would be the presence or absence of BPA.

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