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

Gandra, 20 de julho de 2021

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Toxic effects of the resin-matrix cements on fibroblast or mesenchymal cells: An integrative review

Trabalho realizado sob a Orientação de Prof. Doutora Orlanda Torres

Co-orientação do Prof. Doutor Júlio Souza

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Agradecimentos

Em primeiro lugar, quero agradecer à minha família, sem eles, não teria sido possível realizar este sonho.

Aos meus pais que me apoiaram incondicionalmente em cada momento. Obrigada, Mãe, por seres uma referência, mulher lutadora e trabalhadora, espero tornar-me como tu um dia. Para ti pai, por veres o lado positivo de tudo nos piores momentos e seres uma alegria na minha vida.

Para o meu irmão e sobrinhos, por fazerem que cada momento ao seu lado se torne em memórias inesquecíveis.

Aos meus avós, por me terem dado tanto amor e confiado em mim. Amo-vos.

Para as minhas amigas de toda vida, espero desfrutar de mais 21 anos ao vosso lado, vivendo a vida como se fosse acabar, sendo jovens para sempre.

Para os meus amigos, que tornaram esta fase tão agradável apesar de todas as dificuldades. É a família que pude escolher. Especialmente a Maria, o melhor binómio que pude ter, o meu pilar neste caminho desde o primeiro momento. Obrigado por todos os momentos vividos, estando sempre presente quando precisei. Seremos sempre "*team*"M&M.

Agradeço também à minha orientadora, a Prof. Orlanda Torres, e ao meu co-orientador Prof. Júlio Souza, obrigado por toda a atenção e disponibilidade desde o primeiro momento.

Finalmente, muito obrigado a esta instituição e a todos os professores que durante estes anos me ensinaram o amor por esta profissão.

Abstract

Purpose: The objective of this study was to perform an integrative review on the toxic effects the resin-matrix cements and their products in contact with fibroblasts, epithelial, or mesenchymal cells.

Method: A bibliographic review was performed on PubMed using the following search terms: "cytotoxicity" AND "fibroblast" OR "epithelial" OR "mesenchymal" AND "polymerization" OR "degree of conversion" OR "methacrylate" OR "monomer" AND "resin cement" OR "resin-based cement".

Results: The initial search in the available database yielded a total of 277 articles of which 21 articles were included in this review. A reduction of the viability of mouse fibroblasts ranged between 13 and 15% was recorded for different resin-matrix cements after light curing exposure for 20s. The viability of human fibroblast was recorded at 83.11% after light curing for 20 s that increased up to 90.91% after light curing exposure for 40 s. Most of studies linked the highest toxicity levels when the cells were in contact with Bis-GMA followed by UDMA, TEGDMA and HEMA.

Conclusions: Resin-matrix cements cause a cytotoxic reaction when in contact with fibroblasts or mesenchymal cells due to the release of monomers from the polymeric matrix. The amount of the monomers released from the resin matrix and their cytotoxicity depends on the polymerization parameters.

Key words: cytotoxicity; fibroblast; polymerization; degree of conversion; resin cement

Resumo

Objetivo: O objetivo deste estudo foi realizar uma revisão sistemática integrativa dos efeitos tóxicos dos cimentos da matriz de resina e dos seus produtos em contacto com fibroblastos, células epiteliais ou mesenquimais.

Método: Foi efetuada uma revisão bibliográfica em PubMed utilizando os seguintes termos de pesquisa: "*cytotoxicity*" AND "*fibroblast*" OR "*epithelial*" OR "*mesenchymal*" AND "*polymerization*" OR "*degree of conversion*" OR "*methacrylate*" OR "*monomer*" AND "*resin cement*" OR "*resin-based cement*".

Resultados: A pesquisa inicial na base de dados disponível identificou um total de 277 artigos dos quais 21 artigos foram incluídos nesta revisão sistemática. foi registada em 83% após polimerização dos cimentos por 20 s. No entanto, a viabilidade dos fibroblastos aumentou para aproximadamente 90% após polimerização dos cimentos por 40 s. A maioria dos estudos apresentaram resultados com níveis mais altos de toxicidade quando as células estiveram em contacto com moléculas de Bis-GMA em comparação ao contacto com moléculas de UDMA, TEGDMA e HEMA.

Conclusões: Os cimentos com matriz resinosa proveram uma reação citotóxica quando em contacto com fibroblastos ou células mesenquimais devido à libertação de monómeros da matriz polimérica. A quantidade de monómeros libertados da matriz de resina depende não só da composição química dos cimentos mas também dos diversos parâmetros de polimerização.

Palavras chave: cytotoxicity; fibroblast; polymerization; degree of conversion; resin cement

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List of abbreviations

Bis-GMA: Bisphenol-A-diglycidylmethacrylate

UDMA: Urethane dimethacrylate

TEGDMA: Triethylene glycol dimethacrylate

HEMA: Hydroxyl ethyl methacrylate

CQ: Camphorquinone

nm: Nanomolar

mm: Millimetre

LCUs: Light-curing units

LED: Light-emitting diode

RLCs: Resin luting cements

S: Seconds

mW/cm^2: Milliwatts per centimeter squared

w/w: Weight for weight

DMEM: Dulbecco´s modified eagle medium

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide

RBOC: Resin-based orthodontic cements

DC: Degree of conversion

CO_2: Carbon dioxide

FOLC: Fuji Ortho LC

OPAL: Opal Band Cement

OBPLC: Ortho Band Paste LC

TBP: Transbond Plus

HGF: Human gingival fibroblast

TiO_2- nt: Titanium dioxide nanotubes

S03: Self- Curing resin-based cement with the addition of 0.3wt% of TiO_2-nt

S06: Self- Curing resin-based cement with the addition of 0.6wt% of TiO_2-nt

S09: Self- Curing resin-based cement with the addition of 0.9wt% of TiO_2-nt

D03: Dual-Curing resin-based cement with the addition of 0.3wt% of TiO_2-nt

D06: Dual-Curing resin-based cement with the addition of 0.6wt% of TiO_2-nt

D09: Dual-Curing resin-based cement with the addition of 0.9wt% of TiO_2-nt

HPDLF: Human periodontal ligament fibroblast

RBC: Resin-based cement

SADRC: Self-adhesive dual-cured resin cement

SARC: Self-adhesive resin cement

PV5: Panavia V5

PSA: Panavia SA

MLA: Multilink Automix

RUN: RelyX Unicem

RUL: RelyX Ultimate

SCP: Speed Cem Plus

QHT: Quartz tungsten halogen

PAC: Plasma arc curing

CQ/TA: Camphorquinone/Ternary amine

PPD: Phenyl-propanedione

TPO: Diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide

h: Hour

mg/ml: Milligrams per millilitre

ºC: Degree Celsius

ELISA: Enzyme-Linked Immunosorbent Assay

ISO: International Organization for Standardization

Min: Minutes

ROS: Reactive oxygen species

1. Introduction

Resin-matrix cements play a key role on the cementation of indirect restorations such as prosthetic crowns, on-lay and in-lay restorations [1–3].However, the organic matrix of resin-matrix cements is composed of several monomers that can cause adverse reactions to human cells and tissues [4–6]. Adverse reactions can occur at the gingival margins and at the dentin-pulp complex regarding the restorative location, chemical composition, amount, and release rate of monomers and their derivatives [3,7,8]. Furthermore, inadequate polymerization results in a low conversion of monomers into polymers and therefore the residual monomers are the major cytotoxic concern [9]. The cytotoxic effects of resin-matrix cements influence the behaviour of soft and hard tissues around tooth or an implant abutment that determine the long-term clinical performance of the restorations [5,10].

The organic matrix of a resin-matrix cement is a combination of high molecular weight monomers such as bisphenol-A-diglycidylmethacrylate (Bis-GMA) and urethane dimethacrylate (UDMA) and low molecular weight monomers including triethylene glycol dimethacrylate (TEGDMA) and hydroxyl ethyl methacrylate (HEMA) [3,4,8]. The extremely high viscosity of Bis-GMA resin requires the addition of significant amounts of TEGDMA as a reactive diluent to provide a flowable mixture for restorative cementation [11]. Current photo initiator system of resin-matrix cements involves camphorquinone (CQ) associated with a tertiary amine to promote at 460 nm wavelength [12]. The ability of light-curing units to deliver enough energy at appropriate light absorption range for the respective photo initiator systems is crucial to optimize the physical properties of light-activated dental materials [13]. The resin-matrix cement has also an inorganic content ranging from 60 up to 89wt% [12,14]. The major inorganic compounds are barium fluoroaluminoborosilicate glass, strontium calcium aluminosilicate glass, zirconia, ytterbium fluoride, quartz, and colloidal silica [2]. The size of inorganic particle varies from micro- (around 1-10 μm) down to nano-scale (40-60 nm) dimensions [5,14]. The mechanical properties and viscosity can be controlled by the addition of inorganic fillers although the organic matrix also determine such properties.

After cementation, the excess of resin-matrix cement is initially removed with either foam pellets or by light curing for two seconds to achieve an initial polymerization followed by removal

with a hand instrument of the consequently slightly hardened cement [16,17]. In the subgingival (> 1.5 mm) and interdental area, excess of resin-matrix cement is difficult to eliminate, and therefore remnant cement is often found surrounding tissue and restoration, acting as a potential cause of inflammatory reactions [10,18]. On implant-supported restorations, intraoral cementation is often performed for compensation of divergent implant angulations or when zirconia implants are used, which are mainly one-piece designs [19,20]. However, excess or thick layers of resin-matrix cements can easily occur even though the removal procedure is applied.

1.1 Objective and hypothesis

The main aim of the present study was to perform an integrative review on the toxic effects of resin-matrix cements and their products in contact with fibroblasts, epithelial, or mesenchymal cells. It was hypothesized that the organic matrix components of the resin cement do induce toxic effects in contact fibroblasts, epithelial, or mesenchymal cells.

2. Method

2.1. Information sources and search strategy

A bibliographic review was performed on PubMed (via National Library of Medicine) considering such database includes the major articles in the field of dentistry and biomaterials. The present search of articles was performed in accordance with previous integrative or systematic review articles. The following search terms were applied: "cytotoxicity" AND "fibroblast" OR "epithelial" OR "mesenchymal" AND "polymerization" OR "degree of conversion" OR "methacrylate" OR "monomer" AND "resin cement" OR "resin-based cement". Also, a hand-search was performed on the reference lists of all primary sources and eligible studies of this systematic review for additional relevant publications. The inclusion criteria encompassed articles published in the English language, within the last 10 years (from January 2011 up to January 25th, 2021), dealing with the toxic effects of resin-matrix cements and their products in contact with fibroblast, epithelial, or mesenchymal cells. The eligibility inclusion criteria used for article searches also involved: *in vitro* studies on cell culture; meta-analyses; randomized controlled trials; animal assays; and prospective cohort studies. The exclusion criteria were the following: papers without abstract; case report with short follow-up period; narrative review; traditional systematic review; and studies on other cell types. Studies based on publication date were not restricted during the search process.

2.2. Study selection and data collection process

The study selection and data collection were performed into three steps. Studies were primarily scanned for relevance by title, and the abstracts of those that were not excluded at this stage were assessed. Three of the authors (JCMS, MG, OT) independently analyzed the titles and abstracts of the retrieved, potentially relevant articles meeting the inclusion criteria. The total of articles was compiled for each combination of key terms and therefore the duplicates were removed using Mendeley citation manager. The second step comprised the evaluation of the abstracts and non-excluded articles, according to the eligibility criteria on the abstract review. Selected articles were individually read and analyzed concerning the purpose of this study. At last, the eligible articles received a study nomenclature label, combining first author names and year of publication. The following variables were collected for this review: authors' names, journal, publication year, purpose, study design, cell culture method, cell types, analyses, and main outcomes (Table 1). PICO question was adjusted to the issue where "P" was related to the cell

types while "I" referred to the methods of analyses. Data of the reports were harvested directly into a specific data-collection form to avoid multiple data recording regarding multiple 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 initial search in the available database yielded a total of 277 articles of which 209 duplicate articles were eliminated. Of the remaining 68 articles, the titles and abstracts were read seeking concordance with the inclusion criteria of the present study and then 44 studies were discarded because they focused only on the chemical composition of the organic matrix. The evaluation of titles and abstracts resulted in the selection of 24 potentially review articles of which 3 articles were excluded. The results of the selection of articles are shown in Figure 1.

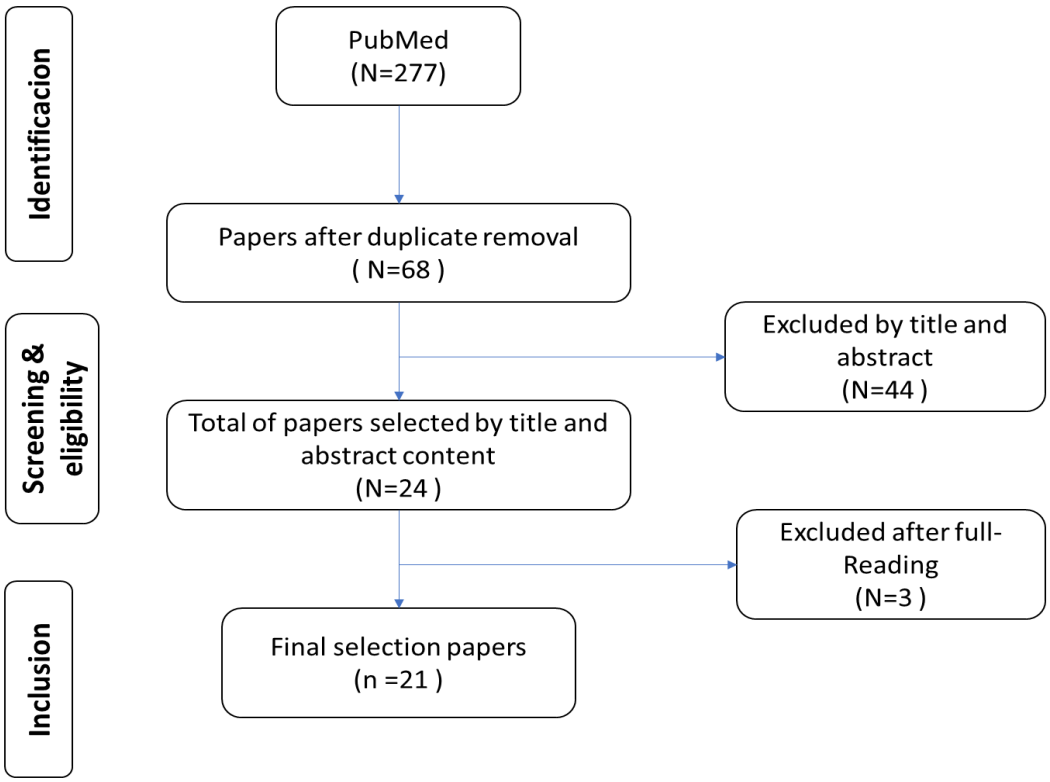

Figure 1 Flow diagram of the search strategy used in this study.

Of the 21 articles included in this review, 12 (57.14%) *in vitro* studies evaluated the cytotoxicity induced by resin-matrix cements in contact with mouse fibroblast [4,6,8,10–18]. One *in vitro* study examined the effects of four different self-adhesive resin cement materials on cell viability and apoptosis of mouse fibroblasts after direct and indirect light-curing exposure [4]. Seven *in vitro* studies (33.33%) investigated the toxic effect of resin-matrix cements on human fibroblasts [5,14,25,28–32]. One article evaluated the cytotoxicity of self-adhesive dual-cured resin cement (SADRC) polymerized beneath three different cusp inclinations of zirconia crowns

with different light-curing time [28]. Only two studies (9.52%) compared the cytotoxicity of resin-matrix cements on human and mouse fibroblasts [10,33].

The main outcomes are shown in Table and described as follow:

- Several studies in human and mouse fibroblast demonstrated that the cytotoxicity highest effects were recorded for Bis-GMA molecule followed by UDMA, TEGDMA, and HEMA [5,14,26];
- The polymerization with LED units decreased the viability of fibroblast cells when compared to a polymerization via halogen light curing units [12,21,22]. An increase in the light-curing time decreased the cytotoxicity [26];
- The heat treatment pre-photopolymerization at 60° also reduced the cytotoxicity effects [6]. The viscosity of the resin-matrix cement has an important role in cell viability since a low viscosity provides a high oxygen content around the cementation site leading to a poor degree of conversion of monomers [12]. That increases the toxicity of the resin-matrix cement:
- The cusp inclinations of zirconia can decrease the light transmission through the restoration toward the resin-matrix cement [34]. A lack of light curing transmission can negatively affect the polymerization of the resin-matrix cement. Additionally, the opacity of monolithic zirconia restorations can limit the amount of light transmitted through the material [35,36].
- The roughness of the resin-matrix cement also affected the cell viability. Mean values of roughness between 0.2 to 0.8 μm decreased the cytotoxicity in human fibroblasts [5].

Table 1 Relevant data of the studies selected on the toxic effect of resin- matrix cements

Author (year)	Purpose	Study design	Chemical composition/ Materials (Manufacturers)	Light curing/Degree of conversion	Biological response
Ergun et. Al (2011) [26]	The effect of reduced curing time of five resin luting cements (RLCs) polymerized by high-power LED curing unit on the viability of a cell of L-929 fibroblast cells	- *in vitro.* - *Mouse* L929 fibroblast - *MEM* (incubated for 2–5 min at 37°C) -MTT assay	A) Methacrylated phosphoric ester, Dimethacrylate, Acetate, Stabiliser,Initiator (RelyX Unicem, 3M ESPE Dental Products, St. Paul, USA) B) Bis-GMA, TEGDMA, UDMA (Duolink, Bisco, Inc. Schaumburg, IL, USA) C) Bis – GMA, UDMA, HDDMA, Silane photoinitator (Lute-It, Pentron Clinical Technologies, L.L.C., Wallingford, USA) D) EBPADMA, TEGDMA Bis–GMA, (Illusion, Bisco, Inc. Schaumburg, IL, USA) E) Bis–GMA, TEGDMA, (Rely X ARC, 3M ESPE Dental Products, St. Paul, USA)	LED LCU: 1200mW/ cm^2 20 or 40 s	Decrease of curing time significantly enhances the cytotoxicity.
Mahasti et. Al (2011) [23]	Compare the cytotoxicity of two resin cements (Panavia F2 and Rely X Plus) versus zinc phosphate cement (Harvard) using rat L929-fibroblasts in vitro.	- *in vitro* - *Mouse* L929 fibroblast - MTT assay	A) Hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic aliphatic dimethacrylate, silanated silica filler, silanated colloidal silica, dl-campherquinone, catalysts, initiators, silanated barium glass filler, surface treated sodium fluoride, accelerators, pigments (Panavia F2, Kuraray America, Inc., New York, USA) B) Paste A: Radiopaque fluoro aluminosilicate (FAS glass) opacifying agent, HEMA, water, proprietary reducing agent Paste B: nonreactive zirconia silica filler methacrylated polycarboxylic acid, HEMA, BisGMA, water, potassium per sulphate (Rely X	LED LCU: 550mW/ cm^2 40 s	Cytotoxicity differs significantly in Panavia F2 and Rely X Plus cements with respect to time ($p< 0.001$) while this factor not affect the cytotoxicity of Harvard cement. Harvard cement is probably the most cytotoxic cement.

			Plus, 3M ESPE Dental Products, St. Paul, USA) C)zinc oxide, magnesia, phosphoric acid (Harvard, Hoffmann Dental, Hoppegarten, Germany)		
Cörekçi et. Al (2014) [12]	Evaluate the cytotoxicity of four resin-based orthodontic cements (RBOC) as a function of degree of conversion (DC) and the light curing unit (LCU)	*-In vitro.* *-Mouse L929 fibroblast* *-MTT assay (200* µL MTT solutions, overnight at 37C and 5% CO2)	A) Dimethacrylate, UDMA, polyacrylic acid, (Fuji Ortho LC, GC Corp., Tokyo, Japan) B) UDMA, Bis-GMA, TEG–DMA, benzoyl peroxide (Opal Band Cemen, Opal Orthodontics, South Jordan, Utah, USA) C) Bis-GMA (Ortho Band Paste LC, Bisco, Inc. Schaumburg, Illinois, USA) D) Glycerol 1,3- dimethacrylate, citric acid dimethacrylate oligomer (Transbond Plus, 3M Orthodontics, Maplewood, Minnesota, USA)	<u>Light curing:</u> A) plasma-emulating LED: 2585 mW/cm^2 (3s) B) Conventional LED:1030 mW/cm^2 (20s) <u>Degree of conversion</u> A) OPAL: 73.7% B) FOLC 62.7% C) OBPLC: 66.1% D) TBP: 59.8 %	A) Cements, LCUs, and interaction between cements and LCUs were found to play a statistically significant role in cytotoxicity ($p < 0.0001$) B) Opal band cement (OPAL) plasma LED: 60–90% cell viability C) Interaction between cement and LCU had no statistically significant role on DC ($p > 0.05$) . The correlations between cell viability and DC were positive for three RBOCs.
TRUMPAITE-VANAGIENE et. al (2015) [32]	A) evaluate the cytotoxicity of luting cements B) test if pre-washing reduces the cements' cytotoxicity	*-in vitro* *-HGF* *-MTT (*1, 6 and 12 h)	A) o-phosphoric acid, zinc oxide, magnesium oxide (Hoffmann's ZP, Hoffmann Dental Manufactur, Berlin, Germany) B) 2-hydroxyethylmethacrylate, polyacrylic acid, urethanedimethacrylate (Fuji Plus RMG, GC CORPORATION, Tokyo, Japan) C) methacrylate monomers containing phosphoric acid groups, methacrylate monomers, initiator components, stabilizers. (RelyX Unicem RC, 3M ESPE, St. Paul, MN, U.S.A.)		the luting cements studied, Hoffmann's ZP cement indicated less cytotoxicity than Fuji Plus RMGI or and RelyX Unicem RC. Pre-washing of luting cements reduced the cytotoxicity
Ramos-Tonello et. Al (2017) [22]	Investigate the influence of Titanium dioxide nanotubes (TiO_2-nt) addition to self-adhesive resin cement on the degree of conversion, water	*-in vitro* - NIH/3T3 fibroblasts *-MTT assay*	Dimethacrylate monomers,methacrylated aliphatic amine(RelyX U200, Seefeld, Germany)	The DC analysis: A) 3min: 0.3% TiO_2 (S03 = 26.76%; D03 = 26.11%) and 0.9% TiO_2	Cytotoxicity assays revealed that reinforced cements were biocompatible

	sorption, and water solubility, mechanical and biological properties			(S09 = 25.62%; D09 = 26.05%) B) 6 min: 0.3% TiO_2 (S03 = 34.63%; D03 = 33.9%) and 0.9% TiO_2 (S09 = 40.4%; D09 = 39.89%) C) 9 min: 0.3% TiO_2 (S03 = 41.03 %; D03 = 46.30%) and 0.9% TiO_2 (S09 = 42.15%; D09 = 44.12%) D) 12 min: 0.3% TiO_2 (S03 = 45.6%; D03 = 47.20%) and 0.9% TiO_2 (S09 = 45.47 %; D09 = 44.58 %)	
Sun et. Al (2018) [14]	Evaluate the potential cytotoxicity of self-adhesive resin cements with or without light irradiation	*-in vitro* *-HPDLFs* *-DMEM assay*	A) Resin matrix: triethyleneglycol dimethacrylate, 2-propenoic acid, 2-methyl 1,1-(1-[hydroxymetil]-1,2-ethanodlyl) ester dimethacrylate, 1-benzyl-5-phenyl-barbic-acid, 1,12-dodecane dimethacrylate, tert-butyl peroxy-3,5,5-trimethylhexanoate (RelyX U200, 3M ESPE AG, St. Paul, Germany) B) Resin matrix: bis-phenol-A-diglycidylmethacrylate, glycerol dimethacrylate, glycerophosphoric acid dimethacrylate (Maxcem Elite, Kerr, Orange, USA) C) Resin matrix: dimethacrylate, 2-hydroxyethyl methacrylate, acid monomers (Multilink Speed, voclar-vivadent, Schaan, Liechtenstein)	LED LUCs: 800 mW/cm^2 20s	The composition and light irradiation of self-adhesive resin cements could affect cell proliferation and cell apoptosis induction of HPDLFs

Zhang et Al. (2019) [28]	Evaluate the cytotoxicity of self-adhesive dual-cured resin cement (SADRC) polymerized beneath three different cusp inclinations of zirconia with different light curing time	*-in vitro* *-human gingival fibroblast* *-DMEM assay*	Resin matrix: dimethacrylate, 2-hydroxyethyl methacrylate, acid monomers (Multilink Speed, voclar-vivadent, Schaan, Liechtenstein)	LES LCUs: 1200 mW/cm^2 for 20 s and 40 s	A) A zirconia restoration with a thickness of 1.0 mm, when the cusp inclination is smaller than 20°, the cytotoxicity of SADRC conforms to ISO standard, regardless of the light curing time is 20 s or 40 s. B) When the cusp inclination of zirconia reaches or exceeds 30°, the cytotoxicity of polymerized SADRC did not conform to ISO standard
Klein- junior et. Al (2019) [7]	The influence on cytotoxicity of heat treatment applied before photopolymerization, while mixing three self-adhesive resin cements, in an NIH/3T3 fibroblast cell culture, based on cell viability measures	*-in vitro* - NIH/3T3 mouse fibroblasts *-MTT assay (24h and 7 days, 37° and 60°)*	A) Dimethacrylate monomers, 1,12-dodecanediol dimethacrylate methacrylated aliphatic amine (RelyX U200, (3M ESPE, Saint Paul, Minnesota, USA) B) Dimethacrylate, HEMA (Multilink N, Ivoclar Vivadent, Schaan, Liechtenstein) C) Bis-GMA, dimethacrylate monomer (Bis Cem, Bisco Inc., Schaumburg, Illinois, USA)	Light curing time: 20s	Heat treatment at 60°C should be considered as a strategy to reduce cytotoxicity of self-adhesive resin cements.
Oguz et. Al (2019) [6]	Evaluate the cytotoxicity of resin-based luting cements on fibroblast cells using different polymerization protocols	*-in vitro* *-NIH/3T3 mouse fibroblast* *-MTT assay*	<u>CONVENCIONAL RESISN COMPOSITE CEMENT</u> A) TEGDMA/bis-GMA (RelyX ARC, 3M ESPE, St Paul, MN, USA) B) bis-GMA, TEGDMA, UDMA, (Variolink N, Ivoclar Vivadent, Schaan, Liechtenstein) <u>SELF-ADHESIVE RESIN CEMENT</u> C) methacrylated, dimethacrylate, acetate, (Rely X Unicem, 3M ESPE, St Paul, MN, USA) D) UDMA, TEGDMA, polyethylene glycol dimethacrylate, (Multilink Speed, Ivoclar Vivadent, Schaan, Liechtenstein)	A)Photopolymerization with direct light application B) photo-polymerization over ceramic C) resin nano-ceramic discs D) auto-polymerization	Cytotoxicity of dual-polymerized resin cements was material-dependent and decreased gradually up to 7 days. Photo-polymerization plays an important role in reducing the cytotoxic effects

Rohr et. Al (2020) [5]	Investigate the effect of cement type and roughness on the viability and cell morphology of human gingival fibroblasts (HGF-1).	*-In vitro* -HGF-1 - *MEM*	A) Bis-GMA, TEGDMA, (Panavia V5, Kuraray, Okayama, Japan) B) Bis-GMA, TEGDMA, HEMA (Panavia SA plus, Kuraray Noritake Dental Inc., Okayama, Japan) C)Bis-GMA, HEMA, 2-dimethylaminoethyl methacrylate ethyoxylated bisphenol A dimethacrylate, UDMA, HEMA (Multilink Automix, Ivoclar Vivadent, Schaan, Liechtenstein) D) UDMA, TEGDMA, (SpeedCem Plus, Ivoclar Vivadent, Schaan, Liechtenstein) E) methacrylate monomers containing phosphoric acid groups, methacrylate monomers (RelyX Ultimate, 3M ESPE, St Paul, MN, USA) F) phosphoric acid modified methacrylate monomers, bifunctional methacrylate methacrylate monomers (RelyX Unicem 2 Automix, 3M ESPE, St Paul, MN, USA)		The composition of resin composite cements significantly affects the cell viability of HGF-1. High viability for PSA RUN = MLA SCP = PV5. RUL (p,0.05) and for P2500 = P400. P180 (p,0.001). Cell morphology did not vary among the materials but was affected by the surface roughness.
Şişmanoğlu (2020) [4]	The effects of four different self-adhesive resin cement materials on cell viability and apoptosis after direct and indirect exposure were evaluated using different cell culture techniques	*-In vitro* - NIH/3T3 mouse fibroblast - *MTT assay (viability):* Cells were incubated in a highly humidified atmosphere containing 5% CO2 at 37°C - Annexin-V-FITC/PI staining (apoptosis)	A) Urethane dimethacrylate, 2-hydroxyethyl methacrylate (BeautiCem SA Cemen, Shofu, Kyoto, Japan) B) Bis-GMA, TEGDMA, hydrophobic aromatic dimethacrylate, HEMA (Panavia SA Cement Plus, Kuraray Noritake Dental Inc., Okayama, Japan) C) dimethacrylate monomers, 1,12-dodecanediol dimethacrylate methacrylated aliphatic amine (RelyX U200) D) Bis-GMA, dimethacrylates, 2-hydroxyethyl methacrylate (TheraCem)	Light curing: 1250 mW/cm^2	BeautiCem SA caused significantly more severe cytotoxic and apoptotic (24.1%) effects than other cements tested

4. Discussion

The toxicity of the organic matrix of resin cements against fibroblasts and mesenchymal cells has been reported in literature. Different factors related to the polymerization of the resin-matrix cements affect their toxicity such as light-curing exposure time, mode, intensity, and distance. Considering the findings reported by the selected studies, the hypothesis that the organic matrix components of the resin cements do induce toxic effects in contact fibroblasts or mesenchymal cells has been confirmed.

4.1. Resin-matrix cements

The resin-matrix composite cements are composed of an organic matrix and silanized fillers particles [37]. Bis-GMA is the predominant base monomer in resin-matrix cements due to its physical properties although it is mixed with other dimethacrylates, such as UDMA, TEGDMA or other monomers [11]. The mixture of Bis-GMA with other monomers is industrially performed to reach optimum physical properties such viscosity, elastic modulus, strength etc. The fillers used are composed of different glass-ceramic or ceramic materials such as barium fluoroaluminoborosilicate glass, strontium calcium aluminosilicate glass, zirconium silicate glass, zirconia, quartz, colloidal silica 20% to 80% by weight, ytterbium fluoride and other glass fillers, as seen in Table 1 [5,15,38]. The total filler content typically ranges from 60 up to 89 wt%. The size of inorganic particle can be found from micro- (around 1-10 μm) down to nano-scale (40-60 nm) dimensions [2,39].As seen in Table 1, the resin-matrix cements most commonly used in the previous studies were the following: Panavia SA Plus™, RelyX Unicem™, RelyX U200™, and Multilink Speed™. Panavia SA Plus™ is composed of Bis-GMA, TEGDMA, HEMA, dl-Camphorquinone and 40 vol% fillers (silanated barium glass filler, silanated colloidal silica) with a particle size between 0.02-20 μm. RelyX Unicem™ is composed of phosphoric acid modified methacrylate monomers, Sodium toluene-4- sulphinate, sodium persulfate, tert-butyl 3,5,5-trimethylperoxyhexanoate (initiators) and 43 vol% fillers (alkaline (basic) fillers, silanated fillers) with a particle size between 12.5 μm [5]. RelyX U200™ comprises also methacrylate monomers and 70wt% filler content with 12.5μm mean particle size such as silanated silica, sodium persulfate, titanium dioxide, calcium hydroxide). Multilink Speed ™ involves methacrylate monomers and 57wt% filler content with 5 μm mean particle size such as barium glass fillers, ytterbium trifluoride, silicon dioxide [14].

The most common photoinitiator system is the camphorquinone (CQ) coupled to a tertiary amine (CQ/TA). Camphorquinone molecules absorb the light at wavelength of 468nm (absorption peak) and start to chemically interacts with the tertiary amine to form a photoexcited complex [40–42]. At that state, CQ abstracts a hydrogen atom from the tertiary amine, producing free radicals. The formed free radicals react with the C=C bonds of monomers, resulting in the formation of new radicals with a much longer chain than before propagating radicals. The same process continues through the chain reaction until the reaction process is accomplished [41]. Other initiators are currently incorporated in resin-matrix cements such as phenyl-propanedione (PPD) and diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide (TPO). TPO absorption spectrum extends from 380 up to about 425nm while PPD absorption spectrum extends from below 350 up to approximately 490 nm [42]. Thus, resin-matrix cements that only contain CQ/TA system, only require light curing at the blue spectral range (420-540 nm) while resin-matrix cements containing CQ plus TPO and/or PPD require light curing in both the blue (420-540 nm) and violet (360-420 nm) [43].The degree of light-induced conversion of monomers to polymers is influenced by various parameters, such as the intensity of the light around the wavelength triggering level, the photoinitator system, the irradiation exposure time, concentrations, types, organic matrix composition, fillers, and mixtures of photoinitators, co-initiators, stabilizers [40–43]. Adequate polymerization is the most important factor in maximizing the physical properties, clinical performance and biocompatibility of resin-matrix cements [26]. However, monomers display considerable residual unsaturation after the polymerization procedure and therefore the degree of conversion ranges from 55 up to 75% [35]. That does not imply that remaining monomer molecules are left in the resin, since each one of two methacrylate groups per dimethacrylate molecule could still be covalently bonded to the polymeric structure [36]. Substances released from the resinous matrix due to incomplete polymerization or resin degradation may cause adverse effects to the surrounding tissues [38].

The initial light exposure causes a rapid increase in the conversion of monomers, resulting in a viscous gel. Such rapid increase in viscosity hinders the migration of active radical components that would be responsible for the further chemically induced polymerization [36]. The dual-cured resin cements play an important role in reduce the cytotoxic effects [12,21,22]. Also, different types of light curing units have been proposed for the polymerization of light activated restorative materials including conventional quartz tungsten halogen (QTH),

intermittent light, plasma arc curing (PAC), light-emitting diode (LED) or laser-assisted irradiation. Solid-state LEDs use junctions of doped semiconductors (p-n junctions) based on gallium nitride to emit blue light. The spectral output of blue LEDs falls between 450 and 490 nm at an irradiance of 1000 mW/cm^2 [26,14] [12]. [26]. In the selected studies, the irradiance of light curing was assessed between 780 and 1250 mW/cm^2 for 20 or 40 s following the manufacturer´s instructions [4,7,12,14,26,28]. The opaque nature of zirconia-based restorations can limit the amount of light transmitted through the resin-matrix cement [36]. Yttria-stabilized tetragonal zirconia polycrystal (3Y-TZP) causes absorption and diffusion of light, therefore decreasing degree of conversion of monomers [44,35,36]. It should also be noted that thickness of the material may not be the only parameter for the attenuation of light curing [35]. The amount of light transmission depends on the microstructure and thickness of YTZP restorations [36].

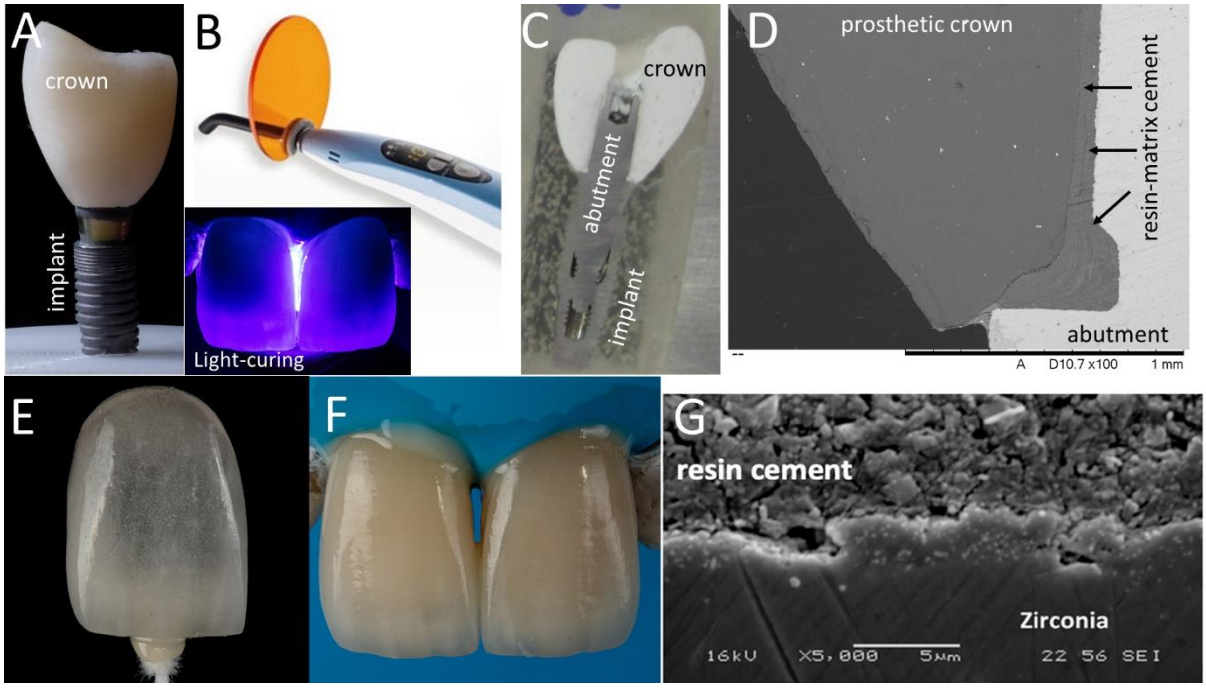

Figure 2 Schematics of cementation implant´s crown and the resin-matrix cement light curing through a zirconia veneer. (A) Prosthetic crown and endosseous implant. (B) Light curing of the resin-matrix cement. (C) Sagittal cross-sectioned slices of the crown and implant. (D) SEM images of the cross-sectioned slices of the crown and implant (E) Zirconia veneer and the (F) resin-matrix cement application. (G) SEM images of the zirconia to resin-matrix cement interface. (Adapted from Sulaiman et. Al [36]*)*

Resin-matrix cements are frequently categorized by mechanism of matrix formation: (1) self- or auto-curing; (2) light activated-curing; and (3) dual-curing [1,35,37]. Three divisions can also be recognized based on previous bonding procedure: (1) total etch; (2) self-etching; (3) self-

adhesive [1]. Total-etch bonding systems comprises the use of a phosphoric acid etching procedure followed by the primer and adhesive application onto tooth tissue prior to the cementation [26,35,45]. Total-etch bonding systems shows the highest bond strength to enamel surfaces due to an increased retention. Self-etching bonding systems involves the use of an acidic primer to partially dissolve the enamel or dentin tissues and therefore the smear layer is incorporated into the hybrid layer. The bonding agent can be mixed or not with the acidic primer [4,6,46]. Self-adhesive resin cements do not require the use of adhesive systems due to the viscosity and flowability of the resin cement although that can be a limitation to fill the micro- and nano-scale retentive regions onto the tooth surfaces. Self-etch resin cement have evolved as a result of the desire of clinicians to simplify the luting procedures for cementation and to shorten their window of contamination [47]. A common molecule in most bonding agents is 2-hydroxyethyl methacrylate (2-HEMA) which provide a primary hybrid layer via chain cross-link in the dentin or enamel rough surfaces [38]. Resin-matrix cements are available as powder/liquid, encapsulated, or paste-to-paste systems [2]. Light-cured resin-matrix cements are cured after initial placement while dual-cured cement polymerize along the time [48].

4.2 Toxicity

Several *in vitro* studies conducted in contact with mouse fibroblasts showed that the heat treatment applied before light curing procedure affected the cytotoxicity of three self-adhesive resin cements: RelyX U200™ (3M ESPE, USA), Multilink N™ (Ivoclar Vivadent, Liechtenstein), and BisCem™ (Bisco Inc., USA). Specimens were prepared in three different forms: (1) no heat treatment while mixing the pastes (control); (2) warm air stream (37°C); and (3) j hot air stream (60°C). All specimens were subsequently light cured for 20 s using a VALO Cordless™ light-emitting diode curing unit (Ultradent, USA). The results showed that heat treatment had a significant effect in preventing the increase of cytotoxicity. Specimens treated with a hot air stream (60°C) showed 7day cell viability rates of 14.7 % RelyX™ for, 15.3% for Multilink N™, 13.8% for BisCem™ that was similar to those recorded on nonheated specimens at 24h (14.9% for RelyX™, 13.9% for Multilink N™, and 15.52% BisCem™). Such findings suggest that heat treatment promoted a higher cell viability rates when compared with nonheated resin cements. Thus, heat treatment before light curing significantly increased monomer conversion rates to above the levels recorded for traditional methods [7]. Another study reported that the presence

of oxygen can prevent complete conversion of monomer to polymer at the surface of the material leading to the release of cytotoxic monomers [9] . The thickness of oxygen layer on the surface is directly affected by viscosity of resin-matrix material since a low viscosity results in low cell survival [9]. The addition of nano-scale TiO_2 can contributed to modifying the chemical and physical characteristics of resin-matrix cements [49]. The addition of small TiO_2-nt content (0.3 up to 0.9 wt %) to self-adhesive resin cement had a positive effect on the degree of conversion, flexural strength, elastic modulus, and microhardness [49]. Indirect cell viability of TiO_2-nt-reinforced cement was quite similar to non-treated specimens revealing non-toxicity [22].

Regarding the chemical composition of resin-matrix cements, previous studies reported a highest cytotoxicity on Bis-GMA against human and mouse fibroblast demonstrated followed UDMA, TEGDMA, and HEMA molecules [5,14,26]. Different *in vitro* studies conducted in human fibroblasts showed that resin-matrix cements significantly reduce the cell viability and increase reactive oxygen species (ROS) production [14]. High ROS concentrations have harmful effects on cells due to the promotion of oxidative stresses. Oxidative stresses activate the related pathways that control cell survival and death and therefore progressive oxidative stresses promote ROS accumulation in proteins, lipids, and nucleic acids. That may result in biological differences in cell behaviour such as gene expression changes, cell transformation, and mutagenesis [29]. A study in mouse fibroblast describes that resin-matrix cements caused varying toxic degrees which increased with the exposure time, once the highest viability reduction occurred at the end of 72 h exposure [29]. After 72h exposure, the lowest cell viability was found in cell cultures in contact with BeautiCem™ resin cement (54.8%) followed by Panavia SA™ (67%). Cell culture in contact with RelyX U200™ showed the highest viability rate of around 95%.

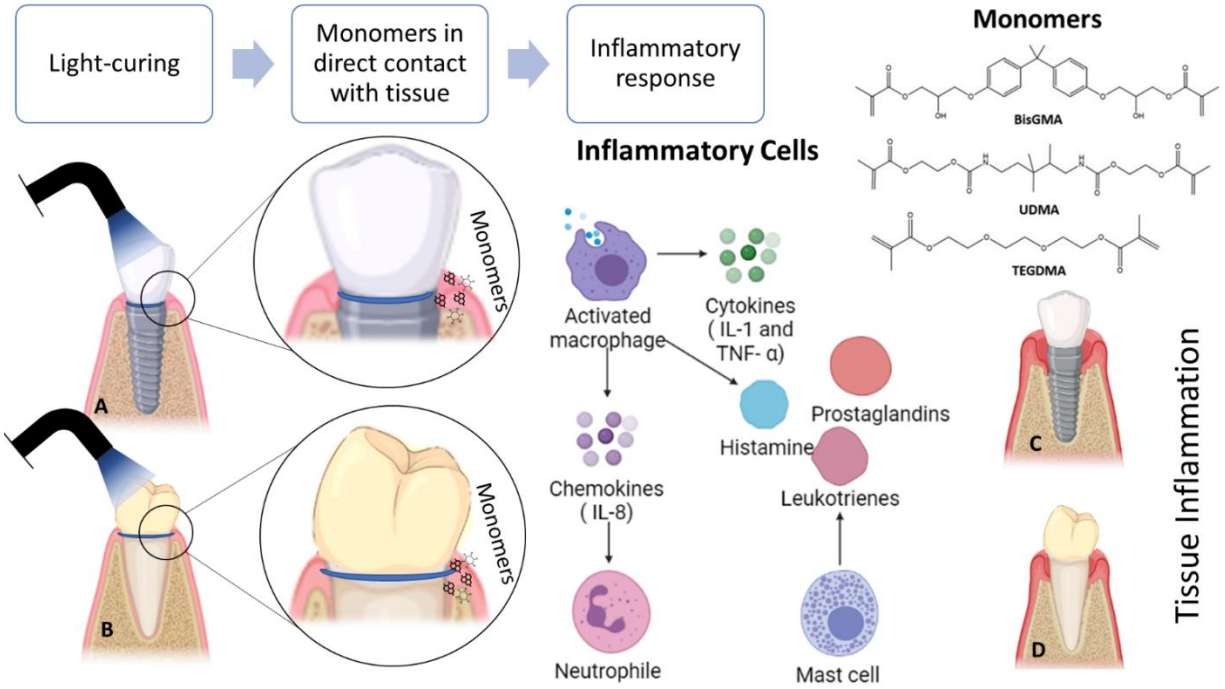

Figure 3. Cells involved in inflammatory reactions around soft tissues when in contact with methacrylate-based monomers. Prosthetic crown cemented on (A) endosseous implant and on (B) tooth, with overflowing cement in contact with the surrounding tissue during polymerization. Prosthetic crown on (C) endosseous implant and (D) tooth with presence of inflammatory reactions around soft tissues.

The most common method used in the selected studies for studying cellular viability was MTT, which is a reasonable representative of the activity of cell mitochondrial dehydrogenases [9]. This assay measures the capability of live cells to reduce 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Sigma) to insoluble blue-to-purple formazan crystals. At each time point (24 and 7 days), the culture medium was removed and 10% MTT solution (5 mg/ml) was added to each well. Then, cells were incubated at 37°C until the formation of blue-to-purple formazan crystals. For the solubilization of formazan crystals, 100 µl dimethyl sulfoxide was added to each well, and absorbance was measured at 570 nm wavelength. The percentage of viable cells was calculated and compared to the results obtained with the negative control (cells cultured in DMEM) [7]. Materials that cause more than 30% reduction in cell viability are considered cytotoxic according to ISO recommendations [4].

The roughness of resin-matrix composite cements also influence the viability of human gingival fibroblasts (HGF). The viability and morphologic aspects of HGF-1 cells were investigated after contact with three conventional cements (Panavia V5 ™ [PV5], Multilink Automix ™ [MLA], RelyX Ultimate ™ [RUL]) and three self-adhesive cements (Panavia SA plus ™ [PSA], SpeedCem plus ™ [SCP], RelyX Unicem 2 Automix ™ [RUN]). Additionally, the roughness effect was assessed.

Self-adhesive resin cement was light cured from both sides for 60 s each using a Elipar DeepCure-STM light-curing unit (3M ESPE, Germany) and then stored in an incubator (CTS T-4025, CTS Clima Temperatur Systeme GmbH, Germany) at 37°C for 15 min. Three different surface morphological aspects were mimicked regarding clinical situations: 1) polishing the cement margin with a rough red proxoshape diamond bur (silica carbide paper P180), 2) polishing with a coarse polisher (P400), and 3) polishing with a fine polisher (P2500). The results demonstrated the highest cell viability (>75%) on surfaces with a roughness between 0.2 and 0.8 µm [5].

A previous study simulates three different cusp inclinations (30, 20 and 0°) of zirconia to evaluate the *in vitro* cytotoxicity of self-adhesive dual-cured resin cement (Multilink Speed™, Ivoclar Vivadent™, Liechtenstein™) [28]. Zirconia cusp inclination at 30° commonly refers to the tooth cusps of premolars, and the molar cusp inclination is close to 20°, while the 0° indicates the labial surface of incisors. The length and width of zirconia were 10 mm, and the thickness was set at 1 mm. All the bonding surfaces of monolithic zirconia specimens were previously grit-blasted with 50 µm alumina particles. Multilink Speed™ resin-matrix cement was mixed according to the manufacturer's instruction into custom silicon rubber molds and then covered with the previously prepared monolithic zirconia specimens. The resin-matrix cement was then light cured through zirconia specimen using a light curing unit (Elipar S10, 3M ESPE, USA) with an intensity of 1200 mW/cm^2 for 20 or 40 s. For a zirconia restoration with 1 mm thickness, when the cusp inclination is smaller than 20°, the cytotoxicity of resin-matrix cement are in agreement with the ISO recommendation when the cusp inclination is smaller than 20°, regardless of the light curing time for 20 or 40 s. However, the cytotoxicity of the resin-matrix cement increased when the cusp inclination of zirconia reaches or exceeds 30° [28]. Another study showed the highest cytotoxic effect on Multilink Speed™ among all tested materials regardless the polymerization method at different cell culture time points (1, 2 and 7 days). The cell survival rate ranged from 53.1 up to 60.5% at 7 days [6]. The purpose of another study was to determine whether the differences in overlying materials can affect the degree of conversion of self-adhesive or dual-cured resin-matrix cements. Three kinds of self-adhesive, or dual-cured resin cements were used: G-CEM LinkAce™ (GC America Inc, USA), Maxcem Elite™ (Kerr Corporation, USA), and BisCemVR™ (Bisco, USA). Light curing was applied at 714.8 mW/cm^2 for 40 s through zirconia (LAVATM Plus™; 3M Deutschland GmbH, Germany) and lithium disilicate (IPS e.max Press HT ingots; Ivoclar-Vivadent AG, Liechtenstein) blocks with 1-mm thickness and

A2 shade was formed using zirconia. Specimens light cured through translucent (lithium disilicate) and semitranslucent (zirconia) showed higher degree of conversion when compared to the groups with opaque veneering material. The translucency aspects of the restorative materials can be a significant variable determining the polymerization magnitude of self-adhesive or dual-cured resin-matrix cements [44]. The resin-matrix cements light-cured through lithium disilicate or zirconia showed higher degree of conversion than that shown by resin cements cured under opaque veneering material at any measurement time [44]. The shorter exposure time can lead to an insufficient degree of conversion of the resin cement mainly at the bottom of restoration. The low degree of conversion can lead to reduced physical properties and poor biocompatibility when ceramic restoration thickness increased from 2 mm [45]. In that previous study, five dual polymerizing luting resin cements were assessed: RelyX Unicem™ (RU, 3M ESPE Dental Products, USA); Duolink™ (DK, Bisco, Inc. Schaumburg, USA); Lute-It™ (LT, Pentron Clinical Technologies, L.L.C., USA); Illusion™ (IN, Bisco, Inc. Schaumburg, USA); Rely X ARC™ (RA, 3M ESPE Dental Products, USA). Light curing was performed with irradiance intensity of 1200 mW/cm^2 and at 430-480 nm wavelength using a Elipar Freelight 2™ LED unit (3M ESPE, Germany). To simulate clinical conditions, the tested materials were irradiated from the top through the ceramic discs (10 x 2 mm, IPS Empress 2; Shade 1A, Ivoclar, Vivadent, Liechtenstein) by contacting the end of the light guide on the restorative surface using a LED light curing unit for 20 s (50% of the recommended exposure time) or 40 s (100% exposure time). The results for 20s exposure to the light were: 88.9% for RelyX Unicem™; 37.3% for Duolink™; 28.1% for Lute-It™; 90.8% for Illusion™; 83.8% for Rely X ARC™. The results of exposure light for 40 s were the following: 75.5 % for RelyX Unicem™; 60.4% for Duolink™; 57.8% for Lute-It™; 88.7% for Illusion™; 90.9% for Rely X ARC™. The cytotoxicity of the tested materials was dependent on the exposure time. Materials polymerized for 20s (except for RelyX Unicem™ and Illusion™) had reduced cell survival rates when compared to the specimens polymerized for 40 s. Those findings lead to the assumption that 20s light exposure time is not sufficient for the polymerization of resin-matrix cements [26].

5. Conclusions

This integrative review reported previous findings regarding the cytotoxic effects of resin-based cements when in contact with fibroblasts or mesenchymal cells. Within the limitations of the selected studies, the following conclusions can be drawn:

- Resin-matrix cements cause a cytotoxic reaction against fibroblasts or mesenchymal cells when the materials were not properly polymerized regarding exposure time, light-curing intensity, veneer material microstructure, or irradiance distance. The low degree of conversion of the monomers promoted a release of significant amount of residual toxic monomers.
- Resin-matrix cements light-cured through lithium disilicate or translucent zirconia showed the highest degree of conversion of monomers when compared to that shown by resin cements polymerized under opaque veneering materials. Also, an increased thickness of the veneer material above 2mm negatively affected the degree of conversion of monomers in the organic matrix. The cusp inclination above 30° also decrease the light transmission through the veneer material towards the resin-matrix cement and therefore increased the toxicity of the resin-matrix cement.
- The viscosity of resin-matrix cements had an important role in the degree of conversion of monomers and then on the cell viability since a low viscosity decreased the oxygen on the restorative surface of the tooth. That leads to a poor degree of conversion of monomer into polymer. Furthermore, pre-photopolymerization heat treatment increased the degree of conversion and decreased cytotoxicity.
- Regarding the chemical composition of resin-matrix cements, previous studies reported a highest cytotoxicity on Bis-GMA against human and mouse fibroblast demonstrated followed UDMA, TEGDMA, and HEMA molecules.
- The roughness variation also affected the cell viability. Low roughness mean values between 0.2 and 0.8 μm decreased cytotoxic effects of resin-matrix cements on cells;
- Further studies should assess the effects of different veneer materials' microstructure since novel zirconia or glass-ceramic materials have been recently developed. Also, toxic effects of resin-matrix cements should be assessed against epithelial cells. At last,

different content of Bis-GMA, UDMA, TEGDMA, and HEMA molecules should be studied when in contact with different cell lines.

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