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Release and toxicity of glass ionomer and resin-modified glass ionomer cements in dentistry. A systematic review.

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INDEX

List of symbols and abbreviations

ZOE=Zinc oxide eugenol

GIC=Glass ionomer cements

RMGIC=Resin modified glass ionomer cement

HEMA=Hydroxyethyl methacrylate

TEGDMA=Triethylene glycol dimethacrylate

UDMA=Urethane dimethacrylate

SCE=Sister chromatid exchange

IC50=Inhibitory concentration 50

TD=Tertiary dentin

MDA=Malonaldehyde

OTM=Tail moment according to Olive

CAs=Chromosomal aberrations

PRI=Proliferation rate index

HGF=Human gingival fibroblasts

LDH=Lactate dehydrogenase release

hOLCs=Human odontoblast like cells

IS=Ionoseal

VB=Vitrebond

CG=Compoglass

KF=Ketac fil

RX=Rely X

1.ABSTRACT

Introduction: The combination of resin and glass ionomer cements provides enhanced resistance to microleakage while retaining the advantages of the conventional GICs such as the fluoride release and the bonding to the tooth. However, it is important to assess that the resin-modified glass ionomer cements are not more toxic than the conventional glass ionomer cements. The aim of the study was to evaluate the toxicity of the RMGICs and compare it to the one of the conventional GICs. The biocompatibility of the materials will also be tested. Finally, the toxicity of the monomers (HEMA and TEGDMA) responsible for the toxicity of this cement will be evaluated.

Material and Methods: An electronic search about the toxicity of the resin modified glass ionomer cements and their monomers was performed on the databases Pubmed, Scopus and Web of science until December 2022.

Results: Of the 560 potentially eligible papers, 9 complied the inclusion criteria and were included in the present review. The different variables assessing the toxicity of the resin modified glass ionomer cements showed cellular morphological changes, significantly higher lactate dehydrogenase (LDH) release, growth inhibition of the cells, increase in the frequency of SCE and chromosomal aberrations and decrease of the proliferation. The variables studying the toxicity of the monomers displayed a reduction in cellular viability, increase of pro apoptotic caspase-3 activity, cellular morphological changes, and DNA migration. The variables studying the biocompatibility do not present augmentation of inflammation, bacterial presence, tertiary dentin formation, odontoblastic changes, and tissue disorganization.

Conclusion: The RMGICs seem to display a higher cytotoxicity and genotoxicity than the GICs due to the toxicity of the monomers HEMA and TEGDMA. However, the biocompatibility of the RMGICs does not seem to be compromised.

Further In vivo and clinical studies are required.

2.KEY WORDS

I. Patients
II. Cells
III. Tissues
IV. Fibroblasts
V. Odontoblasts
VI. Connective tissue
VII. Dental pulp
VIII. Resin modified glass ionomer cements
IX. RMGIC
X. Monomers
XI. hydroxyethyl methacrylate
XII. HEMA
XIII. glass ionomer cements
XIV. GIC
XV. Cytotoxicity
XVI. Toxicity
XVII. Biocompatibility
XVIII. Genotoxicity

3.INTRODUCTION

3.1. Generalities

Dental cements are widely used in the field of dentistry. They can be defined as substances that harden to act as a base, liner, filling material, or adhesive to bind devices and prostheses to tooth structure or to each other's (1). Nowadays, there are many cements used depending on the type of treatment and their composition. The proper selection of the dental cements is a crucial element in the long-term success of the restoration (2).

Most of the time, the hardening of a cement results from the mixture between a powder and a liquid. The objectives of cements are to maintain the restoration in position and prevent microleakage. In order to fulfill these objectives certain requirements must be met: The material must: maintain and protect the tooth tissues; presents a high resistance to tension and pressure; provide lasting bond between the tooth tissues and restorations. It should be biocompatible; impermeability should be ensured, and a layer of minimal thickness should be provided. Moreover, the cements must be easy to use; have low solubility; be radiopaque; have optimal working and curing time. In addition, high resistance, a good viscosity to ensure a complete distribution and a good esthetic are also requirements for the perfect cement. The removal of excess should be as easy as possible (3). Unfortunately, none of the cements used nowadays manage to fulfil all these characteristics.

The cements can be classified according to their uses, composition, and properties. Among the use we can divide them between the temporary cements and the definitive cements.

3.1.1 Definitive cements.

Concerning the definitive cements, we can find:

• The resin-based cements are composed of an organic matrix and filler materials. The organic matrix contains acidic monomers, di-methacrylate monomers (Bis-GMA, UDMA or TEGMA) (4). They serve as luting agents for indirect restorations but are contraindicated if a cement is containing eugenol, like the zinc oxide eugenol (ZOE), currently used in the temporary cementation. Furthermore, these cements show very good aesthetic properties, good physical properties when compared to other cements and a good resistance to compression. However, they do not release fluor, they are expensive, they can be the cause of a possible post operative sensitivity and toxicity, they are hard to manipulate and remove the excess (5,6).

• The zinc polycarboxylate is composed of zinc oxide, bismuth and aluminum oxide as the powder component. The liquid component consists of aqueous polyacrylic acid. This cement demonstrates a good biocompatibility, prevents microleakage and therefore, it reduces pulp sensitivity. However, it has some disadvantages, including the low pH after mixing and the potential for decementation (7).

• The zinc phosphate cements are also available in a powder/liquid form. The powder mainly consists of zinc oxide and magnesium oxide, whereas the liquid is composed of phosphoric acid and water. These cements have a wide range of applications, including the cementation of bridges and crowns (metal or ceramic), orthodontic appliances, cavity liner, etc. The advantages of the use of zinc phosphate cements encompass their easiness to handle, their good biological properties and the good clinical experience. However, these compounds show several disadvantages such as non-adhesive properties, possible pulp irritations due to the acidity, they are soluble in oral fluids, and the absence of antibacterial action (4).

• The glass ionomer and resin-modified glass ionomer cements are also options when it comes to definitive cementation. A more detailed description of these materials is provided in the following paragraphs.

3.1.2 Temporary cements.

The main temporary cements used are the zinc oxide eugenol and the calcium hydroxide.

• The zinc oxide eugenol is a cement composed of zinc oxide, eugenol, and olive oil. It is used in the temporary filling restorations, the cementation of provisional restoration and cavity liner. However, it cannot be used if a resin-based cement is contemporaneously used in the definitive cementation, because it slows down its polymerization and reduce the bond strength (3). The characteristics of this material include the sedation of the pulp, the good clinical experience, a neutral pH and a good marginal seal. The weak mechanical properties, its solubility, the possible cytotoxicity due to the eugenol presence and the low strength constitute its disadvantages (8–10).

• Calcium hydroxide is a temporary cement formed by the reaction of a catalyst paste, containing calcium hydroxide and zinc oxide, with a base paste containing calcium tungstate, calcium phosphate and zinc oxide (11). Its main characteristic is its capacity to stimulate the formation of reparative dentin formation (12). In addition, it provides protection to the pulp against thermal shock and exhibits antibacterial action. Due to these properties, calcium hydroxide is suitable for the lining of cavity, indirect and direct pulp capping, and temporary root canal filling. However, its high solubility, the low mechanical strength, poor sealing, and limited adhesion have hindered its long-term durability and restricted its application (13).

3.2. Glass ionomer cements.

Glass-ionomer cements (GIC) were invented in 1969 in London by Wilson and Kent (14).These materials are composed by an aqueous polyalkenoic acid and fluoroaluminosilicate. Moreover, to be considered a true GIC, the materiel needs to present an acid-base reaction with continuing fluoride release (14). This acid-base reaction initiates when the acid is mixed to the glass powder. During the hardening stage, the silica and phosphate ions, released from the glass,

condensate and form a gel matrix (15). The adhesion appears to be via mechanical interlocking of cement in dentinal tubules and the development of an ion-exchange layer adjacent to the dentine (14).

The release of fluoride is considered one of the most significant characteristics of these cements. According to Sidhu (14), it is believed that the continuous and sustained fluoride release GICs contributes to their caries-inhibiting effect. Fluoride, which is not a matrix-forming species and is incorporated as a flux during the manufacturing process of the glass powder. It can diffuse out the set cement and affect the tissue immediately surrounding the tooth as well as the adjacent surfaces. However, the naturally occurring fluoride is typically depleted within the first few months. Nevertheless, depending on the concentration gradient, the cement can absorb additional fluoride from the surrounding environment. As the GIC acts as a fluoride reservoir throughout the restoration, it enables long-term effectiveness of the material. (16).

The biocompatibility of the cement has also been extensively investigated (14,17). According to these studies, many of the properties exhibited by the cement fall within a biocompatible range. Even if the initial pulp reactions to some products seem to terminate over time, the long-term effects of direct application of GICs on pulp tissue remain uncertain.

Glass ionomer cements can be available in various forms, including hand-mixed powder-liquid, capsule, and paste systems. The initial setting of GICs typically occurs within 2-3 minutes, but complete hardening through chemical reaction may take up to 48 hours (18). Furthermore, it is crucial to follow the recommendations given by the manufacturer regarding powder-liquid ratio in order not to impair the cement’s properties.

Due to the unique properties of this material such as: the fluoride release, the biocompatibility, a direct bonding to the tooth, a good marginal adaptation, and an elasticity similar to the dentin, the GICs are used for a wide range of applications in dentistry. They can be used in direct restorations of teeth, as cavity liner, luting of indirect restorations, luting of crowns and bridges.

However, this material does not have ideal properties for any kind of restorations. Indeed, the limitations of this material are related to the poor mechanical (low wear resistance) and physical (slow setting rate and high solubility) properties, as well as the moisture sensitivity. These characteristics have restricted the GICs from certain clinical applications such as the use as a filling material in stress bearing areas (posterior teeth) (14,18). To improve the features of these cements, the formulations of the glass powder were changed by adding to the polyalkenoic acid: resin, hydroxyapatite, fibers, zirconia, nano sized particles, and other metals (15). Those advances have improved and sometimes worsen the mechanical and chemical properties.

3.3. Resin-modified glass ionomer cements.

Developed in 1991, to overcome the disadvantages associated to the glass ionomer cement like low physical properties and moisture sensitivity, the resin modified glass ionomer cement (RMGIC) is a material that has the same composition of the conventional GIC (glass powder, water, polyacid) with the addition of hydroxyethyl methacrylate (HEMA) or other monomers and initiators, such as camphorquinone, that are involved in the polymerization reaction. Therefore, the acid-base reaction of a glass ionomer cement is supplemented by a polymerization reaction (14,15). Most of the RMGICs have a light-activated polymerization setting reaction.

The combination of resin and GICs provides enhanced resistance to microleakage while retaining the advantages of the conventional GICs such as the fluoride release and the bonding to the tooth. Furthermore, according to Pegoraro et al. (4), the RMGICs are easier to handle and can be bounded to composite. The addition of resin also allows a better aesthetic for the restoration.

Despite the moisture sensitivity has improved with the addition of resin, it remains a limiting factor. Indeed, even though the resin networks reduce the susceptibility of RMGICs to moisture, this material is still prone dehydration and

has the ability to absorb water from the environment (14). Ultimately, this will affect some properties such as the strength or the color stability.

Moreover, the biocompatibility of this cement is compromised by the release of the HEMA component or other monomers (15). HEMA could diffuse in human dentine and a study has shown its cytotoxic effect on pulp's cells (17). Aranha et al. (19) have demonstrated these effects on an odontoblast cell-line. In addition, the dental personal may suffer from the exposure to HEMA. Indeed, the volatility of this component may cause an inhalation and exposure to the eyes and skins.

To ensure safe usage of resin-modified glass ionomer cements and minimize risks, it is important to follow proper handling procedures: :

- Ensure adequate ventilation in the workspace.
- Wear gloves and use appropriate instruments when manipulating the material.
- Avoid the direct contact between RMGICs and patient's mucosa.
- Use a liner to prevent the diffusion of monomers to the pulp.
- Ensure thorough curing of the cement to minimize HEMA release.
- Remove any excess of cements carefully.

3.4. Toxicity of dental cements.

Assessing the biocompatibility of dental materials has always been important to protect both the patient and the dental professionals. It also aids practitioners in selecting the appropriate dental cement (20). Numerous studies, including the one conducted by Bajantri et al (21) have demonstrated that most of the cements used in dentistry caused a cytotoxicity on soft tissues (fibroblasts and pulp) and hard tissue (odontoblast). These cytotoxic effects can potentially impact the long-term outcomes of restorations or prosthesis rehabilitation.

However, not all the cements have showed the same cytotoxicity. The adverse effects caused by the cements are due to the leachable components of the material (22). Therefore, the cytotoxicity of each cement depends on its

composition. The release of ions such as zinc, fluoride, the acidity of the cements or even the release of eugenol in the case of ZOE cements are thought to be the cause of this cytotoxicity.

Concerning the glass ionomer cements, it was initially believed that fluoride was the toxic agent (23). However, other studies showed that the presence of fluoride in GICs is below the level required to cause cytotoxicity. Instead, the cytotoxicity of glass ionomer cements is attributed to other major components (20). A study (24) showed that there was no statistical significance between pulpal response to glass ionomer, zinc polycarboxylate or zinc phosphate cement. Moreover, the toxicity of this cement seems to greatly decrease as the setting time increases.

Some cements have been found to be less cytotoxic compared to others, and their toxicity decreases after a few days. On the other hand, resin-based cements and resin-modified glass ionomer cements have shown higher levels of cytotoxicity. Indeed, those types of cement contain monomers such as HEMA, TEGDMA, BisGMA, etc. These monomers produce a significant cytotoxicity, when exposed to the cells of the gingiva, fibroblasts, dental pulp, periodontal ligaments as well as on the bone's cells (17,25). Many studies (26,27) demonstrated that the hydrophilic monomers HEMA and TEGDMA are found in higher amounts compared to BisGMA or UDMA. According to Cao et al. (28), the higher the number of unreacted substances present in the cured material, the greater the toxicity. Therefore, it is important to adhere to the recommended polymerization times provided by the manufacturer.

The rapid diffusion of these monomers and the ability penetrate the dentin, allow them to come in contact with the pulp cells and have several effects on them. A study showed that monomers (particularly HEMA) could cause major disruption to functioning cells inhibiting proliferation and others biological activities (17,29). Monomers can also affect the differentiation of pulp fibroblast cells into odontoblasts. In addition, they can also inhibit the expression of collagen 1, osteonectin and dentin sialoprotein. Therefore, the formation of mineral nodules within the tooth would be reduced (30). A study conducted on mice (31) have demonstrated that monomers can induce an immunological reaction by

binding to endogenous proteins, potentially leading to the production of autoantibodies. This immune reaction may be responsible for adverse effects and allergies, such as contact dermatitis. Monomers like TEGDMA and HEMA appear to be also able to promote apoptosis in macrophage cell lines and peripheral blood mononuclear cells (32,33). In addition, these effects can have a role in the generation and continuation of hypersensitivity. The process of apoptosis should be considered when evaluating the biocompatibility of a material. Additionally, certain monomers, including HEMA and TEGDMA, have been shown to cause genotoxic effects. These effects include an increase in micronucleus formation and cell-cycle arrest, mediated at least in part by the oxidative DNA damage (34). The genotoxicity from these monomers is supported by an increase of the sister chromatid exchange (SCE) and chromosomal aberrations in cultures of human peripheral blood lymphocytes (35).

4.Justification and hypothesis.

Justification

The glass ionomer cements are widely used in dentistry, but they have certain limitations, as described in the chapter 4.2. Adding resin to enhance is a way to improve these materials (15). The resin-modified glass ionomer cements, indeed, exhibit better physical and mechanical properties. However, if the biocompatibility of the resin-modified glass ionomer cements is compromised, their usage may be less favorable.

Therefore, it is crucial to assess the safety of these materials for both the patient and the dental personnel. Using the conventional glass ionomer cements as a control will allow us to have a reference in terms of acceptable biocompatibility. Additionally, the two types of cements share similarities in terms of composition and the acid-base reaction occurring. Understanding their potential side effects will help the dentists to choose the appropriate cement and can also aid the practitioner in diagnosis or prevention of complications.

The current scientific literature lacks systematic reviews comparing the toxicity at the level cellular or genetic of those two cements. A status report from Sidhu et al. (36) assessed the biocompatibility of GICs but did not specifically compare the two cements. Moreover, several studies have been published since the publication date of that paper in 2001. However, a recent systematic review from Malacarne et al. (37) assessed the genotoxicity of the glass ionomer cements including the resin-modified glass ionomer cement but did not address other types of effects.

With the aforementioned information, it is justified to conduct a systematic review of the literature to evaluate the biocompatibility, the cytotoxicity, and the potential genotoxicity of both the glass ionomer cement and the resin-modified glass ionomer cement.

Hypothesis

The hypothesis of our work considerate that the resin-modified glass ionomer cements exhibit toxic effects and a worse biocompatibility than the conventional glass ionomer cements.

5.Objectives.

General objective

1. Assess the toxicity of the resin modified glass ionomer cement in comparison with the conventional glass ionomer cement.

Specific objectives

1. Determine the toxicity, complications and effects of the monomers entering in the composition of the RMGIC.
2. Evaluate the biocompatibility and safety of use of the resin-modified glass ionomer cements compared to the one of the conventional GICs.

6.MATERIAL AND METHODS.

This systematic review was conducted following the declaration of the PRISMA guide (Preferred Reporting Items for Systematic reviews and Meta-Analysis) (38).

6.1. Identification of the PECO question.

The Medline-PubMed (United States National Library of Medicine), Web of science and Scopus databases were used to search for indexed articles on the biocompatibility of the glass ionomer cements and the resin-modified glass ionomer cements, published up to December 2022 to answer the following question: *In patients or cells exposed to glass ionomer cements, the resin-modified glass ionomer cements have a worse toxicity when compared to the conventional glass ionomer cements ?*

This study question was established according to the structured PECO question. The question format was established in the following way:

- **P** (population): Patients or human cells exposed to glass ionomer cements.
- **E** (exposure): Exposition to resin-modified glass ionomer cements.
- **C** (comparison): Exposition to conventional glass ionomer cements.
- **O** (outcome):
  - O1: Cytotoxicity, Genotoxicity of the resin-modified glass ionomers.
  - O2: Toxicity related to monomers in the composition of the resin-modified glass ionomer cements.
  - O3: Biocompatibility and secondary effects of the resin-modified glass ionomer cements.

6.2. Eligibility criteria

The inclusion criteria were:

- **Study types**: In vivo toxicological studies, in vitro toxicological studies, Observational studies: Case-control studies, Prospective and retrospective cohort studies, cross-sectional studies, Case reports.
- **Type of population**: Patients that were exposed to glass ionomer cements (resin modified and/or conventional), Human cells exposed to glass ionomer cements. Other human experimental models exposed to glass ionomer cements.
- **Type of exposure**: Direct exposure of the patients or human cells to the cements.
- **Type of result variables:** Studied variables include data about the cytotoxicity, the genotoxicity and/or the biocompatibility of the glass ionomer cements and resin-modified glass ionomer cements. As secondary variables, studies including data about the secondary effects of the resin-modified glass ionomer cements. As a tertiary variable, studies related with the toxicity of the monomers in the composition of the resin-modified glass ionomer cements.

The exclusion criteria were systematic reviews, meta-analysis, reviews, letters or comments to the editors and expert reports. Those studies that used animal cells as experimental model. The studies that were treating only other resin-based cements or resin containing materials such as composite were also excluded. The studies evaluating cements that are not commercially available or experimental were also excluded. The studies reporting the cytotoxicity of the fluoride release or other components than monomers were excluded as well. The studies using control groups that were not in the inclusion criteria were removed. Were excluded the studies assessing the toxicity of the cements in other fields than dentistry.

There were no restrictions concerning the date of publication of the articles.

6.3. Information sources and data search.

Research was realized in the following databases: PubMed, Scopus and Web of science. In order to perform this research, the following key words were used: “patients”, “cells”, “experimental models”, “tissues”, “fibroblasts”, “odontoblasts”, “connective tissue”, “dental pulp”, “resin modified glass ionomer cements”, “RMGIC”, monomers, “hydroxyethyl methacrylate”, “HEMA”, “glass ionomer cements”, “GIC”, “conventional glass ionomer cements”, “cytotoxicity”, “toxicity”, “biocompatibility”, “genotoxicity”, “cell death”, “cell damage”, “toxicity test”, “cytotoxicity test”, “inflammation”, inflammatory response”. Booleans operators (AND, OR) were used to combine the key words. Concerning the research on PubMed, controlled terms (MeSH) were used to get the best results.

The research strategy on PubMed was:

((((((((((patients[MeSH Terms]) OR (cells[MeSH Terms])) OR (tissues[MeSH Terms])) OR (fibroblasts[MeSH Terms])) OR (odontoblasts[MeSH Terms])) OR (connective tissue[MeSH Terms])) OR (models, experimental[MeSH Terms])) OR (dental pulp)) AND (((((resin modified glass ionomer cements) OR (RMGIC)) OR (hydroxyethyl methacrylate)) OR (HEMA)) OR (monomers))) AND ((((glass ionomer cements[MeSH Terms])) OR (GIC)) OR (conventional glass ionomer cements))) AND ((((((((((cytotoxicity) OR (toxicity)) OR (cellular damage)) OR (biocompatibility)) OR (cell death[MeSH Terms])) OR (inflammation[MeSH Terms])) OR (acute toxicity test[MeSH Terms])) OR (chronic toxicity test[MeSH Terms])) OR (cytotoxicity test, immunologic[MeSH Terms])) OR (cytotoxicity, immunologic[MeSH Terms])) OR (genotoxicity)) OR (inflammatory response))

The research was made the 05/01/2023 and 98 articles were found.

The research strategy on Scopus was:

(ALL ("patients" OR "cells" OR "tissues" OR "fibroblasts" OR "odontoblasts" OR "connective tissue" OR dental AND pulp OR "models, experimental") AND ALL (resin AND modified AND glass AND ionomer AND cement OR rmgic OR hydroxyethyl AND methacrylate OR monomers) AND ALL ("glass ionomer cement" OR gic OR conventional AND glass AND ionomer AND cement) AND ALL (cytotoxicity OR toxicity OR cellular AND damage OR biocompatibility OR genotoxicity OR "cell death" OR "acute toxicity test" OR "chronic toxicity test" OR "cytotoxicity test, immunologic" OR "cytotoxicity, immunologic" OR "inflammation" OR inflammatory AND response)) AND (EXCLUDE (DOCTYPE , "re"))

The research was made the 05/01/2023 and 307 articles were found.

The research strategy on Web of Science was:

(((TS=(patients OR cells OR tissues OR fibroblasts OR odontoblasts OR connective tissue OR dental pulp OR models, theoretical)) AND TS=(resin modified glass ionomer cement OR RMGIC OR hydroxyethyl methacrylate OR monomers)) AND TS=(glass ionomer cement OR GIC OR conventional glass ionomer cement)) AND TS=(cytotoxicity OR toxicity OR cellular damage OR biocompatibility OR genotoxicity OR cell death OR acute toxicity test OR chronic toxicity test OR cytotoxicity test, immunologic OR cytotoxicity, immunologic OR inflammation OR inflammatory response)

The research was made the 05/01/2023 and 154 articles were found.

To complete the research, a review of the bibliographical references of each study was performed. A cross study of articles potentially interesting for the study was done.

6.4. Process of study selection.

The study selection was realized by two reviewers (MM) and (MIM). During the first step, the articles from the different databases were imported with a software (Mendeley) and duplicated articles were eliminated. Then the titles of the articles were reviewed to eliminate the irrelevant articles. The next step consisted of reviewing the summary and abstract of the remaining articles and do a selection according to the type of study, the type of intervention and the results variables. In the final step, the articles were filtered through the full reading of each one of them. Data were extracted to confirm the eligibility of an article. During each step, a third reviewer (CC) could be consulted if the two reviewers weren't able to resolve disagreement through discussion.

6.5. Data extraction.

The following information was extracted by the studies that entered in the inclusion criteria and disposed in tables according to the experimental models (Humans, cells). The tables are organized in function of the authors of the study (with date of publication), time of exposure to the cements, experimental model, number of participants or sample, type of study (In vivo toxicological studies, in vitro toxicological studies, observational studies), type of variables and methods used, type of toxicity (cytotoxicity, genotoxicity…), effects of the cements (Inflammation, presence of microorganisms…).

Principal variables:

Cytotoxicity: Cellular viability will be estimated by fraction unaffected, which is the proportion of cells that were not affected from the agent and derived from the following equation: fraction unaffected = ODx/ODc. ODx represented the test optical density, and ODc represented the control optical density. The results will be expressed in percentage. Another variable indicative for the cellular toxicity is the inhibitory concentration 50 (IC50), that will be expressed as a range of concentration when showed. The TC50 will also be taken in account to measure

the cytotoxicity and will be expressed in concentration. The relative percentage of Lactate Dehydrogenase released from the cells will be taken in consideration as a necrosis parameter. Apoptosis will be reported with the caspase 3 activity. The difference in the cell morphology after the exposure to the material will also be taken in account to measure the cytotoxicity. Concerning the oxidative stress, the reactive oxygen species (ROS) will be reported as fluorescence intensity or in percentage, depending on the data availability. Malonaldehyde (MDA) levels are an oxidative indicator of the membrane integrity, they will be reported in percentage or as nmol/mgprot.

Genotoxicity: To understand the genotoxicity, will be taken in account the results of DNA migration by tail moment according to Olive (39) (OTM). The frequency of Sister chromatid exchange (SCE), Chromosomal aberrations (CAs) per metaphases and the assessment of proliferation rate index (PRI) are also going to be taken in account.

Secondary variables:

Toxic effects of the monomers present in the composition of the RMGIC: Monomers mainly HEMA are one of the main causes of the reported toxicity of these cements. The variables that will be applied are the same reports in principal variables but applied to the monomers.

Tertiary variables:

Secondary effects of the possible toxicity of the glass ionomer cements and resin-modified glass ionomer cements: To understand the levels of toxicity in pulp for those studies which reported it, we have also taken in consideration histological variables such as: inflammation; odontoblastic changes; tissue disorganization; reactionary dentin formation and presence of microorganisms.

6.6 Quality and risk of bias assessment.

The assessment of the risk of bias was done by two reviewers (MM) and (MIM), to analyze the methodological quality of the articles included.

For the assessment of the observational studies, the Newcastle-Ottawa scale (40) was used. A study with more than 6 stars on the Newcastle-Ottawa scale was a “low risk of bias” study. A study with less than 6 stars on the Newcastle-Ottawa scale was a “high risk of bias” study.

To assess the risk of bias of the in vitro studies, the modified scale of ARRIVE and CONSORT (41) was used. We also used the QUIN tool (42), specifically used to assess the risk of bias of in vitro studies conducted in dentistry.

6.7 Data synthesis.

To summarize and compare the data extracted from the different articles, the mean value of the principal variables and the error (standard error of the mean and standard deviation) were regrouped when possible, according to the study group.

7.RESULTS.

7.1 Study selection and flow chart.

A total of 559 Articles were obtained from the initial search process:

PubMed (n=98), Scopus (n=307) and the Web of Science (n=154). Moreover, one article was obtained through snowballing search, checking the reference lists of journal articles among the identified articles with the handsearching. After the

elimination of the duplicates and the screening by titles and abstracts, 23 articles were identified as potentially eligible. The full text articles were obtained and evaluated, in order to get the total of articles included in the present systematic review. As a result, 9 articles met the inclusion criteria and were chosen (Fig.1). The excluded articles as well as the reason of their exclusions are presented in table 1.

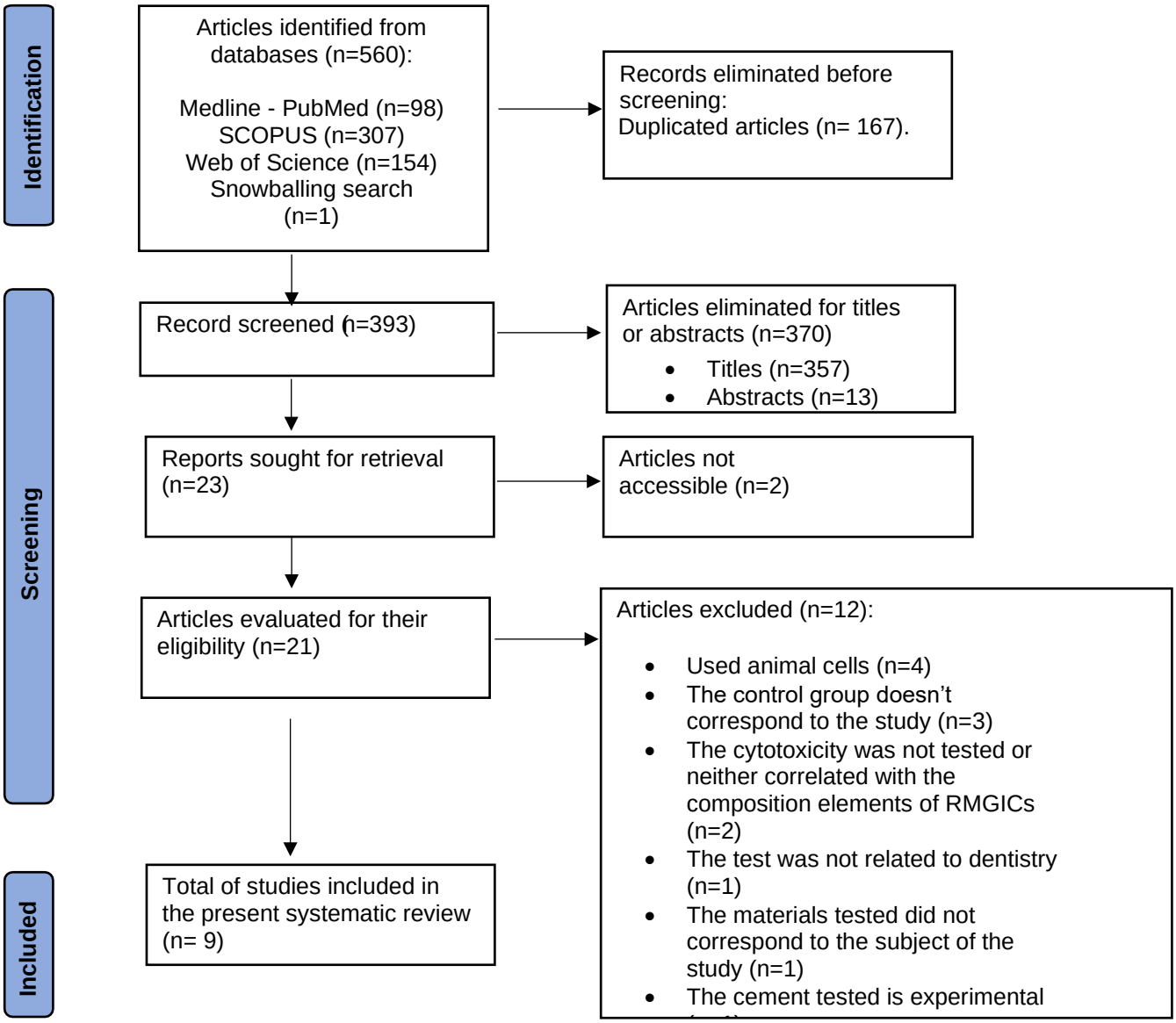

Fig. 1. Diagram of the flow chart and process of the articles selection during the systematic review.

Table 1. Articles excluded.

AUTHORS AND YEAR	TITLES	REASONS OF EXCLUSION
Costa C. 2011 (43)	Pulp response after application of two resin modified glass ionomer cements (RMGICs) in deep cavities of prepared human teeth	The control group was a calcium hydroxide cement.
Alizadehgharib S 2017 (44)	Effects of the methacrylate/acrylate monomers HEMA, TEGDMA, DEGDA, and EMA on the immune system	Most of the monomers evaluated don't enter in the composition of RMGIC.
Geurtsen W. 1998 (29)	Residual monomer additive release and variability in cytotoxicity of light-curing glass-ionomer cements and compomers	The comparison is made with compomers.
Potiprapanpong W. 2021 (45)	Monomer Conversion, Dimensional Stability, Biaxial Flexural Strength, Ion Release, and Cytotoxicity of Resin-Modified Glass Ionomer Cements Containing Methacrylate-Functionalized Polyacids and Spherical Pre-Reacted Glass Fillers	The cement tested is an experimental cement.
Lucksanasombool P. 2002 (46)	Effects of glass ionomer cements on bone tissue	The test was realized for use in orthopedic surgery.
Kanjevac T. 2012 (47)	Cytotoxic effects of glass ionomer cements on human dental pulp stem cells correlate with fluoride release	The cytotoxicity was correlated with the fluoride release.
Williams D. 2013 (48)	2-Hydroxyethyl methacrylate inhibits migration of dental pulp stem cells	The cytotoxicity was not tested.
Dos Santos R. 2012 (49)	Evaluation of cytotoxicity and degree of conversion of glass ionomer cements reinforced with resin	Used animal cells.
De Souza Costa C. 2003 (50)	In vitro cytotoxicity of five glass-ionomer cements	Used animal cells.
Souza P. 2006 (51)	In vitro cytotoxicity and in vivo-biocompatibility of contemporary resin-modified glass-ionomer cements	Used animal cells.
Sasanaluckit P. 1993 (52)	Biocompatibility of glass ionomer cements	Used animal cells.
Stanislawski L. 1999 (53)	Factors responsible for pulp cell cytotoxicity induced by resin-modified glass ionomer cements	The comparison was made with another cement and a metal reinforced GIC.

7.2 Analysis of the characteristics of the reviewed studies.

Among the 9 articles selected for the present systematic revision, 5 assessed the biocompatibility / toxicity of the resin-modified glass ionomer cements (35,54–57), 4 described the effects and toxicity of the monomers in the resin-modified glass ionomer cements (58–61). All the articles assessing the toxicity of the resin-modified glass ionomer cements offered a comparison with at least one conventional glass ionomer cements. 7 articles are in vitro studies (35,55,56,58–61), and 2 articles are controlled clinical trials (54,57).

Concerning the controlled clinical trials, the histological response of the pulp to the materials tested was evaluated. A total of 56 teeth (human premolars) were evaluated.

Concerning the in vitro studies, all the cells exposed to the materials are human cells. One article (56) used mouse cells in addition to the human cells but for the present systematic study only the results of the tests performed on the human cells were taken into consideration.

The articles (55,56,58,60) used human gingival fibroblasts to test the materials. Bakopoulou et al. (35) used human lymphocytes. The study of Baldion et al. (59) used human odontoblasts like cells and Kleinsasser et al. (61) used human parotid gland tissue and lymphocytes.

Table 2. General characteristics of the study

Authors and year	Study type	Materials tested	Experimental models	Time of exposure	Type of toxicity reported	Variables studied
Eskandarizadeh A. 2015 (54)	Controlled clinical trial	RMGIC (Vivaglass) GIC (Ionocid) Calcium hydroxide (Dycal)	30 human premolars	5 and 30 days	NONE	Odontoblastic changes Inflammatory response Tertiary dentin (TD) formation Presence of microorganisms
Ribeiro A. 2020 (57)	Controlled clinical trial	RMGIC (Riva LC) GIC (Riva SC) Calcium hydroxide (Dycal)	26 human premolars + 4 controls	7 and 30 days	NONE	Inflammatory reaction Tissue disorganization Reactionary dentin formation Bacteria
Rodriguez I. 2013 (55)	In vitro study	RMGIC (Vitrebond) GIC (Ketac-molar)	Human gingival fibroblasts (HGF)	72 hours	Cytotoxicity	Morphological changes (Phase contrast microscopy) Lactate dehydrogenase release (LDH)
Bakopoulou A. 2009 (35)	In vitro study	RMGIC (RelyX Lutting and Vitrebond) GIC (Ketac Cem and Fuji I) Resin cements (Variolink and Panavia)	Normal cultured human lymphocytes	72 hours	Genotoxicity Cytotoxicity	Sister chromatid exchange (SCE) Chromosomal aberrations (CAs) Assessment of proliferation using proliferation rate index (PRI)
Leyhausen G. 1998 (56)	In vitro study	RMGICS (Ionoseal, Vitrebond, Compoglass) GIC (Ketac fil)	Human primary fibroblasts attached to the gingiva (HGF)	48h	Cytotoxicity	Morphology and growth characteristics by phase contrast microscopy.

Teti G. 2015 (58)	In vitro study	HEMA TEGDMA	Human gingival fibroblasts (HGF)	24 – 48 – 72 hours	Cytotoxicity	Cell viability assay by optical density (percentage of untreated cells) Expression of protein markers for apoptosis and autophagy (by western blot) Morphological changes (by transmission electron microscopy)
Baldion P. 2021 (59)	In vitro study	HEMA TEGDMA	Human odontoblast like cells (hOLCs)	3, 6, 9, 12, 18 and 24 hours	Cytotoxicity	Cell viability evaluation by calcein Lactate dehydrogenase release assay Oxidative damage assessment (ROS production, Malonaldehyde (MDA) levels) Capsase-3 activity
Falconi M. 2007 (60)	In vitro study	HEMA	Human gingival fibroblasts (HGF)	24 – 72 – 96 hours	Cytotoxicity	HGF viability by MTT assay Morphological changes by FEISEM
Kleinsasser N. 2006 (61)	In vitro study	TEGDMA UDMA HEMA	Human parotid gland tissue and lymphocytes		Cytotoxicity Genotoxicity	DNA migration by tail moment according to Olive (39) (OTM)

7.3 Assessment of methodological quality and risk of bias

The two controlled clinical trials (54,57) presented a score on the Newcastle-ottawa (40) superior or equal at 6 (table 4), therefore they can be considered as “low-risk” of bias studies.

Concerning the in vitro studies (35,55,56,58–61), 4 studies showed a moderate risk of bias (55,56,60,61) whereas the three other studies (35,58,59) presented a low risk of bias according to the modified ARRIVE and CONSORT scale (table 3) (41).

Table 3: Measurement of the risk of bias of in vitro studies with the modified Arrive and Consort scale (acceptability range 21-28)

Author/Year	Title	Abstract	Introduction	Introduction	Methods: Study design	Methods: Experimental procedures	Methods: Sample Size	Methods: Statisitcal method	Results	Discussion	Declaration of potential conflicts and disclosure of liability	Publication in a peer-reviewed journal	Risk of bias
Rodriguez I. 2013 (55)	1	2	3	1	2	3	2	3	3	1	1	1	=23
Bakopulou A. 2009 (35)	1	3	2	3	3	3	3	3	3	2	1	1	=27
Leyhausen G. 1998 (56)	1	2	3	2	2	2	2	3	2	1	1	0	=22
Teti G. 2015 (58)	1	3	3	2	2	3	3	2	2	2	1	1	=25
Baldion P. 2021 (59)	1	3	3	3	2	2	3	2	3	2	1	1	=26
Falconi M. 2007 (60)	1	2	3	2	2	3	3	3	2	2	0	0	=23
Kleinsasser N. 2006 (61)	1	2	2	2	3	2	3	2	3	2	0	0	= 22

	Case definition	Representativity	Controls selection	Controls definition	Comparability	Comparability	Exposure check	Same method for both groups	Drop-out rate	Total
Eskandarizadeh A. 2015 (54)	☆	-	☆	-	☆	☆	☆	☆	-	6
Ribeiro A. 2020 (57)	☆	-	☆	☆	☆	☆	☆	☆	-	7

Fig. 2. Measurement of the risk of bias of non-randomised observational studies with the Newcastle-Ottawa scale

7.4 Synthesis of the results.

7.4.1 Toxicity of the resin-modified glass ionomer cements.

Regarding the toxicity of the resin-modified glass ionomer cements, 3 studies (35,55,56) displayed data over the toxicity of the resin-modified glass ionomer cements when compared to the conventional glass ionomer cements.

The in vitro studies (35,55,56) each used different methods and variables to evaluate the toxicity of resin-modified glass ionomer and glass ionomer, therefore it was not possible to calculate a mean value between each study.

In Rodriguez et al. (55) study, 52.2±20.4 % (median 52.9%) of the human gingival fibroblasts exposed to glass ionomer cements have the spindle shape of normal living cells. For the human gingival fibroblasts exposed to resin modified glass ionomer cement, only 3.9±5.0 % (median1.5%) of the cells showed a normal morphology.

The lactate dehydrogenase release was also evaluated. The cells exposed to RMGIC have a significantly higher LDH release mean 38.46% ± 7.29 (median 31,6%) than the cells exposed to GIC 11.04% ± 21.69 (median 3%).

In Leyhausen et al. (56) article, three resin modified glass ionomer cements were tested: Ionoseal, Vitrebond, Compoglass. In addition, one glass ionomer cement was tested, Ketac fil. Human gingival fibroblasts were incubated

for 48h with two extracts: the first 24-h extract (day 1/24 h) and the second 24-h extract on day 9 (day 9/24h).

For the first 24-h extract (day 1/24 h), the cells exposed to Ionoseal (IS) had a growth of 88% ± 4.7, cells exposed to Vitrebond (VB) had a growth of 15.7% ± 13.9, the fibroblasts exposed to compoglass (CG) had a growth of 107% ± 9 and the cells exposed to glass ionomer cement Ketac fil (KF) had a growth of 107% ± 19.5.

For the second 24-h extract on day 9 (day9/24), cells exposed to IS showed a growth of 100.9% ± 5.8, the cells exposed to VB exhibited a growth of 68.8% ± 6.8 and the cells exposed to the KF had a growth of 103% ± 4.

The study of Bakopoulou et al (35) determined the genotoxicity of two resin modified glass ionomer cements (Rely X and Vitrebond) compared to two glass ionomer cement (GC Fuji I and Ketac Cem), using the frequencies of Sister chromatid exchange (SCE), Chromosomal aberrations (CAs), and Proliferation rate index of lymphocyte primary culture.

The controls of this study had a mean SCE of 8.6, a mean PRI of 2.67 and a mean CAs of 0.33.

The original extract of glass ionomer cement GC Fuji I (FJ) had a mean value of frequencies of SCE of 11.06. The proliferation rate index mean value was 2.60. It produced a mean of 2.6 per 100 metaphases for the Chromosomal aberrations.

The original extract of Ketac Cem (KC) had a mean value of frequencies of SCE of 10.53. The proliferation rate index (PRI) mean value was 2.65. It produced a mean value of 9.3 per 100 metaphases for Chromosomal aberrations (CAs).

Severe cytogenetic effects were caused by the eluates of the two RMGICs, Rely X (RX) and Vitrebond (VB). RX had to be diluted at a ratio of 1:16, whereas VB eluates needed to be diluted at a ratio of 1:128.

At that dilution, RX eluates caused an increase in the frequencies of Sister Chromatid Exchange (mean 15.15). At the same dilution, RX eluates also induced a statistically significant increase CAs (mean 20,3 CAs per 100 metaphases). The mean PRI values of the RX was 2.29.

On the other hand, VB at a dilution of 1:128 not only caused a significant increase in SCE (mean 17.5) and a decrease in PRI values (mean 1.73), but also a considerable increase in the frequencies of CAs (mean of 209 CAs per 100 metaphases).

Table 4. Toxicity of the resin modified glass ionomer cements.

Authors (with date)	Variables	Results		
		RMGIC		**GIC**
Rodriguez I. 2013 (55)	***Morphology of HGFs***	3.9%±5.0 (median 1.5%) had a normal morphology.		52.2%±20.4 (median 52.9%) had normal morphology.
	Lactate dehydrogenase release (LDH)	**Cells exposed to RMGIC:** Mean 38.46% ± 7.29 (median 31.6%)		**Cells exposed to GIC:** Mean 11.04% ± 21.69 (median 3%)
Leyhausen G. 1998 (56)	***Growth of HGFs***	First 24-h extract (day 1/24 h)	**RMGICs:** Ionoseal (IS): Growth of 88%±4.7. Vitrebond (VB): Growth of 15.7%±13.9. Compoglass (CG): Growth of 107%±9.	**GIC:** Ketac fil (KF): Growth of 107%±19.5.
		Second 24-h extract on day 9 (day9/24)	**RMGICs:** IS: Growth 100.9%±5.8. VB: Growth 68.8%±6.8. CG: ND	**GIC:** KF: Growth 103%±4

Bakopoulou A. 2009 (35)	***Frequencies of sister chromatid exchange (SCE)***	**RMGICs:** Rely X (RX): Increased SCE exchange 20.65±1.16; 16.40±0.80; 8.40±0.55 (Mean 15.15) Vitrebond (VB): Increased SCE exchange 19.3±1.12; 17.20± 1.42; 16.10± 0.89 (Mean 17.5)	**GICs:** GC Fuji I (FJ): mean value 11.06 Ketac Cem (KC) mean value 10.53.
	Chromosomal aberrations (Cas)	**RMGICs:** RX: Increased CAs 28 per 100 metaphases; 11 per 100 metaphases; 1 per 100 metaphases (Mean 20.3 CAs per 100 metaphases) VB: Increased CAs; 278 per 100 metaphases; 90 per 100 metaphases; 259 per 100 metaphases (Mean 209 CAs per 100 metaphases)	**GICs:** FJ: mean 2.6 CAs for 100 metaphases KC: mean 9.3 CAs for 100 metaphases
	Proliferation rate index (PRI)	**RMGICs** RX: decreased PRI: 1.91 2.13 2.83 Mean value: 2.29 VB: decreased PRI: 1.65 1.85 1.70 Mean value: 1.73	**GICs** FJ: Mean value 2.60 KC: Mean value 2.65

7.4.2 Toxicity of the monomers entering in the composition of the resin-modified glass ionomer cements.

In the present systematic review, 4 studies described the potential toxicity of some of the monomers contained in the composition of the resin modified glass ionomer cements (58–61).

In the three studies that measured the cells viability (58–60), Teti G et al (58) found that the IC50 of the resin monomers HEMA and TEGDMA (when tested on the human gingival fibroblasts) were respectively of 3.79 mmol/L and 3.46 mmol/L. Falconi et al (60) found a TC50 (concentration responsible for 50% of cell death) for HEMA (when tested of human gingival fibroblast) of 5.83 mmol/L.

The cell viability was also expressed on percentage. The study (59) that determined the viability of human odontoblasts like cells (hOLCs) found that at 3mM being equal to 3 mmol/L, after 24 hours, the cell viability of the hOLCs is reduced to 81% for HEMA and 86% for TEGDMA. The study (60) found similar results on the viability of HGF exposed to HEMA. After 24h, 72h and 96 h of exposure, at 3 mmol/L the cell viability is respectively reduced to 85%, 40% and 35% showing a significant cytotoxicity.

The apoptosis of the cells is another variable measured by the caspase 3 activity in 2 studies (58,59). Both studies showed an increase in pro-apoptotic capsase-3 activity whether it is for hOLCs or HGFs and for both monomers tested (HEMA(58,59) and TEGDMA(58)).

The HGFs exposed to HEMA and TEGDMA exhibited morphological changes over time (58,60).
Falconi et al (60) exposed the HGFs to 3mmol/L of HEMA during 24h, 72h, and 96h. After 24h of exposure, HGFs showed a morphology comparable to the untreated cells with a fibroblastic shape. However, after 72 h of HEMA exposure, the number of HGFs was moderately reduced, and cells lost the fibroblastic morphology. Finally, after 96 h of exposure to the monomer, there was a notable reduction in the number of cells, and the fibroblastic morphology had almost disappeared.

In Teti G et al study (58), the HGFs were exposed to HEMA and TEGDMA.
For the HGFs exposed to HEMA, after 24h of exposure, cells showed a fibroblast-like morphology with autophagic vesicles and empty vacuoles in the cytoplasm. After 48h of HEMA exposure, a well-preserved morphology was still present but there was an increased number of autophagic vesicles in the cytoplasm. Finally, at 72h of HEMA exposure, cells showed a round or polygonal morphology with several autophagic vesicles in the cytoplasm, condensed masses of chromatin in the nucleus, and enlarged nuclear envelope.
Concerning the HGFs exposed to TEGDMA. After 24h of exposure, the mitochondria and the cells were damaged, while the nucleus was well preserved. After 48 h of TEGDMA exposure, condensed chromatin masses, damaged mitochondria, and enlarged Golgi apparatus were observed. After 72 h of TEGDMA exposure, cells showed a clear morphological pattern of necrosis. Those morphological changes are proof of the cytotoxicity of the monomers.

The work of Bladion et al (59) also assessed the LDH release and oxidative damage (ROS and MDA levels) in hOLCs exposed to HEMA and TEGDMA. Concerning the LDH release, the monomers induced membrane damage and resulted in the release of LDH after only 3 hours of treatment. When the cells were exposed to HEMA at a concentration of 3mmol/L for 24 hours, the LDH release was 36%. In the case of TEGDMA at the same concentration and duration, the LDH release was 24%.
Regarding the ROS production, the greatest production occurred after 3 h of exposure to TEGDMA for each concentration, however in an effort to make the comparison easier with the other variables measured at a concentration of 3mmol/L only the ROS production at 3mmol/L will be taken in account: 16×10^3 ROS. ROS production levels were the highest after 6 h in HEMA treated hOLCs: 7×10^3 ROS for 3mmol/L. Exposure of the cells to monomers beyond 6 h did not induce any changes in ROS activity.
The MDA levels after 9 h of HEMA exposure, were significantly increased: 1.9nmol for 3mmol/L. At 9 h of treatment for TGDMA: 1.9 nmol for 3 mmol/L.
Those increase of LDH release, ROS production and MDA levels display the cytotoxicity of the monomers.

Finally, the study (61) evaluated the genotoxicity of several monomers when tested on human parotid gland tissues and lymphocytes.

In parotid gland tissue cells, with TEGDMA concentrations at 10^{-5} M the mean OTM is 3.0, UDMA at 10^{-7} M have a mean OTM of 4.1, and HEMA at 10^{-3} M have a mean OTM of 5.1, significant enhancements of DNA migration in comparison to the negative control (Mean OTM: 2.3) were achieved.

In lymphocytes, the lowest concentrations with significant DNA migration were 10^{-3} M for TEGDMA with a mean OTM of 3.4, 10^{-7} M for UDMA with a mean OTM of 2.6, and 10^{-5} M for HEMA had a mean OTM of 2.3. The negative control had a mean OTM of 1.7. Those results demonstrate the genotoxicity of the monomers.

Table 5. Toxicity of the monomers entering in the composition of the resin-modified glass ionomer cements.

Variables	Authors with year	Doses	Results	
			HEMA	**TEGDMA** and **UDMA**
Cells viability	Teti G. 2015 (58)	-	IC50 of HGFs: 3.79mmol/L	IC50 of HGFs for **TEGDMA:** 3.46mmol/L
	Falconi M. 2007 (60)	3mmo/L	TC50 on HGFs: 5.83 mmol/L. 24h: cell viability reduced to 85% 72h: cell viability reduced to 40% 96h: cell viability reduced to 35%	
	Bladion P. 2021 (59)	3mmol/L	24h: Cell viability of the hOLCs reduced to 81%	24h: Cell viability of the hOLCs reduced to 86% for **TEGDMA**.
Apoptosis	Teti G. 2015 (58)	3mmol/L	Increase of caspase-3 activity.	Caspase-3 activity increase for **TEGDMA.**
	Bladion P. 2021 (59)	3mmol/L	Increase of caspase-3 activity.	
Morphological changes	Teti G. 2015 (58)	3mmol/L	24h: cells showed a fibroblast-like morphology. 48h: Well-preserved morphology still present, increased number of autophagic vesicles in the cytoplasm. 72h: Round or polygonal morphology, several autophagic vesicles in cytoplasm, condensed masses of chromatin in the nucleus, and enlarged nuclear envelope.	For HGFs exposed to **TEGDMA:** 24h: Nucleus well preserved, but the cells showed slight damage. 48h: Condensed chromatin masses, damaged mitochondria, and enlarged Golgi apparatus. 72h: Cells showed a clear morphological pattern of necrosis.

	Falconi M. 2007 (60)	3mmol/L	24h exposure to **HEMA**: Morphology comparable to the untreated cells with a fibroblastic shape. 72h: Number of HGFs reduced, cells lost the fibroblastic morphology. 96h: Reduction in the number of cells, fibroblastic morphology had almost disappeared.	
LDH release	Bladion P. 2021 (59)	3mmol/L	24h: LDH release was 36%.	24h for **TEGDMA:** LDH release was 24%.
ROS production			After 6h: 7 x 10^3 ROS	After 3h for **TGDMA:** 16 x 10^3 ROS
MDA levels			After 9 h MDA levels: 1.9nmol	After 9h **TEGDMA** MDA levels: 1.9nmol
DNA migration by tail moment	Kleinsasser N. 2006 (61)	TEGDMA: 10^{-5} M and 10^{-3} M UDMA: 10^{-7} M HEMA: 10^{-3} M and 10^{-5} M	In parotid gland tissue: **HEMA** at 10^{-3} M mean OTM of 5.1 Negative control mean OTM: 2.3 In lymphocytes: **HEMA** at10^{-5} M mean OTM of 2.3. Negative control mean OTM of 1.7.	In parotid gland tissue: **TEGDMA** at 10^{-5} M mean OTM is 3.0 **UDMA** at 10^{-7} M mean OTM of 4.1 Negative control mean OTM: 2.3 In lymphocytes: **TEGDMA** at10^{-3} M mean OTM of 3.4 **UDMA** at 10^{-7} M mean OTM of 2.6 Negative control mean OTM of 1.7.

7.4.3 Biocompatibility and secondary effects of the resin-modified glass ionomer cements.

Regarding the biocompatibility, safety of use and secondary effects of the resin-modified glass ionomer cements. The controlled clinical trial of Eskandarizadeh et al and Ribeiro et al (54,57) showed:

After 5 and 7 days, over 10 specimens of RMGIC, 3 specimens presented no inflammation, 5 showed a mild inflammation, 2 a moderate inflammation and none displayed severe inflammation. In comparison with the GIC, over 10 specimens, 4 showed no inflammation, 5 a mild inflammation, 1 a moderate inflammation and none displayed severe inflammation.

In terms of bacterial presence, over 10 specimens of RMGIC, 8 showed no presence of bacteria and 2 showed a presence of bacteria. For the GIC, over 10 specimens, all showed no presence of bacteria.
Concerning the formation of tertiary dentin, for the 10 specimens of RMGIC, 9 exhibited no formation and 1 exhibited a mild formation. The 10 GICs showed no formation of tertiary dentin.

For the odontoblastic changes over the 5 specimens of RMGIC, 3 showed mild odontoblastic changes and 2 a moderate to severe odontoblastic changes. Among the 5 GIC, 1 showed no changes, 3 showed mild odontoblastic changes and 1 showed moderate to severe odontoblastic changes.
Concerning the tissue disorganization, among the 5 specimens of RMGIC, 1 demonstrated no tissue disorganization and 4 showed a mild tissue disorganization. For the 5 samples of GIC, 2 showed no tissue disorganization and 3 a mild tissue disorganization.

After 30 days, among the 10 specimens of RMGIC tested for the inflammation, 6 showed no inflammation and 4 a mild inflammation. For the 10

specimens of GIC, 8 showed no inflammation, 1 showed a mild inflammation and 1 a severe inflammation.

Concerning the presence of bacteria: for the 10 specimens of RMGIC, 8 showed no presence of bacteria and 2 showed a mild presence of bacteria. In the GIC, 9 had no presence of bacteria and 1 a mild presence of bacteria.

For the formation of tertiary dentin, In RMGIC, 5 showed no formation and 5 showed a mild formation of tertiary dentin. For the GIC, 8 specimens had no formation of tertiary dentin and 2 showed a formation of tertiary dentin.

Regarding the odontoblastic changes, over the 5 teeth exposed to RMGIC, 2 showed no changes, 1 specimen had mild changes and 2 samples moderate to severe changes. Over the 5 GIC, 4 presented no changes and 1 specimen presented mild changes.

Finally concerning the tissue disorganization, among the 5 specimens exposed to RMGIC, 3 showed no tissue disorganization and 2 a mild tissue disorganization. Among the 5 specimens exposed to GIC, 4 had no tissue disorganization and 1 a mild tissue disorganization.

Table 6. Effects of the resin modified and conventional glass ionomers on the pulp.

Histological event	Material	Periods								Total of specimen
		5-7 days				30 days				
		No	Mild	Moderate	Severe	No	Mild	Moderate	Severe	
Inflammatory response	RMGICs	3	5	2	0	6	4	0	0	20
	GICs	4	5	1	0	8	1	0	1	20
Presence of bacteria	RMGICs	8	2	0	0	8	2	0	0	20
	GICs	10	0	0	0	9	1	0	0	20
Odontoblastic changes	RMGICs	3	0	2	0	2	1	2	0	10
	GICs	1	3	1	0	4	1	0	0	10
Formation of tertiary dentin	RMGICs	9	1	0	0	5	5	0	0	20
	GICs	10	0	0	0	8	2	0	0	20
Tissue disorganization	RMGICs	1	4	0	0	3	2	0	0	10
	GICs	2	3	0	0	4	1	0	0	10

RMGICs used: Riva Light Cure and Vivaglass
GICs used: Riva Self Cure and Ionocid

8.DISCUSSION.

The present systematic review exhibits information, based on the scientific evidence, related to the toxicity of the resin-modified glass ionomer cements compared to the conventional glass ionomer cements. The objective of this study was, in a first place, to assess the toxicity of the resin modified glass ionomer cement in comparison with the conventional glass ionomer cement. In a second time this study evaluates the toxicity related to the monomers entering in the composition of the RMGIC. Finally, the biocompatibility of the resin-modified glass ionomer cement was determined.

8.1 Toxicity of the resin-modified glass ionomer cements.

Among the results of the present systematic review, the 3 in vitro studies describing the toxicity of the resin-modified glass ionomer cements (35,55,56) showed similar results. Despite the different variables and different cell types used, they all conclude that the RMGICs tested cause greater alterations to the cells than the conventional glass ionomer cements tested. To determine the dose of cements used for each study, the authors used the manufacturer recommendations to get results as close as they could be in clinical situations.

The study from Rodriguez et al. (55) explains that the alterations cause necrosis of the cells. Indeed, the increase of lactate dehydrogenase release in the culture medium indicates that the membrane stability has been disrupted. The cells exposed to RMGIC have a LDH release significantly higher (31.6%) than the cells exposed to GIC (3%). Other similar studies on animal models showed the same results than the selected studies. De Souza et al (50) in vitro assays on odontoblasts cell line MDPC-23, confirms through a 72h evaluation with a cell viability assessment (through a methyltetrazolium assay) that the RMGICs tested have a cytotoxic effect. Oliva A. et al also demonstrate that the RMGIC Vitremer 3M, exhibits a great cytotoxicity toward the osteoblastic cells compared to the

GICs (62). The same studies attribute these adverse reactions to leaching of a component named HEMA.

Furthermore, the morphological changes and growth inhibition observed respectively in two studies (55,56) confirm similar observation. Only a small percentage of human fibroblasts exposed to resin modified glass ionomer cement (3.9%) present a normal cellular morphology when more than half of the cells exposed to glass ionomer cements (52.2%) have a normal morphology. The study from Costa et al (50) and Aranha et al (63) found comparable results. Indeed, the odontoblast-cell line exposed to RMGICs exhibited alterations characterized by a changed rounded morphology and disruption of the plasma membrane in these studies.

The study from Bakopoulou et al (35) demonstrate another type of toxicity. Hence, the significant increase of the frequencies of SCE with a mean value of 15.15 for RX and mean value of 17.5 for VB, shows the extensive genotoxic effects of the RMGICs. The significant increase of CAs in cultures of peripheral blood lymphocytes (mean value of 20.3 CAs per 100 metaphases for RX and mean value of 209 CAs per 100 metaphases for VB) and the decrease of the relevant PRI values in a dose-dependent manner is also a proof of the genotoxicity that exhibits the resin modified glass ionomer cements, whereas the GICs caused only minor cytogenetic effects. The major cytogenetic toxicity of several RMGICs compared with that of conventional GICs is supported by other studies (64,65). Stea et al. (64) measure the SCE of human peripheral lymphocytes when exposed to conventional glass ionomer (Ketac cem) and resin-modified glass ionomer (Vitrebond). They conclude that VB displays a strong cytotoxicity that results in genotoxicity even one week after the polymerization. The study (65) reports that VB also have a cytotoxic and genotoxic effects on hamster ovary cells with a reduction in cell viability up to 40% and significant DNA migration when compared to conventional glass ionomer cements. Once again, the methacrylate monomers such as HEMA and TEGDMA seem to be responsible. The papers (34,61) also demonstrate that HEMA and TEGDMA, have been found to cause increase in micronucleus formation and cell-cycle arrest causing genotoxic effects.

Among all the studies, the resin modified glass ionomer cement VB seems to be the most cytotoxic and genotoxic of all the cements studied. This can be explained by the high concentration of components elutable from the polymerized material (resinous monomers) contained in the VB. Several studies also place the VB as a cytotoxic cement (66). The pronounced effects caused by RMGICs and more particularly VB could be attributed to monomeric HEMA, found to be released immediately, despite the light-curing of this material according to the manufacturer's recommendation (67,68). Therefore, the results of the present systematic review and in numerous other studies indicate that the cytotoxic alterations induced by VB must be taken seriously and should be considered when deciding about the material to use.

As previously mentioned, most of the authors in the present scientific literature relate the toxicity of the resin-modified glass ionomer cements described in the previous paragraphs, to their monomers mainly HEMA and TEGDMA present in their composition in various concentration. In support of this conclusion, chromatography tests have demonstrated the release of HEMA and TEGDMA from RMGICs (67,69). However, the quantity of released dental monomers a patient may be exposed has yet to be determined. According to other studies (70,71), HEMA and TEGDMA monomers are able to diffuse into the pulp at significant concentration. HEMA could reach concentrations as high as 1.5–8 mmol/L in the pulp. Whereas TEGDMA concentration after its diffusion through the dentin in deep cavities could be around 4 mmol/L.

The fact that the selected studies and those present in the current scientific literature used different cell types and different commercial brands of cements to determine the toxicity, reinforce the value of the results. However, these results imply the need to proceed to an extended investigation over the materials used in dentistry.

8.2 Toxicity of the monomers entering in the composition of the resin-modified glass ionomer cements.

The toxicity of those resin monomers has been analyzed in several ways. They seem to reduce the cell viability like explained in the studies (59,60). The results are similar after 24h with a mean viability of 83% for fibroblasts exposed to HEMA and a viability of 86% for cells exposed to TEGDMA for a concentration of 3mmol/L for both monomers. Moreover, the viability of the cells decreases over time of exposure. The two studies found an IC50 for HEMA of 3.79mmol/L and an IC50 for TEGDMA of 3.46mmol/L, the TC50 for HEMA was: 5.83 mmol/L. A study from Bouillaguet et al (72) reported a TC50 of HEMA of 3.6 mmol/L in 3T3 fibroblasts. The study from Rakich et al (73) demonstrated a TC50 value of 10 mmol/L for HEMA. This difference can be explained by the difference of sensitivities between the cell types. However, the fact that there is a percentage of cell death, but also other living cells suggest that the monomers HEMA and TEGDMA can activate some cell protective mechanisms. Gallorini et al. (74) displayed, the activation of the Nrf2-controlled enzymatic antioxidants allows the cells to re-establish homeostasis and counteract the cell damage. However, with medium or high concentrations of monomer, the protective mechanisms of the cells can be hindered, and cell death may happen.

The significant increase of caspase-3 activity in cells exposed to HEMA (58,59) and TEGDMA (58) is a sign of apoptosis of the cells. This can be explained by the fact that HEMA and TEGDMA can interact with the plasma membrane. However, the monomers do not appear to induce lysis of the membrane and are able to penetrate the cytoplasm to reach mitochondria. They can then deteriorate the function of these cellular components and cause metabolic dysfunction or even apoptosis.

Moreover, the morphology of the cells exposed to HEMA and TEGDMA is also another variable showing the cytotoxicity of the monomers. Indeed, after few hours of exposition, the cells show an altered morphology, a decrease in their number and even pattern of necrosis (58,60). It seems that the cytotoxicity of these methacrylates is mainly generated by radical metabolites and may be reduced by antioxidants (75).

The cytotoxicity of the monomers HEMA and TEGDMA has also been demonstrated by the report of Baldion et al (59) showing the increase of LDH release, ROS production and MDA levels. In addition, the significant increase of LDH release in cells exposed to HEMA and TEGDMA indicate a disruption in the membrane stability.

The results from Kleinsasser et al (61) conclude that the methacrylates TEGDMA, UDMA and HEMA induce a significant DNA migration. Indeed, the values of OTM for each material are significantly higher than the OTM of the negative control with a mean value for each monomer >2 Whether it is in lymphocytes or in parotid gland tissue cells. These genotoxic effects suggest a tumor initiating potency even though there is no predisposition of the cells. The study (76) exhibits comparable results with an OTM >2 for HEMA and TEGDMA indicating the existence of a genotoxicity. The study (77) explains the genotoxicity of TEGDMA by the fact that TEGMA caused cell cycle delays and thereby might influence cell growth and differentiation.

8.3 Biocompatibility and secondary effects of the resin-modified glass ionomer cements

Another aim of this study was to evaluate the biocompatibility of the RMGICs and compare it to the biocompatibility of GICs.

The findings from trials conducted by Eskandarizadeh et al and Ribeiro et al (54,57) provide valuable insights into variables such as the inflammatory response, bacterial presence, formation of tertiary dentin, odontoblastic changes, and tissue disorganization associated with the use of RMGIC as a liner in deep cavities.

Concerning the inflammatory response, the results from our study show that after 5 and 7 days, most specimens present some degree of inflammation regardless of the cement type. Nevertheless, a higher proportion of RMGIC specimens presented mild to moderate inflammation compared to GIC specimens. Therefore, it appears that in the early stages RMGICs seem to induce

a stronger inflammation than GICs. Overall, the rate of severe inflammation was low for both groups and traduce an acceptable level of biocompatibility for both materials. The study of Mousavinasab et al. (78) shares the same results with a significantly higher pulp inflammation at 7 days than at 30 and 60 days. This can be explained by the fact that these residual monomers (HEMA and TEGDMA) are present within the material the first few days after the lining (29).

Concerning the presence of bacteria, the results show a lack of bacteria and only a small proportion exhibiting mild presence. However, this bacterial presence is most likely due to an inadequate isolation during the restorative procedure and do not come from a leaking in the cavities.

The tertiary dentin formation is an important parameter to evaluate the long-term effects of a material. Our study showed that there were no differences statistically relevant between the GICs and RMGICs. This indicates that the RMGICs have only a limited impact on the pulpal response. Moreover, a study (79) showed that time is more important than the material regarding the tertiary dentin formation with an average of 28 days needed for TD formation. Our studies during only 30 days, the time was not long enough to properly measure the TD formation. Nonetheless, the study (78) that measured the TD formation for 7 days, 30 days and 60 days display similar results than the selected studies with a lack of TD formation regardless of the material used.

Other variables such as the odontoblastic changes and the tissue disorganization provoked by the cements provide insights on the impact of these materials on the pulp tissue and therefore the biocompatibility. These two variables, as the previous ones, show an overall acceptable level of biocompatibility whether it is for RMGICs or GICs.

8.4 Limitations of the study

The present review clearly lacks in vivo studies or clinical studies to really assess the toxicity of the resin modified glass ionomer cements and their

monomers in the clinical situation. Over the 9 studies selected 7 were in vitro studies.

In the studies (54,57), It is worth noting that the selected studies present analysis based on a very small number of samples which may limit the value of the results.

Another limitation was the huge variety of cement tested all coming from different commercial brands and all with different composition and concentration of resin monomers. Even if we find a higher toxicity in the resin-modified glass ionomer cements when compared to conventional glass ionomer cements, some RMGICs showed a toxicity much higher than others. Therefore, each cement must be evaluated individually with in vivo studies to assess the clinical implications.

The important number of variables used to evaluate the toxicity of the cements or resin monomers is also a limitation. Indeed, it makes the comparison difficult and gives more interpretations that can lead to possible mistakes.

Different type of cells was used to assess the toxicity. However, the lethal concentration of a specific dental material can vary with different cell lines and between the same types of cells obtained from different donors.

Furthermore, there is a lack of clinical studies with a longer follow up of subjects exposed to these materials and more studies assessing the toxicity of these materials in clinics are needed to assess the safety of these materials.

9.CONCLUSION.

General Conclusions.

1. In vitro, the resin-modified glass ionomer cements display a higher cytotoxicity and genotoxicity than the conventional glass ionomer cements.

Specific conclusions.

2. The resin monomers composing the RMGICs appear to be the cause of the reported toxicity and therefore cause a cytotoxicity and genotoxicity.

3. Despite the toxicity reported in vitro, the biocompatibility of the RMGICs on the clinical aspect does not seem to be compromise. Further investigations evaluating each cement individually and in clinical situation must be performed.

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12.ANNEXES

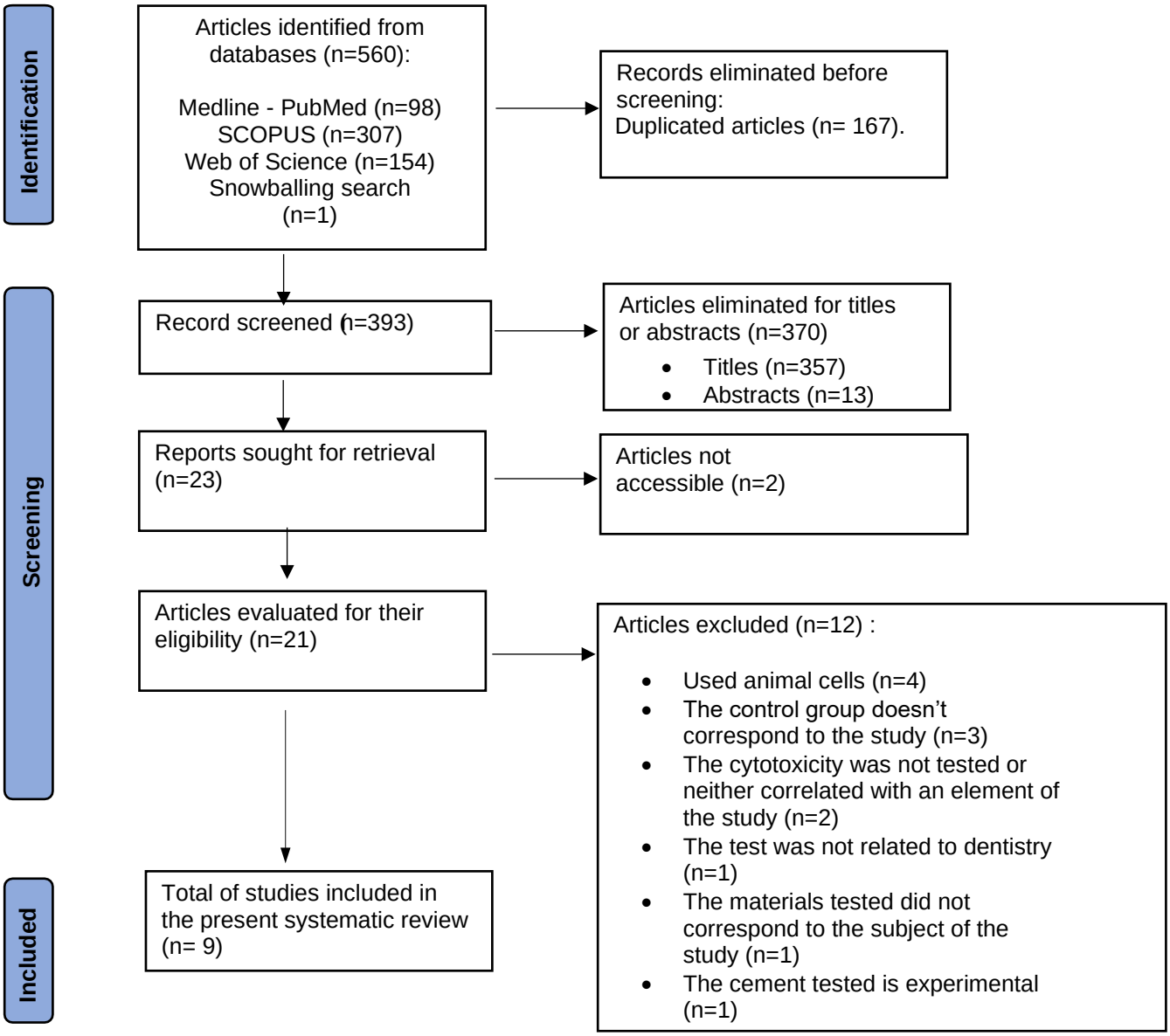

Fig. 1. Diagram of the flow chart and process of the articles selection during the systematic review.

Table 1. Articles excluded.

AUTHORS AND YEAR	TITLES	REASONS OF EXCLUSION
Costa C. 2011 (43)	Pulp response after application of two resin modified glass ionomer cements (RMGICs) in deep cavities of prepared human teeth	The control group was a calcium hydroxide cement.
Alizadehgharib S 2017 (44)	Effects of the methacrylate/acrylate monomers HEMA, TEGDMA, DEGDA, and EMA on the immune system	Most of the monomers evaluated don't enter in the composition of RMGIC.
Geurtsen W. 1998 (29)	Residual monomer additive release and variability in cytotoxicity of light-curing glass-ionomer cements and compomers	The comparison is made with compomers.
Potiprapanpong W. 2021 (45)	Monomer Conversion, Dimensional Stability, Biaxial Flexural Strength, Ion Release, and Cytotoxicity of Resin-Modified Glass Ionomer Cements Containing Methacrylate-Functionalized Polyacids and Spherical Pre-Reacted Glass Fillers	The cement tested is an experimental cement.
Lucksanasombool P. 2002 (46)	Effects of glass ionomer cements on bone tissue	The test was realized for use in orthopedic surgery.
Kanjevac T. 2012 (47)	Cytotoxic effects of glass ionomer cements on human dental pulp stem cells correlate with fluoride release	The cytotoxicity was correlated with the fluoride release.
Williams D. 2013 (48)	2-Hydroxyethyl methacrylate inhibits migration of dental pulp stem cells	The cytotoxicity was not tested.
Dos Santos R. 2012 (49)	Evaluation of cytotoxicity and degree of conversion of glass ionomer cements reinforced with resin	Used animal cells.
De Souza Costa C. 2003 (50)	In vitro cytotoxicity of five glass-ionomer cements	Used animal cells.
Souza P. 2006 (51)	In vitro cytotoxicity and in vivo-biocompatibility of contemporary resin-modified glass-ionomer cements	Used animal cells.
Sasanaluckit P. 1993 (52)	Biocompatibility of glass ionomer cements	Used animal cells.
Stanislawski L. 1999 (53)	Factors responsible for pulp cell cytotoxicity induced by resin-modified glass ionomer cements	The comparison was made with another cement and a metal reinforced GIC.

Table 2. General characteristics of the study

Authors and year	Study type	Materials tested	Experimental models	Time of exposure	Type of toxicity reported	Variables studied
Eskandarizadeh A. 2015 (54)	Controlled clinical trial	RMGIC (Vivaglass) GIC (Ionocid) Calcium hydroxide (Dycal)	30 human premolars	5 and 30 days	NONE	Odontoblastic changes Inflammatory response TD formation Presence of microorganisms
Ribeiro A. 2020 (57)	Controlled clinical trial	RMGIC (Riva LC) GIC (Riva SC) Calcium hydroxide (Dycal)	26 human premolars + 4 controls	7 and 30 days	NONE	Inflammatory reaction Tissue disorganization Reactionary dentin formation Bacteria
Rodriguez I. 2013 (55)	In vitro study	RMGIC (Vitrebond) GIC (Ketac-molar)	Human gingival fibroblasts (HGF)	72 hours	Cytotoxicity	Morphological changes (Phase contrast microscopy) Lactate dehydrogenase release (LDH)
Bakopoulou A. 2009 (35)	In vitro study	RMGIC (RelyX Lutting and Vitrebond) GIC (Ketac Cem and Fuji I) Resin cements (Variolink and Panavia)	Normal cultured human lymphocytes	72 hours	Genotoxicity Cytotoxicity	Sister chromatid exchange (SCE) Chromosomal aberrations (CAs) Assessment of proliferation using proliferation rate index (PRI)
Leyhausen G. 1998 (56)	In vitro study	RMGICS (Ionoseal, Vitrebond, Compoglass) GIC (Ketac fil)	Human primary fibroblasts attached to the gingiva (HGF)	48h	Cytotoxicity	Morphology and growth characteristics by phase contrast microscopy.

Teti G. 2015 (58)	In vitro study	HEMA TEGDMA	Human gingival fibroblasts (HGF)	24 – 48 – 72 hours	Cytotoxicity	Cell viability assay by optical density (percentage of untreated cells) Expression of protein markers for apoptosis and autophagy (by western blot) Morphological changes (by transmission electron microscopy)
Baldion P. 2021 (59)	In vitro study	HEMA TEGDMA	Human odontoblast like cells (hOLCs)	3, 6, 9, 12, 18 and 24 hours	Cytotoxicity	Cell viability evaluation by calcein Lactate dehydrogenase release assay Oxidative damage assessment (ROS production, Malonaldehyde (MDA) levels) Capsase-3 activity
Falconi M. 2007 (60)	In vitro study	HEMA	Human gingival fibroblasts (HGF)	24 – 72 – 96 hours	Cytotoxicity	HGF viability by MTT assay Morphological changes by FEISEM
Kleinsasser N. 2006 (61)	In vitro study	TEGDMA UDMA HEMA	Human parotid gland tissue and lymphocytes		Cytotoxicity Genotoxicity	DNA migration by tail moment according to Olive (39) (OTM)

Table 3: Measurement of the risk of bias of in vitro studies with the modified Arrive and Consort scale (acceptability range 21-28)

Author/Year	Title	Abstract	Introduction	Introduction	Methods: Study design	Methods: Experimental procedures	Methods: Sample Size	Methods: Statisitcal method	Results	Discussion	Declaration of potential conflicts and disclosure of liability	Publication in a peer-reviewed journal	Risk of bias
Rodriguez I. 2013 (55)	1	2	3	1	2	3	2	3	3	1	1	1	=23
Bakopulou A. 2009 (35)	1	3	2	3	3	3	3	3	3	2	1	1	=27
Leyhausen G. 1998 (56)	1	2	3	2	2	2	2	3	2	1	1	0	=22
Teti G. 2015 (58)	1	3	3	2	2	3	3	2	2	2	1	1	=25
Baldion P. 2021 (59)	1	3	3	3	2	2	3	2	3	2	1	1	=26
Falconi M. 2007 (60)	1	2	3	2	2	3	3	3	2	2	0	0	=23
Kleinsasser N. 2006 (61)	1	2	2	2	3	2	3	2	3	2	0	0	= 22

	Case definition	Representativity	Controls selection	Controls definition	Comparatibility	Comparatibility	Exposure check	Same method for both groups	Drop-out rate	Total
Eskandarizadeh A. 2015 (54)	☆	-	☆	-	☆	☆	☆	☆	-	6
Ribeiro A. 2020 (57)	☆	-	☆	☆	☆	☆	☆	☆	-	7

Fig. 2. Measurement of the risk of bias of non-randomised observational studies with the Newcastle-Ottawa scale

Table 4. Toxicity of the resin modified glass ionomer cements.

Authors (with date)	Variables	Results		
		RMGIC		**GIC**
Rodriguez I. 2013 (55)	***Morphology of HGFs***	3.9%±5.0 (median 1.5%) had a normal morphology.		52.2%±20.4 (median 52.9%) had normal morphology.
	Lactate dehydrogenase release (LDH)	**Cells exposed to RMGIC:** Mean 38.46% ± 7.29 (median 31.6%)		**Cells exposed to GIC:** Mean 11.04% ± 21.69 (median 3%)
Leyhausen G. 1998 (56)	***Growth of HGFs***	First 24-h extract (day 1/24 h)	**RMGICs:** Ionoseal (IS): Growth of 88%±4.7. Vitrebond (VB): Growth of 15.7%±13.9. Compoglass (CG): Growth of 107%±9.	**GIC:** Ketac fil (KF): Growth of 107%±19.5.
		Second 24-h extract on day 9 (day9/24)	**RMGICs:** IS: Growth 100.9%±5.8. VB: Growth 68.8%±6.8. CG: ND	**GIC:** KF: Growth 103%±4

Bakopoulou A. 2009 (35)	***Frequencies of sister chromatid exchange (SCE)***	**RMGICs:** Rely X (RX): Increased SCE exchange 20.65±1.16; 16.40±0.80; 8.40±0.55 (Mean 15.15) Vitrebond (VB): Increased SCE exchange 19.3±1.12; 17.20± 1.42; 16.10± 0.89 (Mean 17.5)	**GICs:** GC Fuji I (FJ): mean value 11.06 Ketac Cem (KC) mean value 10.53.
	Chromosomal aberrations (Cas)	**RMGICs:** RX: Increased CAs 28 per 100 metaphases; 11 per 100 metaphases; 1 per 100 metaphases (Mean 20.3 CAs per 100 metaphases) VB: Increased CAs; 278 per 100 metaphases; 90 per 100 metaphases; 259 per 100 metaphases (Mean 209 CAs per 100 metaphases)	**GICs:** FJ: mean 2.6 CAs for 100 metaphases KC: mean 9.3 CAs for 100 metaphases
	Proliferation rate index (PRI)	**RMGICs** RX: decreased PRI: 1.91 2.13 2.83 Mean value: 2.29 VB: decreased PRI: 1.65 1.85 1.70 Mean value: 1.73	**GICs** FJ: Mean value 2.60 KC: Mean value 2.65

Table 5. Toxicity of the monomers entering in the composition of the resin-modified glass ionomer cements.

Variables	Authors with year	Doses	Results	
			HEMA	**TEGDMA** and **UDMA**
Cells viability	Teti G. 2015 (58)	-	IC50 of HGFs: 3.79mmol/L	IC50 of HGFs for **TEGDMA:** 3.46mmol/L
	Falconi M. 2007 (60)	3mmo/L	TC50 on HGFs: 5.83 mmol/L. 24h: cell viability reduced to 85% 72h: cell viability reduced to 40% 96h: cell viability reduced to 35%	
	Bladion P. 2021 (59)	3mmol/L	24h: Cell viability of the hOLCs reduced to 81%	24h: Cell viability of the hOLCs reduced to 86% for **TEGDMA**.
Apoptosis	Teti G. 2015 (58)	3mmol/L	Increase of caspase-3 activity.	Caspase-3 activity increase for **TEGDMA.**
	Bladion P. 2021 (59)	3mmol/L	Increase of caspase-3 activity.	
Morphological changes	Teti G. 2015 (58)	3mmol/L	24h: cells showed a fibroblast-like morphology. 48h: Well-preserved morphology still present, increased number of autophagic vesicles in the cytoplasm. 72h: Round or polygonal morphology, several autophagic vesicles in cytoplasm, condensed	For HGFs exposed to **TEGDMA:** 24h: Nucleus well preserved, but the cells showed slight damage. 48h: Condensed chromatin masses, damaged mitochondria, and enlarged Golgi apparatus. 72h: Cells showed a clear morphological pattern of necrosis.

			masses of chromatin in the nucleus, and enlarged nuclear envelope.	
	Falconi M. 2007 (60)	3mmol/L	24h exposure to **HEMA**: Morphology comparable to the untreated cells with a fibroblastic shape. 72h: Number of HGFs reduced, cells lost the fibroblastic morphology. 96h: Reduction in the number of cells, fibroblastic morphology had almost disappeared.	
LDH release	Bladion P. 2021 (59)	3mmol/L	24h: LDH release was 36%.	24h for **TEGDMA:** LDH release was 24%.
ROS production			After 6h: 7 x 10^3 ROS	After 3h for **TGDMA:** 16 x 10^3 ROS
MDA levels			After 9 h MDA levels: 1.9nmol	After 9h **TEGDMA** MDA levels: 1.9nmol
DNA migration by tail moment	Kleinsasser N. 2006 (61)	TEGDMA: 10^{-5} M and 10^{-3} M UDMA: 10^{-7} M HEMA: 10^{-3} M and 10^{-5} M	In parotid gland tissue: **HEMA** at 10^{-3} M mean OTM of 5.1 Negative control mean OTM: 2.3 In lymphocytes: **HEMA** at10^{-5} M mean OTM of 2.3. Negative control mean OTM of 1.7.	In parotid gland tissue: **TEGDMA** at 10^{-5} M mean OTM is 3.0 **UDMA** at 10^{-7} M mean OTM of 4.1 Negative control mean OTM: 2.3 In lymphocytes: **TEGDMA** at10^{-3} M mean OTM of 3.4 **UDMA** at 10^{-7} M mean OTM of 2.6 Negative control mean OTM of 1.7.

Table 6. Effects of the resin modified and conventional glass ionomers on the plulp.

Histological event	Material	Periods								Total of specimen
		5-7 days				30 days				
		No	Mild	Moderate	Severe	No	Mild	Moderate	Severe	
Inflammatory response	RMGICs	3	5	2	0	6	4	0	0	20
	GICs	4	5	1	0	8	1	0	1	20
Presence of bacteria	RMGICs	8	2	0	0	8	2	0	0	20
	GICs	10	0	0	0	9	1	0	0	20
Odontoblastic changes	RMGICs	3	0	2	0	2	1	2	0	10
	GICs	1	3	1	0	4	1	0	0	10
Formation of tertiary dentin	RMGICs	9	1	0	0	5	5	0	0	20
	GICs	10	0	0	0	8	2	0	0	20
Tissue disorganization	RMGICs	1	4	0	0	3	2	0	0	10
	GICs	2	3	0	0	4	1	0	0	10

RMGICs used: Riva Light Cure and Vivaglass
GICs used: Riva Self Cure and Ionocid

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Front page
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	3-8
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	13
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	15
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	16-17
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	16-17
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	18
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	18
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	18
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	20
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	18-19
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	18

Section and Topic	Item #	Checklist item	Location where item is reported
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	21-22
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	22
Study characteristics	17	Cite each included study and present its characteristics.	23-25
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	26-27
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	26
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	27-37
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	38-43
	23b	Discuss any limitations of the evidence included in the review.	43-44

Section and Topic	Item #	Checklist item	Location where item is reported
	23c	Discuss any limitations of the review processes used.	43-44
	23d	Discuss implications of the results for practice, policy, and future research.	45
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

RELEASE AND TOXICITY OF GLASS IONOMER AND RESIN-MODIFIED GLASS IONOMER CEMENTS IN DENTISTRY. A SYSTEMATIC REVIEW.

Running title: Release and toxicity of glass ionomer and resin-modified glass ionomer cements in dentistry.

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Abstract

Introduction: The combination of resin and glass ionomer cements provides enhanced resistance to microleakage while retaining the advantages of the conventional GICs such as the fluoride release and the bonding to the tooth. However, it is important to assess that the resin-modified glass ionomer cements are not more toxic than the conventional glass ionomer cements.

Aim: The aim of the study was to evaluate the toxicity of the RMGICs and compare it to the one of the conventional GICs. The biocompatibility of the materials will also be tested. Finally, the toxicity of the monomers (HEMA and TEGDMA) responsible for the toxicity of this cement will be evaluated.

Material and Methods: An electronic search about the toxicity of the resin modified glass ionomer cements and their monomers was performed on the databases Pubmed, Scopus and Web of science until December 2022.

Results: Of the 560 potentially eligible papers, 9 complied the inclusion criteria and were included in the present review. The different variables assessing the toxicity of the resin modified glass ionomer cements showed cellular morphological changes, significantly higher lactate dehydrogenase (LDH) release, growth inhibition of the cells, increase in the frequency of SCE and chromosomal aberrations and decrease of the proliferation rate index. The variables studying the toxicity of the monomers displayed a reduction in cellular viability, increase of pro apoptotic caspase-3 activity, cellular morphological changes, and DNA migration. The variables studying the biocompatibility do not present augmentation of inflammation, bacterial presence, tertiary dentin formation, odontoblastic changes and tissue disorganization.

Conclusion: The RMGICs seem to display a higher cytotoxicity and genotoxicity than the GICs due to the toxicity of the monomers HEMA and TEGDMA. However, the biocompatibility of the RMGICs does not seem to be compromised.

Further In vivo and clinical studies are required.

Key words: *Resin-modified glass ionomer, glass ionomer, monomers, toxicity, biocompatibility*

Introduction

Dental cements are widely used in the field of dentistry. They can be defined as substances that harden to act as a base, liner, filling material or adhesive to bind devices and prostheses to tooth or to each other's. Nowadays, there are many cements used depending on the type of treatment and their composition. The proper selection of dental cements is a crucial element in the long-term success of the treatment (1).

Glass ionomer cements (GICs) are a type of cement created in 1969 that presents an acid-base reaction with continuing fluoride release providing a caries-inhibiting effect (2).

In 1991, when hydroxyethyl methacrylate (HEMA) or other monomers, as well as initiators, were added to the composition of GICs, the resin-modified glass ionomer cements (RMGICs) were created. Those additions permitted to improve the low physical properties and moisture sensitivity of the GICs (2,3).

However, the addition of monomers seems to have a negative impact on the toxicity of the RMGICs. A study showed that monomers (particularly HEMA) could cause major disruption to functioning cells inhibiting proliferation and others biological activities (4).

The current scientific literature lacks systematic review comparing the cytotoxicity or genotoxicity of those two cements. Only a status report assessing the biocompatibility of the GICs (5) and a recent systematic review explaining the genotoxicity without explaining other type of toxicities (6) have been found.

The aim of the present systematic review was to systematically review the question: In patients or cells exposed to glass ionomer cements, the resin-modified glass ionomer cements have a higher toxicity when compared to the conventional glass ionomer cements?

This was firstly done by assessing the toxicity of the resin-modified glass ionomer cements and compare it to the one of the glass ionomer cements. Then the toxicity of the monomers (mainly HEMA and TEGDMA) composing the RMGICs was assessed. We finished by determining if the biocompatibility of the materials is affected.

Material and Methods

This systematic review was conducted following the declaration of the PRISMA guide (Preferred Reporting Items for Systematic reviews and Meta-Analysis) (7).

Focus question:

This study question was established according to the structured PECO question. The question format was established in the following way:

- **P** (population): Patients or human cells exposed to glass ionomer cements.
- **E** (exposure): Exposition to resin-modified glass ionomer cements.
- **C** (comparison): Exposition to conventional glass ionomer cements.
- **O** (outcome):
  - O1: Cytotoxicity, Genotoxicity of the resin-modified glass ionomers.
  - O2: Toxicity related to monomers in the composition of the resin-modified glass ionomer cements.
  - O3: Biocompatibility and secondary effects of the resin-modified glass ionomer cements.

Eligibility criteria:

The inclusion criteria were:

- **Study types**: In vivo toxicological studies, in vitro toxicological studies, Observational studies: Case-control studies, Prospective and retrospective cohort studies, cross-sectional studies, Case reports.
- **Type of population**: Patients that were exposed to glass ionomer cements (resin modified and/or conventional), Human cells exposed to glass ionomer cements. Other human experimental models exposed to glass ionomer cements.
- **Type of exposure**: Direct exposure of the patients or human cells to the cements.
- **Type of result variables:** Studied variables include data about the cytotoxicity, the genotoxicity and/or the biocompatibility of the glass ionomer cements and resin-modified glass ionomer cements. As secondary variables, studies including data about the secondary effects of the resin-modified glass ionomer cements. As a tertiary variable, studies related with the toxicity of the monomers in the composition of the resin-modified glass ionomer cements.

The exclusion criteria were systematic reviews, meta-analysis, reviews, letters or comments to the editors and expert reports. Those studies that used animal cells as experimental model. The studies that were treating only other resin-based cements or resin containing materials such as composite were also excluded. The studies evaluating cements that are not commercially available or experimental were also excluded. The studies reporting the cytotoxicity of the fluoride release or other components than monomers were excluded as well.

There were no restrictions concerning the date of publication of the articles.

Information sources and data searches:

Research was realized in the following databases: PubMed, Scopus and Web of science. In order to perform this research, the following key words were used: “patients”, “cells”, “experimental models”, “tissues”, “fibroblasts”, “odontoblasts”, “connective tissue”, “dental pulp”, “resin modified glass ionomer cements”, “RMGIC”, monomers, “hydroxyethyl methacrylate”, “HEMA”, “glass ionomer cements”, “GIC”, “conventional glass ionomer cements”, “cytotoxicity”, “toxicity”, “biocompatibility”, “genotoxicity”, “cell death”, “cell damage”, “toxicity test”, “cytotoxicity test”, “inflammation”, inflammatory response”. Booleans operators (AND, OR) were used to combine the key words. Concerning the research on PubMed, controlled terms (MeSH) were used to get the best results.

The research strategy on PubMed was:

((((((((((patients[MeSH Terms]) OR (cells[MeSH Terms])) OR (tissues[MeSH Terms])) OR (fibroblasts[MeSH Terms])) OR (odontoblasts[MeSH Terms])) OR (connective tissue[MeSH Terms])) OR (models, experimental[MeSH Terms])) OR (dental pulp)) AND (((((resin modified glass ionomer cements) OR (RMGIC)) OR (hydroxyethyl methacrylate)) OR (HEMA)) OR (monomers))) AND ((((glass ionomer cements[MeSH Terms])) OR (GIC)) OR (conventional glass ionomer cements))) AND ((((((((((cytotoxicity) OR (toxicity)) OR (cellular damage)) OR (biocompatibility)) OR (cell death[MeSH Terms])) OR (inflammation[MeSH Terms])) OR (acute toxicity test[MeSH Terms])) OR (chronic toxicity test[MeSH Terms])) OR (cytotoxicity test, immunologic[MeSH Terms])) OR (cytotoxicity, immunologic[MeSH Terms])) OR (genotoxicity)) OR (inflammatory response))

To complete the research, a review of the bibliographical references of each study was performed. A cross study of articles potentially interesting for the study was done.

Process of study selection:

The study selection was realized by two reviewers (MM) and (MIM). During the first step, the articles from the different databases were imported with a software (Mendeley) and duplicated articles were eliminated. Then the titles of the articles were reviewed to eliminate the irrelevant articles. The next step consisted of reviewing the summary and abstract of the remaining articles and do a selection according to the type of study, the type of intervention and the results variables. In the final step, the articles were filtered through the full reading of each one of them. Data were extracted to confirm the eligibility of an article. During each step, a third reviewer (CC) could be consulted if the two reviewers weren't able to resolve disagreement through discussion.

Data extraction:

The following information was extracted by the studies that entered in the inclusion criteria and disposed in tables according to the experimental models (Humans, cells). The tables are organized in function of the authors of the study (with date of publication), time of exposure to the cements, experimental model, number of participants or sample, type of study (In vivo toxicological studies, in vitro toxicological studies, observational studies), type of variables and methods used, type of toxicity (cytotoxicity, genotoxicity…), effects of the cements (Inflammation, presence of microorganisms…).

Quality and risk of bias assessment:

The assessment of the risk of bias was done by two reviewers (MM) and (MIM), to analyze the methodological quality of the articles included.

For the assessment of the observational studies, the Newcastle-Ottawa scale (8) was used. A study with more than 6 stars on the Newcastle-Ottawa scale was a "low risk of bias" study. A study with less than 6 stars on the Newcastle-Ottawa scale was a "high risk of bias" study.

To assess the risk of bias of the in vitro studies, the modified scale of ARRIVE and CONSORT (9) was used.

Data synthesis:

To summarize and compare the data extracted from the different articles, the mean value of the principal variables and the error (standard error of the mean and standard deviation) were regrouped when possible, according to the study group.

Results:

Study selection:

A total of 559 Articles were obtained from the initial search process:

PubMed (n=98), Scopus (n=307) and the Web of Science (n=154). Moreover, one article was obtained through the manual search. After the elimination of the duplicates and the screening by titles and abstracts, 23 articles were identified as potentially eligible. The full text articles were obtained and evaluated, to get the total of articles included in the present systematic review. As a result, 9 articles met the inclusion criteria and were chosen (Fig.1).

Study characteristics:

Among the 9 articles selected for the present systematic revision, 5 assessed the biocompatibility / toxicity of the resin-modified glass ionomer cements (10–14). 4 described the effects and toxicity of the monomers in the resin-modified glass ionomer cements (15–18). 7 articles are in vitro studies (10,11,14–18), and 2 articles are controlled clinical trials (12,13). Concerning the controlled clinical trials, the histological response of the pulp to the materials tested was evaluated. A total of 56 teeth (human premolars) were evaluated.

Concerning the in vitro studies, all the cells exposed to the materials are human cells (Table 1).

Risk of bias:

The two controlled clinical trials (12,13), are considered as “low risk” of bias studies (Fig. 2).

Concerning the in vitro studies (10,11,14–18), 4 studies showed a moderate risk of bias (11,14,17,18) whereas the three other studies (10,15,16) presented a low risk of bias (table 2).

Synthesis of results:

Toxicity of resin-modified glass ionomer cements:

Regarding the toxicity of the resin-modified glass ionomer cements, 3 studies (10,11,14) displayed data over the toxicity of the resin-modified glass ionomer cements compared to the conventional glass ionomer cements.

Human gingival fibroblasts exposed to GICs showed a spindle shape characteristic of normal living cells in 52.2% ± 20.4 (median 52.9%) of the cells. However, when exposed to RMGICs, only 3.9% ± 5.0 (median 1.5%) of the cells displayed a normal morphology (14). The cells exposed to RMGICs exhibited a significantly higher lactate dehydrogenase (LDH) release, with a mean of 38.46% ± 7.29 (median 31.6%), compared to the cells exposed to GICs, which had a mean LDH release of 11.04% ± 21.69 (median 3%) (14).

Three different RMGICs (Ionoseal, Vitrebond, Compoglass) and one GIC (Ketac fil) were tested on human gingival fibroblasts to assess the growth inhibition. fibroblasts exposed to Ionoseal (IS) exhibited a growth of 88% ± 4.7, Vitrebond (VB) showed a growth of 15.7% ± 13.9, Compoglass (CG) had a growth of 107% ± 9, and Ketac fil (KF) displayed a growth of 107% ± 19.5 (11).

The genotoxicity of two resin modified glass ionomer cements (Rely X and Vitrebond) compared to two glass ionomer cement (GC Fuji I and Ketac Cem) was determined. GC Fuji I (FJ) had mean values of frequencies of sister chromatid exchange (SCE) of 11.06, proliferation rate index (PRI) of 2.60 and 2.6 for Chromosomal aberrations. Ketac Cem (KC) had a mean value of frequencies of SCE of 10.53. The proliferation rate index mean value was 2.65. It produced a mean value of 9.3 Chromosomal aberrations.

Rely X (RX) eluates caused an increase in the frequencies of SCE (mean 15.15). It also induced a statistically significant increase CAs (mean 20,3 CAs per 100 metaphases). PRI values of the RX was 2.29. Vitrebond (VB) not only caused a significant increase in SCE (mean 17.5) and a decrease in PRI values (mean 1.73), but also a considerable increase in the frequencies of CAs (mean of 209 CAs per 100 metaphases) (10). (Table 3)

<u>Toxicity of the monomers in the composition of RMGICs:</u>

The potential toxicity of monomers present in resin-modified glass ionomer cements (RMGICs) was examined in four studies (15–18).

Three studies (15–17) measured the impact of monomers on human gingival fibroblasts (HGFs) and human odontoblast-like cells (hOLCs). The IC50 values of the monomers HEMA and TEGDMA for HGFs were 3.79 mmol/L and 3.46 mmol/L, respectively (15). The TC50 for HEMA

of 5.83 mmol/L on hOLCs (17) . Cell viability percentages were also determined, showing that at 3 mmol/L, HEMA reduced hOLC viability to 81% after 24 hours, while TEGDMA reduced it to 86%. HGF viability exposed to HEMA decreased to 85%, 40%, and 35% after 24, 72, and 96 hours, respectively (16,17).

Apoptosis, measured by caspase-3 activity, increased in both hOLCs and HGFs exposed to HEMA and TEGDMA (15,16).

Morphological changes were observed in HGFs over time (15,17). After 72 hours of HEMA exposure, a notable reduction in cell number and loss of fibroblastic morphology occurred. After 72 h of TEGDMA exposure, cells showed a clear morphological pattern of necrosis.

LDH release was induced after 3 hours of exposure to HEMA (36%) and TEGDMA (24%). ROS production was highest at 3 mmol/L of TEGDMA after 3 hours and at 3 mmol/L of HEMA after 6 hours. MDA levels increased significantly after 9 hours of HEMA and TEGDMA exposure (16).

Additionally, the genotoxicity of monomers was evaluated in human parotid gland tissues and lymphocytes. In TEGDMA at 10-5 M, UDMA at 10-7 M, and HEMA at 10-3 M led to an increase of OTM indicating genotoxic effects.

In lymphocytes, an increase of OTM signifying DNA migration was observed at 10-3 M for TEGDMA, 10-7 M for UDMA, and 10-5 M for HEMA (18). (Table 4.)

Biocompatibility and secondary effects of the resin-modified glass ionomer cements:

The biocompatibility and secondary effects of resin-modified glass ionomer cements (RMGICs) were investigated in controlled clinical trials (12,13).

After 5 and 7 days, among 10 RMGIC specimens, 3 showed no inflammation,7 showed inflammation going from mild to moderate. In comparison, among 10 specimens of conventional glass ionomer cement, 4 had no inflammation and 6 reported inflammations from mild to moderate.

Regarding bacterial presence, among 10 RMGIC specimens, 8 had no bacteria, and 2 showed bacterial presence. None of the GIC's specimen showed bacterial presence.

Tertiary dentin formation was observed in only 1 out of 10 RMGIC specimens. None of the 10 GIC specimens showed tertiary dentin formation.

Odontoblastic changes were found in 3 out of 5 RMGIC specimens going from mild to severe changes. Among the 5 GIC specimens, 1 had no changes, 4 showed changes.

Tissue disorganization was observed in 4 out of 5 RMGIC specimens, showing mild disorganization. Among the 5 GIC specimens, 3 had mild disorganization.

After 30 days, among 10 RMGIC specimens, 6 showed no inflammation, and 4 had mild inflammation. For the 10 GIC specimens, 8 had no inflammation, 1 had mild inflammation, and 1 had severe inflammation.

Regarding bacterial presence, among 10 RMGIC specimens, 8 showed no bacteria, and 2 had mild bacterial presence. In the GIC group, 9 showed no bacteria, and 1 had mild bacterial presence.

Tertiary dentin formation was observed in 5 out of 10 RMGIC specimens, exhibiting mild formation. Among the 10 GIC specimens, 8 had no tertiary dentin formation, and 2 showed mild formation.

Regarding the odontoblastic changes, over the 5 teeth exposed to RMGIC, 2 showed no changes, 1 specimen had mild changes and 2 samples moderate to severe changes. Among the 5 GIC specimens, 4 showed no changes, and 1 presented mild changes.

Finally, tissue disorganization was found in 2 out of 5 RMGIC specimens. Among the 5 GIC specimens, 1 had mild tissue disorganization. (Table 5.)

Discussion:

This systematic review examines the toxicity of resin-modified glass ionomer cements compared to conventional glass ionomer cements based on scientific evidence. The study aims to evaluate the toxicity of RMGICs compared to GICs and assess the toxicity of the monomers present in RMGICs. Finally, the biocompatibility of RMGIC is also determined.

Toxicity of the resin-modified glass ionomer cements:

In vitro studies (10,11,14). consistently show that RMGICs cause greater cellular alterations than GICs, the significantly higher LDH release (31%) indicates disrupted membrane stability and potentially necrosis. Those results are confirmed by several Animal studies (19). Morphological changes and growth inhibition of human fibroblasts and odontoblast-cell line exposed to both cements further support the observation that RMGICs have greater cytotoxicity compared to GICs (11,14,20). The adverse reactions are attributed to the leaching of a component called HEMA (21). Genotoxic effects are also observed, with RMGICs causing significant increases in frequencies of SCE and CAs, indicating genotoxicity, while GICs only

have minor cytogenetic effects (10). Methacrylate monomers like HEMA and TEGDMA are identified as responsible for the genotoxic effects. Vitrebond (VB) resin-modified glass ionomer cement appears to be the most cytotoxic and genotoxic among the cements studied. The high concentration of elutable components, particularly resinous monomers, in VB contributes to its pronounced effects (22). The release of HEMA and TEGDMA from RMGICs has been confirmed, and these monomers can diffuse into the pulp at significant concentrations (1.5-8mmol/L for HEMA and 4mmol/L for TEGDMA) (23). The study highlights the need for further investigation into dental materials used in dentistry, considering their toxicity and monomer release.

Toxicity of the monomers entering in the composition of the resin-modified glass ionomer cements:

The toxicity of resin monomers, specifically HEMA and TEGDMA, has been analyzed in several studies. These monomers seem to reduce cell viability, with viability decreasing over time of exposure (16,17). The IC50 values for HEMA (3.79mmol/L) and TEGDMA (3.46mmol/L) indicate their toxic effects on cells. The TC50 values for HEMA vary between 3.6mmol/L and 10mmol/L (24), this difference can be explained by the cell type and their sensitivity. However, the presence of living cells suggests that HEMA and TEGDMA can activate cell protective mechanisms to reestablish homeostasis (25). Exposure to HEMA and TEGDMA seems to increase the caspase-3 activity and therefore induce apoptosis (15,16). These monomers can interact with the plasma membrane and penetrate the cytoplasm, affecting cellular components such as mitochondria and causing metabolic dysfunction. Due to the radical metabolites of the methacrylate, HEMA and TEGDMA induce morphological changes, decreased cell number, and necrosis pattern(15,17,26). LDH release, ROS production, and MDA levels also confirm the disruption of membrane stability caused by these monomers (16). HEMA, TEGDMA, UDMA, and TEGMA induce DNA migration and exhibit genotoxicity (18,27). The genotoxicity of TEGDMA is explained by its ability to cause cell cycle delays, influencing cell growth and differentiation(28).

Biocompatibility and secondary effects of the resin-modified glass ionomer cements:

The biocompatibility of resin-modified glass ionomer cements (RMGICs) compared to conventional glass ionomer cements (GICs) was evaluated in this study (12,13).

Concerning the inflammatory response, the results show that after 5 and 7 days, a higher proportion of RMGIC specimens presented mild to moderate inflammation compared to GIC specimens. Therefore, it appears that in the early stages RMGICs seem to induce a stronger inflammation than GICs. Overall, the rate of severe inflammation was low for both groups and traduce an acceptable level of biocompatibility for both materials. Mousavinasab M et al (29) shares similar results with a significantly higher pulp inflammation at 7 days than at 30 and 60 days. This can be explained by the fact that the residual monomers (HEMA and TEGDMA) are present within the material the first few days after the lining (30). Concerning the presence of bacteria, the results show a bacterial presence in only a small proportion of specimens. This bacterial presence is most likely due to an inadequate isolation during the restorative procedure and do not come from a leaking in the cavities. Our study showed that there were no differences statistically relevant between the GICs and RMGICs in terms of tertiary dentin formation. Moreover, a study (31) show that time is more important than the material in the tertiary dentin formation with an average of 28 days needed for TD formation. Our studies during only 30 days, the time was not long enough to properly measure the TD formation. Nonetheless, the study (29) that measured the TD formation for 7 days, 30 days and 60 days display similar results than our studies with a lack of TD formation regardless of the material used. Overall, those results traduce an acceptable biocompatibility of RMGICs.

Despite the large number of In vitro studies, and large number of variables used, we can conclude that the RMGICs have an in vitro higher cytotoxicity and genotoxicity than the GICs. This toxicity is due to the toxicity of the monomers composing the RMGICs. However, the biocompatibility of the RMGICs does not seem to be compromised. Current evidence is limited by the few numbers of in vivo studies.

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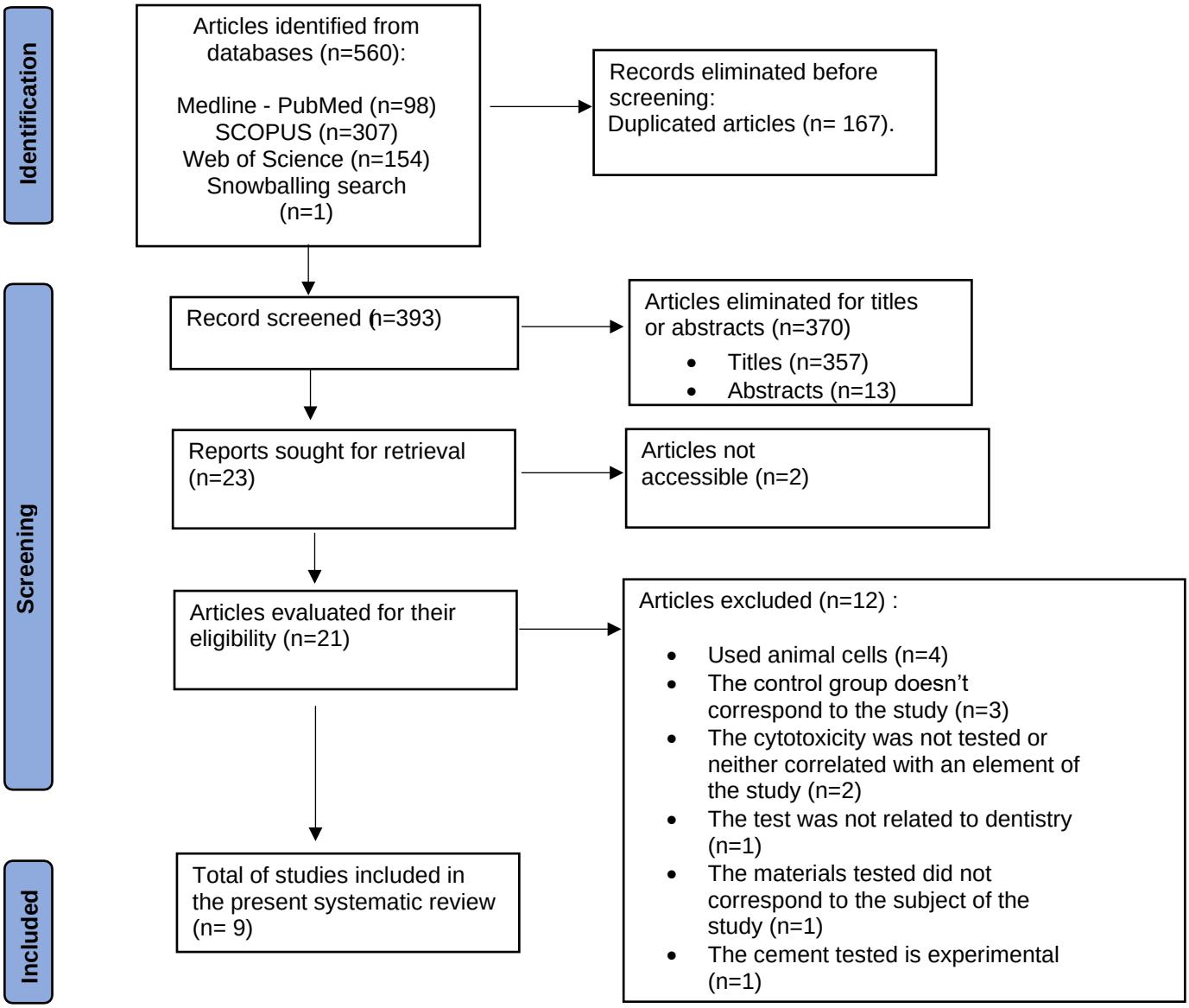

Fig. 1. Diagram of the flow chart and process of the articles selection during the systematic review.

Table 1. General characteristics of the study

Authors and year	Study type	Materials tested	Experimental models	Time of exposure	Type of toxicity reported	Variables studied
Eskandarizadeh A. 2015 (54)	Controlled clinical trial	RMGIC (Vivaglass) GIC (Ionocid) Calcium hydroxide (Dycal)	30 human premolars	5 and 30 days	NONE	Odontoblastic changes Inflammatory response TD formation Presence of microorganisms
Ribeiro A. 2020 (57)	Controlled clinical trial	RMGIC (Riva LC) GIC (Riva SC) Calcium hydroxide (Dycal)	26 human premolars + 4 controls	7 and 30 days	NONE	Inflammatory reaction Tissue disorganization Reactionary dentin formation Bacteria
Rodriguez I. 2013 (55)	In vitro study	RMGIC (Vitrebond) GIC (Ketac-molar)	Human gingival fibroblasts (HGF)	72 hours	Cytotoxicity	Morphological changes (Phase contrast microscopy) Lactate dehydrogenase release (LDH)
Bakopoulou A. 2009 (35)	In vitro study	RMGIC (RelyX Lutting and Vitrebond) GIC (Ketac Cem and Fuji I) Resin cements (Variolink and Panavia)	Normal cultured human lymphocytes	72 hours	Genotoxicity Cytotoxicity	Sister chromatid exchange (SCE) Chromosomal aberrations (CAs) Assessment of proliferation using proliferation rate index (PRI)
Leyhausen G. 1998 (56)	In vitro study	RMGICS (Ionoseal, Vitrebond, Compoglass) GIC (Ketac fil)	Human primary fibroblasts attached to the gingiva (HGF)	48h	Cytotoxicity	Morphology and growth characteristics by phase contrast microscopy.
Teti G. 2015 (58)	In vitro study	HEMA	Human gingival fibroblasts (HGF)	24 – 48 – 72 hours	Cytotoxicity	Cell viability assay by optical density (percentage of untreated cells)

		TEGDMA				Expression of protein markers for apoptosis and autophagy (by western blot) Morphological changes (by transmission electron microscopy)
Baldion P. 2021 (59)	In vitro study	HEMA TEGDMA	Human odontoblast like cells (hOLCs)	3, 6, 9, 12, 18 and 24 hours	Cytotoxicity	Cell viability evaluation by calcein Lactate dehydrogenase release assay Oxidative damage assessment (ROS production, Malonaldehyde (MDA) levels) Capsase-3 activity
Falconi M. 2007 (60)	In vitro study	HEMA	Human gingival fibroblasts (HGF)	24 – 72 – 96 hours	Cytotoxicity	HGF viability by MTT assay Morphological changes by FEISEM
Kleinsasser N. 2006 (61)	In vitro study	TEGDMA UDMA HEMA	Human parotid gland tissue and lymphocytes		Cytotoxicity Genotoxicity	DNA migration by tail moment according to Olive (39) (OTM)

	Case definition	Representativity	Controls selection	Controls definition	Comparatibility	Comparatibility	Exposure check	Same method for both groups	Drop-out rate	Total
Eskandarizadeh A. 2015 (54)	☆	-	☆	-	☆	☆	☆	☆	-	6
Ribeiro A. 2020 (57)	☆	-	☆	☆	☆	☆	☆	☆	-	7

Fig. 2. Measurement of the risk of bias of non-randomised observational studies with the Newcastle-Ottawa scale

Table 2: Measurement of the risk of bias of in vitro studies with the modified Arrive and Consort scale (acceptability range 21-28)

Author/Year	Title	Abstract	Introduction	Introduction	Methods: Study design	Methods: Experimental procedures	Methods: Sample Size	Methods: Statisitcal method	Results	Discussion	Declaration of potential conflicts and disclosure of liability	Publication in a peer-reviewed journal	Risk of bias
Rodriguez I. 2013 (55)	1	2	3	1	2	3	2	3	3	1	1	1	=23
Bakopulou A. 2009 (35)	1	3	2	3	3	3	3	3	3	2	1	1	=27
Leyhausen G. 1998 (56)	1	2	3	2	2	2	2	3	2	1	1	0	=22
Teti G. 2015 (58)	1	3	3	2	2	3	3	2	2	2	1	1	=25
Baldion P. 2021 (59)	1	3	3	3	2	2	3	2	3	2	1	1	=26
Falconi M. 2007 (60)	1	2	3	2	2	3	3	3	2	2	0	0	=23
Kleinsasser N. 2006 (61)	1	2	2	2	3	2	3	2	3	2	0	0	= 22

Table 3. Toxicity of the resin modified glass ionomer cements.

Authors (with date)	Variables	Results		
		RMGIC		**GIC**
Rodriguez I. 2013 (55)	***Morphology of HGFs***	3.9%±5.0 (median 1.5%) had a normal morphology.		52.2%±20.4 (median 52.9%) had normal morphology.
	Lactate dehydrogenase release (LDH)	**Cells exposed to RMGIC:** Mean 38.46% ± 7.29 (median 31.6%)		**Cells exposed to GIC:** Mean 11.04% ± 21.69 (median 3%)
Leyhausen G. 1998 (56)	***Growth of HGFs***	First 24-h extract (day 1/24 h)	**RMGICs:** Ionoseal (IS): Growth of 88%±4.7. Vitrebond (VB): Growth of 15.7%±13.9. Compoglass (CG): Growth of 107%±9.	**GIC:** Ketac fil (KF): Growth of 107%±19.5.
		Second 24-h extract on day 9 (day9/24)	**RMGICs:** IS: Growth 100.9%±5.8. VB: Growth 68.8%±6.8. CG: ND	**GIC:** KF: Growth 103%±4

Bakopoulou A. 2009 (35)	***Frequencies of sister chromatid exchange (SCE)***	**RMGICs:** Rely X (RX): Increased SCE exchange 20.65±1.16; 16.40±0.80; 8.40±0.55 (Mean 15.15) Vitrebond (VB): Increased SCE exchange 19.3±1.12; 17.20± 1.42; 16.10± 0.89 (Mean 17.5)	**GICs:** GC Fuji I (FJ): mean value 11.06 Ketac Cem (KC) mean value 10.53.
	Chromosomal aberrations (Cas)	**RMGICs:** RX: Increased CAs 28 per 100 metaphases; 11 per 100 metaphases; 1 per 100 metaphases (Mean 20.3 CAs per 100 metaphases) VB: Increased CAs; 278 per 100 metaphases; 90 per 100 metaphases; 259 per 100 metaphases (Mean 209 CAs per 100 metaphases)	**GICs:** FJ: mean 2.6 CAs for 100 metaphases KC: mean 9.3 CAs for 100 metaphases
	Proliferation rate index (PRI)	**RMGICs** RX: decreased PRI: 1.91 2.13 2.83 Mean value: 2.29 VB: decreased PRI: 1.65 1.85 1.70 Mean value: 1.73	**GICs** FJ: Mean value 2.60 KC: Mean value 2.65

Table 4. Toxicity of the monomers entering in the composition of the resin-modified glass ionomer cements.

Variables	Authors with year	Doses	Results	
			HEMA	**TEGDMA** and **UDMA**
Cells viability	Teti G. 2015 (58)	-	IC50 of HGFs: 3.79mmol/L	IC50 of HGFs for **TEGDMA:** 3.46mmol/L
	Falconi M. 2007 (60)	3mmo/L	TC50 on HGFs: 5.83 mmol/L. 24h: cell viability reduced to 85% 72h: cell viability reduced to 40% 96h: cell viability reduced to 35%	
	Bladion P. 2021 (59)	3mmol/L	24h: Cell viability of the hOLCs reduced to 81%	24h: Cell viability of the hOLCs reduced to 86% for **TEGDMA**.
Apoptosis	Teti G. 2015 (58)	3mmol/L	Increase of caspase-3 activity.	Caspase-3 activity increase for **TEGDMA.**
	Bladion P. 2021 (59)	3mmol/L	Increase of caspase-3 activity.	
Morphological changes	Teti G. 2015 (58)	3mmol/L	24h: cells showed a fibroblast-like morphology. 48h: Well-preserved morphology still present, increased number of autophagic vesicles in the cytoplasm. 72h: Round or polygonal morphology, several autophagic vesicles in cytoplasm, condensed masses of chromatin in the nucleus, and enlarged nuclear envelope.	For HGFs exposed to **TEGDMA:** 24h: Nucleus well preserved, but the cells showed slight damage. 48h: Condensed chromatin masses, damaged mitochondria, and enlarged Golgi apparatus. 72h: Cells showed a clear morphological pattern of necrosis.

	Falconi M. 2007 (60)	3mmol/L	24h exposure to **HEMA**: Morphology comparable to the untreated cells with a fibroblastic shape. 72h: Number of HGFs reduced, cells lost the fibroblastic morphology. 96h: Reduction in the number of cells, fibroblastic morphology had almost disappeared.	
LDH release	Bladion P. 2021 (59)	3mmol/L	24h: LDH release was 36%.	24h for **TEGDMA:** LDH release was 24%.
ROS production			After 6h: 7 x 10^3 ROS	After 3h for **TGDMA:** 16 x 10^3 ROS
MDA levels			After 9 h MDA levels: 1.9nmol	After 9h **TEGDMA** MDA levels: 1.9nmol
DNA migration by tail moment	Kleinsasser N. 2006 (61)	TEGDMA: 10^{-5} M and 10^{-3} M UDMA: 10^{-7} M HEMA: 10^{-3} M and 10^{-5} M	In parotid gland tissue: **HEMA** at 10^{-3} M mean OTM of 5.1 Negative control mean OTM: 2.3 In lymphocytes: **HEMA** at10^{-5} M mean OTM of 2.3. Negative control mean OTM of 1.7.	In parotid gland tissue: **TEGDMA** at 10^{-5} M mean OTM is 3.0 **UDMA** at 10^{-7} M mean OTM of 4.1 Negative control mean OTM: 2.3 In lymphocytes: **TEGDMA** at10^{-3} M mean OTM of 3.4 **UDMA** at 10^{-7} M mean OTM of 2.6 Negative control mean OTM of 1.7.

Table 5. Effects of the resin modified and conventional glass ionomers on the plulp.

Histological event	Material	Periods								Total
		5-7 days				30 days				
		No	Mild	Moderate	Severe	No	Mild	Moderate	Severe	
Inflammatory response	RMGICs	3	5	2	0	6	4	0	0	20
	GICs	4	5	1	0	8	1	0	1	20
Presence of bacteria	RMGICs	8	2	0	0	8	2	0	0	20
	GICs	10	0	0	0	9	1	0	0	20
Odontoblastic changes	RMGICs	3	0	2	0	2	1	2	0	10
	GICs	1	3	1	0	4	1	0	0	10
Formation of tertiary dentin	RMGICs	9	1	0	0	5	5	0	0	20
	GICs	10	0	0	0	8	2	0	0	20
Tissue disorganization	RMGICs	1	4	0	0	3	2	0	0	10
	GICs	2	3	0	0	4	1	0	0	10

RMGICs used: Riva Light Cure and Vivaglass
GICs used: Riva Self Cure and Ionocid