Biological effects of resin monomers on oral cell populations: descriptive analysis of literature

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Abstract

Aim Recently, the application of restorative materials containing metacrilate monomers in the conservative and paediatric dentistry has focused on the possible negative effects due to the use of these composites. In particular the release of monomers from reconstructions as a result of an insufficient polymerisation, can spread along the mucosal and dental tissues with potential immunological ed cytotoxic effects. Regarding to the importance of this issue, the aim of this study is to provide a descriptive review of the literature on potential local and systemic interactions of metacrylic and acrylic monomers with the immune system, both *in vitro* and *in vivo.*

Results The most highly used monomers in composite materials applied in conservative dentistry include: 2-hydroessietil-methacrylate (HEMA), triethylene glycol-dimethacrylate (TEGDMA), bisphenol A glycidyl-methacrylate (BisGMA) and urethane-dimethacrylate (UDMA). Different investigations have been performed for better understanding of the potential side effects of metacrylic monomers on immune system cells. Different factors such as cell population, exposure time and parameters more strictly connected to these materials, such as molecular weight, chemical composition and mechanical characteristics, seem to be directly involved in these reactions.

Introduction

In recent years the research in dentistry, particularly in the conservative branch, has focused on the development and application of materials such as resin composites, which can provide both preventive and aesthetic approaches, which can support not only the mechanical adhesion but also the chemical adhesion to the tooth surface. These materials are being used widely also in paediatric dentistry due to the aesthetic demand during childhood. Despite of mentioned advantages of these materials, the resin composites and their respective enamel-dentine adhesive systems can probably have some undesirable effects such as cytotoxicity, genotoxicity and stimulation of immune system. It should be mentioned that although these side effects are not still understood completely, there is a great interest to know, as reported in the recent literature [Geurtsen et al., 1998a, 1998b; Bationo et al., 2016]. It should be noted that resin composites are widely used materials, not only for conservative and endodontic purposes, but also in prosthetic and orthodontic dentistry for cementation or as temporary restorers or reshaping resins [Chieruzzi et al., 201; Chieruzzi et al., 2018]. Despite of their consolidated use, there are several doubts regarding the possible biological alterations of oral tissues, induced from residual monomers released from resin composite, because of their chemical composition [Gerzina et al., 1996; Spahl et al., 1998]. Evaluation of dental materials cytotoxicity on oral tissues, represent an important reference in the recent literature [Balloni et al., 2016; Pagano et al., 2019].

Composite materials

Resin composite contains acrylate and methacrylate monomers as their main constituents [Rakich et al., 1999; Heil et al., 2002; Goon et al., 2006; Aalto-Korte et al., 2007; Ansteinsson et al., 2011; Schmalz et al., 2011; Van Landuyt et al., 2011; Nocca et al., 2014]. Generally, they are formed by a polymeric matrix, a filler (inorganic particles such as crystalline quartz, pyrogenic silica, glasses of barium, zinc and strontium, zirconium, ceramic, lithium-aluminum-borosilicate), coupling agents (such as silanes), catalysts and inhibitors (such as camphorquinone and benzoyl peroxide) [Schmalz et al., 2009]. The matrix is formed by dimethacrylates, whose main

constitutive monomers are bisphenol A glycidyl-methacrylate (BisGMA) (PM: 513 g/mol) [Altintas et al., 2012] and urethane-dimethacrylate (UDMA) (PM: 470 g/mol). Both of them are highly viscous monomers and should be used with diluent monomers such as triethylene glycol-dimethacrylate (TEGDMA) (PM: 286.32 g/mol) and 2-hydroessietil-methacrylate (HEMA) (PM: 130 g/mol) [Stanislawski et al., 2003; Moharamzadeh et al., 2007]; having good hydrophilic properties HEMA can interact with cell membranes lipid bilayer [Schuster et al., 1996] and also perform lipid peroxidation *in vitro* [Fujisawa et al., 1984; Terakado et al., 1984].

Each monomer consists of three portions: the central part of the chain (or spacer group), which influences hydrophilicity, viscosity, volatility and mechanical properties, and the two poles of the chain that can polymerise with other monomers. According to characteristics of polar ends, monomers can be classified as functional (HEMA) and cross-linkers (TEGDMA, BisGMA and UDMA). A further classification is based on the spacer group polarity and affinity for H_2O, and distinguishes the hydrophilic monomers (affinity for H_2O molecules) from the hydrophobic monomers, that can be dissolved in non-aqueous organic solvents (such as acetone or alcohol).

Enamel-dentine adhesives

Adhesive systems are a group of substances that facilitate and promote the adhesion of composite materials to the hard tissues of the tooth [Pagano et al., 2019]. They are considered as amphiphilic compounds, since they contain hydrophilic chemical groups, useful for binding to the dental surfaces and hydrophobic chemical groups, which join to resinous systems. The adhesives are composed of three components: the enamel-dentin conditioner (or etching or etching agent), the adhesion promoter (or primer) and the fluid resin (or bonding). The etching agent is 37% orthophosphoric acid (H_3PO_4) and is able to create retentive microporosity on the tooth surface, by removing the organic components. The primer (HEMA in combination with 5% gluteraldehyde, or NPG-GMA and PMDM or NTG-GMA and 4Meta) penetrates between the collagen fibers by spacing them and promoting the infiltration of the fluid resin. The bonding (2.5% maleic acid or 17% EDTA) acts to infiltrate the dental substrate and to create a link with it, forming the hybrid layer and the resin interdigitations in the dentinal tubules (resin tags). The enamel-dentine adhesive systems are classified based on the marketing age (I-VIII generation) or the clinical procedures (three-steps, two-steps or one-step).

Polymerisation reaction

The reaction that leads to the formation of polymeric chains from the resin monomers, is a radical polymerisation reaction caused by the decomposition of the initiator which, after a physical or chemical activation, generates free radicals. The radicals react with a methacrylate monomer linked to another monomer, thanks to the presence of one reactive carbon, which starts a chain reaction, that ends when all the free radicals are exhausted. However, this polymerisation process does not involve the whole monomers and results in the release of some of them in to the oral cavity, during a variable period of time [Ferracane, 1995; Schmalz et al., 2011; Carol Dixon Hatrick, 2016]. The residual monomers can diffuse in the adjacent tissues and reach to the pulp cavity through dentin they can reach, where they can be reabsorbed and reach the peripheral circulation [Geurtsen, 1998b].

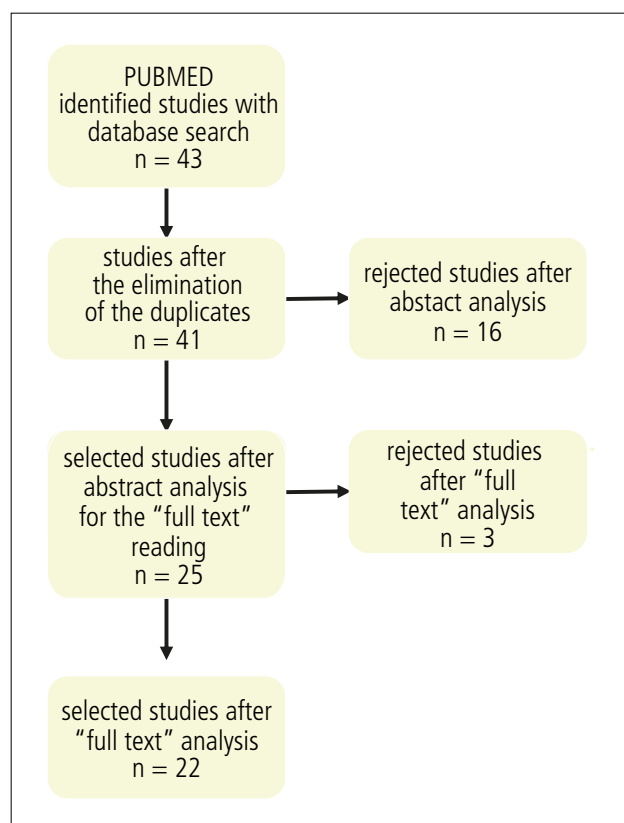The aim of our work is to report a descriptive analysis of

FIG. 1 Flowchart of the research protocol.

the recent literature based on the potential adverse effects of residual monomers on different cellular populations of the oral cavity.

Materials and methods

the aim of this work is to provide a descriptive review of the literature about the potential biological effects of the resin monomers including HEMA, TEGDMA, BisGMA, UDMA, EMA, EGDMA. Specific search strategy has been developed and performed in the electronic database PubMed/Medline during the last 10 years. According to the MeSH database, the chosen matching keywords were: ("hydroxyethylmethacrylate" [Supplementar Concept]) AND "Immune System" [MeSH], ("triethyleneglycoldimethacrylate" [SupplementaryConcept]) AND "Immune System" [MeSH], ("Bisphenol A -GlycidylMethacrylate "[MeSH]) AND" Immune System "[MeSH], ("urethanedimethacrylatelutingresin" [Supplementary Concept] AND "Immune System" [MeSH], ("ethyl methacrylate monomer" [Supplementary Concept]) AND "Immune System" [Mesh], ("ethylene dimethacrylate" [Supplementary Concept] AND "Immune System" [Mesh]. In this review we have included the following types of work: reviews; clinical and experimental studies; original articles in English. The exclusion criteria was represented by studies in a different language from Italian or English. The scientific method used for the identification of the research studies is related to the PICOS protocol, which uses an explicit and reproducible research methodology: Population (P): immune system cells; Intervention (I): resin monomers; Comparison (C):

Authors	Group I:	Group II:	Group III:	Group IV:	Group V:	Total	Reliability
and year of publication	Test substance identification (4)	Test system characterisation (3)	Study design description (6)	Study results documentation (3)	Plausibility of study design and results (2)		
Huang et al., 2015	4	3	4	3	2	16	Reliable without restrinctions
Bolling et al., 2013	4	3	5	3	2	17	Reliable without restrinctions
Eckhardt et al. , 2009	4	3	6	3	2	18	Reliable without restrinctions
Huang et al., 2014	4	3	5	3	2	17	Reliable without restrinctions
Kuan et al., 2013	4	3	5	3	2	17	Reliable without restrinctions
Kuan et al., 2012	4	3	6	3	2	18	Reliable without restrinctions
Ly et al., 2012	4	3	6	3	2	18	Reliable without restrinctions
Schweikl et al., 2014	4	3	6	3	2	18	Reliable without restrinctions

TABLE 1 ToxRTool results and scores with different criteria (test substance identification, test system characterisation, study design description, study results documentation, plausibility of study design and results) for evaluation of in vitro studies.

influences of cytotoxicity and of genotoxicity; Outcome (O): presumable cytotoxic and genotoxic effects; Setting (S): *in vitro* and *in vivo* studies [Maia et al., 2012]. This systematic review is referred to the PRISMA protocol (Fig. 1), that is a set of minimal evidence-based elements to provide a reference in systematic reviews and meta-analysis [Moher et al., 2010]. Furthermore, to evaluate the studies reliability about the cytotoxicity, we used the ToxRTool (Toxicological data Reliability Assessment Tool). This tool provide the criteria for the analysis of the quality of toxicological data and to determine the reliability of the selected articles.

The ToxRTool, developed in Microsoft Excel® software, comprises a list of 21 evaluation criteria for *in vivo* studies and a list of 18 evaluation criteria for *in vitro* ones. These criteria are divided in five groups:.

I. Test substance identification.
II. Test system characterisation.
III. Study design description.
IV. Study results documentation.
V. Plausibility of study design and results.

For each criteria the protocol assigns a score from '1' to '0', whether it is satisfied or not. The reliability of the studies is defined by the total score (a maximum of 21, in vivo, and of 18, in vitro), that result from the addition of the single values of the five group criteria. With a final score of 13 (*in vivo*) and 11 (*in vitro*) or lower the studies are considered unreliable (Table 1).

Results

The initial consultation of the bibliographic metadata in PubMed generates a total of 43 studies with the following combination of matching items.

- 20 studies with the combination of "HEMA" and "Immune System".
- 11 studies with the combination of "TEGDMA" and "Immune System".
- 12 studies with the combination of "BisGMA" and

"Immune System".
- 0 studies with the combination of "UDMA" and "Immune System" in the last 10 years.
- 0 studies with the combination of "EMA" and "Immune System" in the last 10 years.
- 0 studies with the combination of "EGDMA" and "Immune System" in the last 10 years.

Among this research, the studies that were repeated in the different combinations of keywords were removed.

All the titles and the abstracts of the articles retrieved in this research were analysed and selected, according to the eligibility criteria, by two different reviewers. After the reading of the abstracts, 16 studies were rejected, as they analysed other information that were not strictly relevant to the study. The rest of them, according to the inclusion criteria and the applied protocols, were selected for the qualitative analysis, so that the "full text" reading can confirm their eligibility.

The critical analysis of the "full text", following the inclusion and exclusion criteria, led to the selection of 22 studies, 9 of which are related to the effects of the HEMA, 4 of them investigate the TEGDMA, 7 of them the BisGMA and 2 of them refer to the use of the TEGDMA and the HEMA. The studies were analysed and divided according to the basic monomer.

Analysis of the collected information was performed using Microsoft Office Excel 2007 as shown in Table 2.

Hema

The analysis of the 11 studies concerning the HEMA shows the following.
• 5 studies were conducted on the murine macrophage cell line RAW 264.7.

The HEMA on these cells is able to induce apoptosis, increasing oxidative stress induced by an excess of reactive oxygen species (ROS) [Gallorini et al., 2015; Schweikl et al., 2016; Schweikl et al., 2017], or through the activation of the intrinsic p53-dependent and ATM-dependent pathway that causes cell cycle delays and DNA damage [Schweikl et al., 2014]. HEMA is also able to influence the innate immune

Author/s year of publication	Tested monomer	Cell type	Test	Results
Schweikl et al., 2017	HEMA	Murine macrophages, (cell line RAW264.7) *in vitro*	Flow cytometry for ROS identification; Flow cytometry for apoptosis (FITC/PI); Western Blot for SOD-1; GPx1/2; catalase; HO-1.	Hema induces apoptosis by excess of ROS, which affects apoptosis, and induces the expression of catalase, that reflects an oxidative stress.
Schweikl et al., 2016	HEMA	Murine macrophages, (cell line RAW264.7) *in vitro*	Flow cytometry for apoptosis (FITC/PI); Western Blot for NOS, p47, p67, catalase, HO-1.	Hema reduces cell survival. with LPS (trigger pathway) it can induce apoptosis and interfere with the physiological response of tissue, causing an increase in oxidative stress.
Gallorini et al., 2015	HEMA	Murine macrophages, (cell line RAW264.7) *in vitro*	Flow cytometry for ROS identification; Flow cytometry for apoptosis (FITC/PI); Western Blot for Nrf2, Keap1 enzymatic antioxidants.	Hema determines ROS increase, oxidative stress and cell death by apoptosis. It modulates the Nrf2-mediated cellular response, which sensitizes cells to monomers.
Schweikl et al., 2014	HEMA	Murine macrophages, (cell line RAW264.7) *in vitro*	MTT assay; Flow cytometry for ROS identification;Flow cytometry for apoptosis (FITC/PI); Cell cycle analysis; Western Blot for catalase, GPx1/2 e SOD1, ATM, H2AX, p53, p21, PARP-1, LAMIN A/C.	Hema induces apoptosis through the ATM-dependent and p53-dependent pathway and influences the increase in the oxidative stress, associated with the cleavage of pro-caspase 3 (executor of apoptosis).
Alizadehgharib et al., 2018	HEMA	Human monocytes (THP-1 cell line), peripheral blood lymphocytic tumors (primary cell culture) *in vitro* and BALB / c mice *in vivo*	ELISA for IL-1β ed IL-18.	Hema determines the increase in IL-1ß and IL-18 and induces the formation of the NLRP3 inflammasome.
Andersson et al., 2011a	HEMA	BALB / c mice *in vivo*	ELISA for TNF-α, IL-6, anti-OVA IgG e anti-OVAIgM.	Hema induces selective effects on the production of cytokines and antibodies in mice. It also induces IgG production. Mice, after two times exposition, produced less TNF-α in relation to IL-6, compared to controls. Immunisation of mice with OVA / HEMA caused an increased anti-OVA IgG activity compared to anti-OVA anti-IgM activity, compared to controls.
Andersson et al., 2011b	HEMA	BALB / c mice *in vivo*	ELISA for IL-2, TNF-α, anti-OVA IgG, IgM e IgA.	A long-term exposure to minimal amounts of Hema causes decreased production of IL-2 compared to controls.
Andersson et al., 2010	HEMA	Human lymphocytes from peripheral blood (primitive cell culture) *in vitro*	Proliferation assay (thymidine-3H); ELISA for IgG1, IgA, IgM.	Minor concentrations of Hema significantly increase IgG1, but not IgM and IgA, and do not affect cell proliferation. Higher concentrations suppress IgG1 and IgM production and proliferation.
Pawlowska et al., 2010	HEMA	Human lymphocytes from peripheral blood (primitive cell culture) and lung cancer cells (A549 cell line) *in vitro*	Flow cytometry for vitality (PI);Electrophoresis for DNA damages identification; Comet assay;Flow cytometry for apoptosis (FITC/PI); Cell cycle analysis.	Hema induces adverse biological effects, mainly through ROS, with DNA damage concentration dependent, apoptosis and cell cycle delay (at G0 / G1).
Huang et al., 2015	TEGDMA	Murine macrophages, (cell line RAW264.7) *in vitro*	MTT assay; Flow cytometry for apoptosis (FITC/PI); Cell cycle analysis; Electrophoresis for DNA fragmentations; Comet assay; Fluorescence microscopy for nucleus counts; Fluorimetry for caspase-3, -8, -9; Western Blot for caspase -3, -8, -9.	TEGDMA induces cytotoxicity and genotoxicity probably through DNA damage and caspase activation.
Krifka et al., 2010	TEGDMA	Murine macrophages, (cell line RAW264.7) and human cells derived from the pulp (primary cell culture) *in vitro*	Flow cytometry for annexin V-FITC; Western Blot for pERK1/2, p38, pJNK; Flow cytometry for apoptosis (FITC/PI); ELISA for TNF- α, IL-6, IL-10.	Tegdma is related to MAPK activation, inhibition of cytokine release and induction of apoptosis and necrosis.
Eckhardt et al., 2009	TEGDMA	Murine macrophages, (cell line RAW264.7) *in vitro*	Vitality assay (Crystal violet); ELISA for TNF-α, IL-6, IL-10; Flow cytometry for CD14, CD40, CD54, CD80, CD86, MHCI e MHCII.	TEGDMA reduces the production of cytokines and the expression of surface antigens and prevents the development of a competent cellular response.

TABLE 2A. The table collects all the general information about the investigated studies including: authors, publication years, tested monomer, cell types, experimental methods, performed tests and results.

Author/s year of publication	Tested monomer	Cell type	Test	Results
Harorlı et al., 2009	TEGDMA	Human monocytes (THP-1 cell line) *in vitro*	Optical microscopy for apoptosis, necrosis and mitotic phases.	Concentrations of 4 mM and 8 mM of TEGDMA inhibit cell proliferation. Cell viability decreases with increasing concentration. All concentrations have apoptotic and toxic effect on THP-1.
Huang et al., 2016	BisGMA	Murine macrophages, (cell line RAW264.7) *in vitro*	Cytotoxicity assay (LDH); Flow cytometry for apoptosis (FITC/PI); Comet assay; Fluorimetry for caspase -3, -8, -9.	BisGMA induces dose and time dependent cytotoxicity, influenced by the activation of caspases.
Kuan et al., 2013	BisGMA	Murine macrophages, (cell line RAW264.7) *in vitro*	Cytotoxicity assay (LDH); ELISA for PGE2; Western Blot for COX2, cPLA2, ERK, MEK, p38, JNK, cJUN; Fluorimetry for caspase-3, -8, -9.	BisGMA stimulates the production of PGE2 with the expression of COX2 and the phosphorylation of cPLA2 and MAPK.
Kuan et al., 2012a	BisGMA	Murine macrophages, (cell line RAW264.7) *in vitro*	ELISA for IL-1β e IL-6; Fluorimetry for ROS; Nitrite assay; Western Blot for iNOS, p65, IkB, NFkB, Akt, p-p65 (Ser536) e p-Akt (Ser473).	BisGMA induces pro-inflammatory activation of NFkB via PI3K / Akt pathway and the increase in IL-1β, IL-6 and nitric oxide. It also increases the expression of iNOS and intracellular and extracellular ROS in a dose and time dependent manner.
Kuan YH et al., 2012b	BisGMA	Murine macrophages, (cell line RAW264.7) *in vitro*	MTT assay; ELISA for TNF-α; Fluorimetry for CD14, CD40, CD45, CD54, CD80, MHCI, MHCII.	BisGMA induces a dose-dependent cytotoxic effect. It increases the amount of TNF-α and surface antigens.
Li et al., 2012	BisGMA	Murine macrophages, (cell line RAW264.7) *in vitro*	Cytotoxicity assay (LDH); Flow cytometry for apoptosis (FITC/PI); Fluorimetry for caspase 3, 8 e 9; Western Blot for caspase 3, 8 e 9; Electrophoresis for DNA fragmentation; Optical microscopy for nucleus count; Cell cycle analysis	BisGMA induces cytotoxic effect, with apoptosis and necrosis, through activation of Caspases-3, -8 and -9 in a dose and time dependent manner. It has a genotoxic effect, through the increase of the number of DNA strand breaks in a dose dependent manner.
Marovic et al., 2014	BisGMA	Human lymphocytes from peripheral blood, (primary cell culture) *in vitro*	Comet assay.	BisGMA shows genotoxicity with dose and time dependent damages.
Drozdz et al., 2011	BisGMA	Human lymphocytes from peripheral blood (primary cell culture), human cells of acute lymphoblastic leukemia (CCRFCEM cell line), and E. Coli (strain DH5a *in vitro*	Vitality assay (TO/PI); Comet assay; Cell cycle analysis; Electrophoresis for plasmid determination.	BisGMA determines genotoxic effect through oxidative modifications of the DNA bases and through DNA double-strand breaks, responsible for a delay of the cell cycle in the S phase.
Bølling et al., 2013	TEGDMA and HEMA	Murine macrophages, (cell line RAW264.7) *in vitro*	MTT assay; Western Blot for CD14, p-p38, ERK1/2, p-JNK, p-IkB-α, p-NFkB-p65, IL-1β; PCR for TNF-α, IL-1β; ELISA for IL-1β e TNF-α.	HEMA and TEGDMA, at low concentrations, can cause inappropriate cellular responses up to 24 hours after the exposure.
Ginzkey et al., 2015	TEGDMA and HEMA	Human lymphocytes from peripheral blood (primary cell culture), *in vitro*	Comet assay Fluorescence microscopy for nucleus counting; Cell cycle analysis; Optical microscopy for chromosomal alterations; Flow cytometry for apoptosis (FITC/PI).	TEGDMA at 100 µM induces slight decrease in vitality. It induces genotoxic effects at 1mM and a100mM. And it also induces a detectable frequency in CAs and SCEs in a dose dependent manner.

TABLE 2B. The table collects all the general information about the investigated studies including: authors, publication years, tested monomer, cell types, experimental methods, performed tests and results.

system and to modulate the pathway using the redox-sensitive transcription factor Nrf2 [Gallorini et al., 2015]; the HEMA can reduce IL-1β up to 24 h after the exposure of mentioned cells and can interfere with post-transcriptional regulation of cytokine synthesis and release induced by the LPS [Bø' lling et al., 2013].

• 1 study was performed on human monocytes THP-1 cell line and human lymphocytes isolated from peripheral blood (PBMCs).According to the obtained data of these studies, HEMA can influence the immune system by the up-regulation of IL-1β and IL-18 and also by the formation of the NLRP3 inflammasome. The same results have also been demonstrated in *in vivo* investigations performed on BALB/c mice [Alizadehgharib et al., 2018].

• 3 studies have evaluated the effects of HEMA on mice immune system *in vivo*.

In an investigation performed by Andersson et al. [2011a, 2011b] it has been stated that the HEMA can act as an immunological adjuvant by inducing the IgG antibodies production, with selective effects on production of pro-inflammatory cytokines like TNF-α and IL-6. In contrast, long term exposure to low concentrations of HEMA suppresses immunological functions. Another study highlights that HEMA monomers can influence the immune system by increased production of IL-1β and IL-18 and the formation of the NLRP3 inflammasome [Alizadehgharib et al., 2018].

• 1 study was conducted on human B lymphocytes.

Another study by Andersson et al. [2010] showed that the HEMA, at high concentrations, can significantly suppress the production of IgG1, IgM and cell proliferation.

• 1 study was conducted on human lymphocytes isolated from peripheral blood (PBMCs) and on the lung tumor cell line A549.

Pawlowska et al. [2010] stated that HEMA induces high production of ROS, causing DNA damage, apoptosis and cell cycle delays.

• 1 Study was conducted on human peripheral blood lymphocytes (PBMCs).

Ginzkey et al. [2015] stated that HEMA has a genotoxic effect and causes clastogenic responses at low concentrations.

Tegdma

The analysis of 6 TEGDMA studies showed the following.

• 4 studies were conducted on murine macrophage cell line (RAW 264.7).

TEGDMA can have genotoxic effects presented by DNA fragmentation as a result of apoptosis, and also can increase sub-G0/G1 phase in a concentration dependent manner, which can promote the activation of caspases-3, -8 and -9 [Huang et al., 2015], and reduces the release of IL-1β and TNF-α induced by LPS [Bølling et al., 2013]. In another study, elevated amount of apoptosis or necrosis and the decrease in TNF-α was reported, which was accompanied by the reduction of IL-6 and IL-10 and activation of MAPKp38, JNK, and ERK1 / 2 in a time-dependent manner [Eckhardt et al., 2009; Krifka et al., 2010; Bølling et al., 2013]. Furthermore, TEGDMA has been shown to inhibit the ability of an appropriate immune response by reducing not only cytokine release but also the expression of surface antigens CD14, CD40, CD80, CD86, CD54, MHC I and MHCII [Eckhardt et al., 2009].

• 1 study was performed on human peripheral blood lymphocytes (PBMCs).

TEGDMA at low concentrations can induce clastogenic responses thus determining a genotoxic effect, while at high concentrations it can influences cells [Ginzkey et al., 2015].

• 1 study was conducted on the human monocyte cell line THP-1.

Harorli et al. [2009] stated that the cytotoxic effect of the TEGDMA inhibits cell proliferation in a concentration dependent manner, proving its cytotoxic effect.

Bis-GMA

From the analysis of the 7 studies concerning the BisGMA effects, the following results were obtained.

• 5 studies were conducted on the murine macrophage cell line RAW 264.7.

According to the literatures, the BisGMA monomers can stimulate prostaglandin E2 production through the expression of cyclooxygenase-2 (COX2), phosphorylation of cytosolic phospholipase A2 (cPLA2) and mitogen-activated protein kinases (MAPKs). Furthermore, BisGMA in a dose and time dependent manner induces cytotoxicity as a result of activation of caspases caused by the phosphorylation of the cPLA2 and the MAPKs families [Kuan et al., 2013]. The same authors have previously shown that BisGMA, in a dose and time dependent manner, can activate NFkB via PI3K/Akt pathway, which result in an increased production of inflammatory mediators such as IL-1β, IL-6, nitric oxide, increased expression of iNOS, intracellular and extracellular ROS [Kuan et al., 2012a] and surface antigens [Kuan et al., 2012b]. Furthermore, they showed that BisGMA influences an appropriate macrophagic immune response, through an increase of TNF-α.

A study designed by Li et al. [2012] showed that BisGMA can activate caspases-3, -8 and -9 (cytotoxic effect) and determines modifications of the DNA structure (genotoxic effect). Furthermore, the authors have shown that increasing concentrations of BisGMA can cause cell death varying from apoptosis to necrosis. The cytotoxicity and genotoxicity of the BisGMA were also confirmed by Huang et al. (2016): their study revealed that these responses are inhibited by a pre-treatment with Wogonina, a natural flavonoid with an inflammatory, antioxidant and neuroprotective properties which is able to suppress the activation of caspases-3 and -8.

• 1 study was conducted on human peripheral blood lymphocytes (PBMCs).

This study was evaluated the genotoxicity induced by the simultaneous use of resinous composites and adhesive systems containing BisGMA. Both composition and concentration of the materials influence the genotoxic effect [Marovic et al., 2014].

• 1 study was conducted on human peripheral blood lymphocytes (PBMCs) and on a human acute lymphoblastic leukemia cell line (CCRF-CEM). This study showed that the BisGMA determines a genotoxic effect even on a leukemic cell line, causes oxidative changes in the bases of DNA and DNA fragmentation, which are potentially responsible for the prolongation of the S phase of the cell cycle [Drozdz et al., 2011].

Discussion

After a precise analysis of the literature, concerning local and systemic effects of monomers released by resin-based dental composites and dental-enamel adhesives, this descriptive review focused on the potential effects of these substances on the immune system cells.

In a study by Alto Korte et al. (2007), the effects of allergy

patches on dentistry staffs, who worked in the Finnish Institute of Occupational Health between years of 1994 and 2006 were evaluated. The results of this study showed that exposure to resin-based monomers can result in a contact-induced allergic dermatitis. This investigation showed that HEMA is the main allergen affecting nurses and dentists, prosthodontists were affected mostly by EGDAM and finally BisGMA and EMA were indicated as the main monomers affecting patients; additionally, Bando et al. [2014] discovered that HEMA, instead of carrying out an antigenic function, had the peculiarity of being an immunologic adjuvant in the contact-nickel-induced allergic dermatitis in mice.

It is widely known that, once introduced in the oral cavity, resin-based monomers can spread via three ways: by ingestion into the gastrointestinal system, by inhalation into the lungs and by diffusion into the endodontium.

With regards to systemic effects, Hagberg et al. [2005] identified HEMA as one of the probable agents causing occupational pulmonary diseases in dentists while Piirila et al. [1998] pinpointed a relation between acrylate monomers and the onset of a probable non-IgE mediated respiratory hypersensitivity.

The study performed by O'Brien et al. [2014] showed the correlation between Bisphenol A (BPA, main component of BisGMA) and the pathogenesis of asthma in adult mice, by stimulating the mast cells to produce and release proinflammatory mediators which will cause a potential allergic reaction.

Evaluation of local effects showed that once these monomers in the form of restorative materials entered the oral cavity of mice, they can reach the dental pulp, inhibit the odontogenic differentiation and interfere the odontoblasts function [Kwon et al., 2015], furthermore these monomers can have pathological alterations on not only the oral mucosal cells but even on the lingual muscle cells [Schmalz et al., 2011; Davydenko et al., 2018]. Moreover, the potential carcinogenic effect of monomers, finds no unanimous and definitive confirmation in literature, despite it can be found in several scientific publications. More in details, Jontell et al. [1998] and Kleinsasser et al. [2006] showed that exposure to high concentration of monomers can cause modifications in the DNA strands, which could be considered as a risk factor for the development of tumors in human salivary glands.

Many studies show the cytotoxic and genotoxic effects of resin-based monomers such as TEGDMA, HEMA and BisGMA, both in vitro and in vivo. The exposure of cells to these monomers could induce apoptosis, inhibition of proliferation or fragmentation of DNA strands. Furthermore, it has been reported that resin-based monomers, having an effect on all the immune system cells located in the oral cavity, the dental pulp and the epithelium [Jontell et al., 1998], could alter innate and adaptive responses, aggravating systemic and local reactions.

Obviously, in parallel to the effects of monomers, the presence of commensal and pathogenic bacteria in the oral cavity could not be ignored. Especially in those conditions which alter the normal equilibrium of the oral microbiota, bacteria and their toxins, such as the LPS of gram-negative bacteria (pathogens much indicative in deep tooth decays) can cause the onset of inflammation of dental pulp.

It is important to underline that in vivo research have been conducted on BALB/c mice, while in vitro studies have been carried out on different cell types: 13 studies were done on the mouse macrophages cell line (RAW264.7) and 7 on primary culture of human dental pulp cells. The mechanisms by which the monomers can alter cell behaviour have been recognised in different studies, for example BisGMA, operating via activation of caspases (caspase-3, -8 and -9), in mouse macrophages, can induce cell death in form of apoptosis or necrosis [Huang et al., 2015] based on the applied concentration. Moreover, BisGMA stimulates the production of prostaglandin E2 (PGE2), the expression of cyclooxygenase 2 (COX2) and the phosphorylation of the cytosolic phospholipase A-2 (cPLA2) and the MAPKs [Kuan et al., 2013].

It was also noted that BisGMA induces the activation of the transcription factor NF-κB with consequent degradation of IκB and phosphorylation of Akt with activation of mouse macrophages and following increment of TNF-α, nitric oxide, inflammatory cytokines (such as IL-1β and IL-6), expression of iNOS and surface antigens (such as CD11, CD14, CD45, CD54, CD40, CD80 and MHC II) and production of intracellular and extracellular ROS in a dose-dependent pattern [Kuan et al., 2012a, 2012b].

Compared to the BisGMA, HEMA monomers can induce apoptosis in mice macrophages by accelerated production of reactive oxygen species (ROS) and increasing the expression of catalases, proving the presence of oxidative stress (hydroxyl radicals, hydrogen peroxide and superoxide anion) [Schweikl et al., 2016; Schweikl et al., 2017]. Moreover, HEMA induces apoptosis via ATM-depending activation of P53 which is followed by oxidative damage at the DNA level and activation of pro-caspase-3 [Schweikl et al., 2014]. Increased ROS levels can throw oxygen homeostasis out of balance, and this alteration of the cellular oxidation-reduction balance is not only a cause for cellular death, but also one of the main mechanisms altering vital functions of macrophages (such as differentiation, mineralisation, etc.) and innate and adaptive immune responses. It is the cell-mediated response which is capable to inhibit the oxidative stress and support cell viability [Gallorini et al., 2015].

Different studies have shown that exposure of mice macrophages to TEGDMA can result in the imbalance of cellular oxide-reduction and inhibition of the immune response which means cessation of cytokines production and expression of surface antigens like CD14, CD40, CD80, CD86, CD54, MHC I and MHC II [Eckhardt et al., 2009].

TEGDMA, in particular, can reduce the release of IL1-β and LPD-dependent TNF-α, while HEMA only reduces IL-1β and pro-IL-1β. Co-exposition to HEMA and TEGDMA has an additive effect, however monomers do not interfere with the transcription process, but with the post-transcriptional regulation [Bølling et al., 2013]. Above all, TEGDMA can reduce the expression of TNF- , IL-6 (both pro-inflammatory) and anti-inflammatory IL-10 [Eckhardt et al., 2009; Krifka et al., 2010].

As far as the cytotoxic effect of resin-based monomers on human monocytes (THP-1) is concerned, increments in the concentration of TEGDMA reduce cell viability, with possible apoptotic effects unrelated to the concentration of the monomer [Harorli et al., 2009]. HEMA in monocytes affects the immune system instead, inducing the formation of NLRP3 inflammasome with consequent increase of IL-1β and IL-18 [Alizadehgharib et al., 2018].

On human lymphocytes, TEGDMA at high concentration is able to decrease the cell viability [Ginzkey et al., 2015], while HEMA in low concentration can induce an increase of IgG1 but not IgM or IgA. The same monomer has been proved to also alter the activity of mice B lymphocytes, leading to

an increased *in vivo* production of IgG1 antibodies. On the other hand, HEMA in high concentration reduce IgG1 and IgM production and cell proliferation [Andersson et al., 2010]. In addition, HEMA can increase ROS synthesis, which cause apoptosis or cell cycle delay (G0/G1) depending on its concentration [Pawlowska et al., 2010].

BisGMA and TEGDMA can induce their genotoxicity effects on mice macrophages through dose-depending activation of caspase intrinsic pathway (caspase-3, -8 and -9) [Huang et al., 2014; Huang et al., 2015] and increase in the number of micronuclei and fragmentations of DNA strands [Li et al., 2012; Huang et al., 2015]. The genotoxic effects of HEMA are presented by DNA alteration, cell cycle delay, enhancement of the oxidative stress and activation of the ATM protein kinase [Schweikl et al., 2014]. Marovic et al. [2014] reported that BisGMA can cause in DNA damage of human lymphocytes in a dose-dependent manner. Such damages, as proven by Drozdz et al. [2011], can also end up in cell cycle delay (phase S). TEGDMA and HEMA in low concentrations behave as clastogens, inducing mutations which could potentially lead to carcinogenic phenomena [Ginzkey et al., 2015].

In vivo studies on BALB/c mice presented how HEMA affects the immune system, through stimulating of NLRP3 inflammasome formation [Alizadehgharib et al., 2018] and inducing the IgG production (but not IgM). Furthermore, HEMA, as an immunologic adjuvant, stimulates the production of TNF-α, IL-6 e IL-2, showcasing how long-term exposure to even minimal quantities of the monomer can influence general health of mice and suppress specific immune functions [Andersson et al., 2011a, 2011b].

Conclusions

The use of materials in conservative and aesthetic dentistry in the last few years has seen a significant increase of mechanical properties, linked to both the chemical composition of these materials and their improvement with clinical and compositional modifications. These materials, however, attracted the interest of scientists on the possible side-effects of resin-based monomers on the oral cell populations. In this article, the degree of conversion (DC) of a composite material constitutes an important parameter to take into account for its use. This, linked to the number of unconverted monomers, together with other factors, such as PM [Geurtsen et al., 1998b], chemical composition and polymerisation method, should be carefully considered for the selection of materials and clinical application procedures. The investigation of the existing literature, despite not offering univocal and definitive data, showed a potential toxicity risk for resin-based materials, justifying a legitimate concern among scientists. The potential cytotoxic risk seems to be directly related to its contact time with dental tissues. It has been proven, in this sense, that approximately 90% of monomers are released in the first 24 hours following initial polymerisation [Ferracane, 1995]. Hence the need to take into account different time frames for a complete analysis on the cytotoxicity risk. This analysis showed the necessity of further *in vivo* studies in order to have a more complete assessment of the toxicity risk. Mostly, in *in vitro* studies cell viability has been assayed using MTT technique, which is the most common method for investigation of potential cytotoxicity of a specific material [Marinucci et al., 2009; Marinucci et al., 2018. Other techniques, such as SEM analysis evaluation of necrosis or apoptosis and examining

the potential effects of materials on the cell cycle look quite promising for this issue. Furthermore, understanding the potency of these materials for influencing the innate and adaptive immune reactions is also of great importance. The investigated studies in this review article showed that these materials can alter the expression of inflammatory and anti-inflammatory genes and proteins. Critical parameters are the different origin and type of the used cells (human or mice, immortalised or primary). Another parameter that should be considered is the possibility of *in vitro* application of dental barriers between the tested substance and the cell population, so as to have a more accurate simulation of a real clinical situation. On this topic, however, attention should be paid to other factors such as saliva pH, temperature, humidity, etc. Repeatability and reliability of *in vitro* tests, in this case, could undergo alterations.

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