



Influence of Hydrogen Peroxide on Mineralization in Dental Pulp Cells: A Systematic Review

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Background: Dental bleaching agents show the ability to permeate through dental hard tissues, which may lead to pulp tissue changes. This systematic review (PROSPERO register: CRD42020213767) is aimed at understanding the effects of bleaching agents on the process of mineralization of the pulp tissue.

Methods: Only *in vitro* studies evaluating the influence of hydrogen peroxide (HP) on mineralization in dental pulp cells were included. Studies without a non-bleached control group or cells after co-treatment with a bleaching agent other than HP and/or carbamide peroxide were excluded. The primary outcomes evaluated were alkaline phosphatase (ALP) activity and mineralized nodule deposition. The mineralization markers analysis in dental pulp cells and the cell viability were considered secondary outcomes. Two independent authors conducted a systematic search (PubMed/MEDLINE, Scopus, Embase, Cochrane Library, and OpenGrey until January 2021) with no language restrictions and performed data extraction. The quality assessment was appraised according to a modified Joanna Briggs Institute critical appraisal checklist.

Results: The search resulted in 473 studies, and 11 were considered eligible. Overall, a reduction in the process of mineralization was observed among pulp cells after bleaching. A reduction in the ALP activity was reported in the mostly bleached groups using different protocols and analysis periods of nine studies. Regarding mineralized nodule deposition, 6 studies reported a significant reduction from 7 to 21 days among bleached groups. Of those three studies that investigated other mineralization markers, two found a reduction in the expression of dentin matrix acidic phosphoprotein (DMP)-1, dentin sialophosphoprotein (DSPP), and matrix extracellular phosphoglycoprotein (MEPE) among some bleaching gel concentrations. In contrast, one study showed a greater expression of osteopontin (OPN) and osteocalcin (OCN) in 100 $\mu\text{mol/L}$ HP after 5 or 10 min of exposure, and another study showed significant induction of DSPP in concentrations of up to 0.5 mmol/L HP.

Conclusion: Especially, high concentrations of bleaching gel reduce the potential of mineralization in pulp cells in *in vitro* studies; however, different HP concentrations, bleaching protocols, and analysis periods can influence this outcome.

Keywords: dental bleaching, human dental pulp cells, hydrogen peroxide, dentinogenesis, mineralization, odontoblasts

INTRODUCTION

Dental bleaching using hydrogen peroxide (HP), the main active agent of most bleaching products, is considered a popular treatment to achieve esthetical bleaching. This procedure is also considered safe and successful under the supervision of dentists, and it is credited to promote color change by the interaction of oxygen free radicals by HP with intrinsic and extrinsic pigments (1), and has one of the main benefits, fostering positive changes in patients, such as smiling, laughing, and showing teeth without embarrassment (2); however, this procedure is related to having effects on enamel and dentin mineral loss and can lead to pulp tissue changes (3), especially in human mandibular incisor teeth (4), that is still not fully understood and regarded as one of the main concerns about this treatment (3–6).

Bleaching agents with different HP concentrations can diffuse through interprismatic space and promote some enamel surface morphology alterations, for instance, reducing enamel microhardness and increasing the surface roughness (1, 7). Besides that, the reactive oxygen species (ROS) released by HP can permeate through enamel and dentin, reaching the pulp tissue and leading to inflammation, decreasing cellularity and cell metabolism (3, 8–12), protein denaturation (4, 13, 14), and areas of tissue necrosis (5, 6, 12, 15).

From preliminary studies simulating clinical conditions, severe inflammation and necrosis areas in the pulp tissue of rats were observed after one to five bleaching sessions with 3 applications of 15 min each of 35% HP gel, which was accentuated following the number of bleaching sessions (5). These histological observations can be related to the tooth sensitivity reported by the patients after the bleaching (16). After 30 days, the inflammation was ceased, and the pulp tissue was reorganized; however, another study showed an intense production of tertiary dentin directly proportional to the increase in HP concentration (20–35% HP gel) and time of application (5–45 min) of bleaching gel (10). It was also observed the existence of apoptotic cells, which are fundamental for the development of tissues and their recovery after internal and external stimuli (17, 18) and intense cell proliferation in regions below the areas of necrosis (18). Therefore, the pulp tissue can recover after HP cell damage but the long-term consequences of this damage are not fully understood.

A previous study evaluated the immunolabeling of mineralization proteins on bleached molar pulp tissue in rats using a single application of 30 min of the 35% HP (19). The authors observed that osteopontin (OPN) is present in the pulp tissue during the repair process after bleaching and is more immunolabeled after 7 and 15 days (19); however, osteocalcin (OCN) was significantly immunolabeled from 7 days

on, but it was more significant after 30 days of the bleaching treatment (19). The Jun-D transcription factor of odontoblasts was significantly identified after 7 days of similar bleaching treatment with 35% HP, indicating differentiation of these cells (6). Another study also reported the expression of OCN and OPN in cell cultures after 10 min of contact with minimal concentrations of HP (100 $\mu\text{mol/L}$) (20).

Several laboratory studies have reported the effects of distinct concentrations (0.025–0.3 mM or 17.5–35%) of HP on the mineralization of different cell lines (21–23). A favorable outcome of HP on odontoblasts suggests an increase in dentin production capability (21). The studies conducted so far indicate a process involving dentinogenesis and mineralization of pulp tissue or pulp cells after contact with HP. Although several systematic reviews have evaluated the adverse clinical effects of bleaching (16, 24), only one recent systematic review assessed the impact of this procedure on the pulp tissue (12). Thus, the objective of this study is to carry out a systematic review to understand the immediate and long-term effects of bleaching agents on the process of mineralization of the pulp tissue.

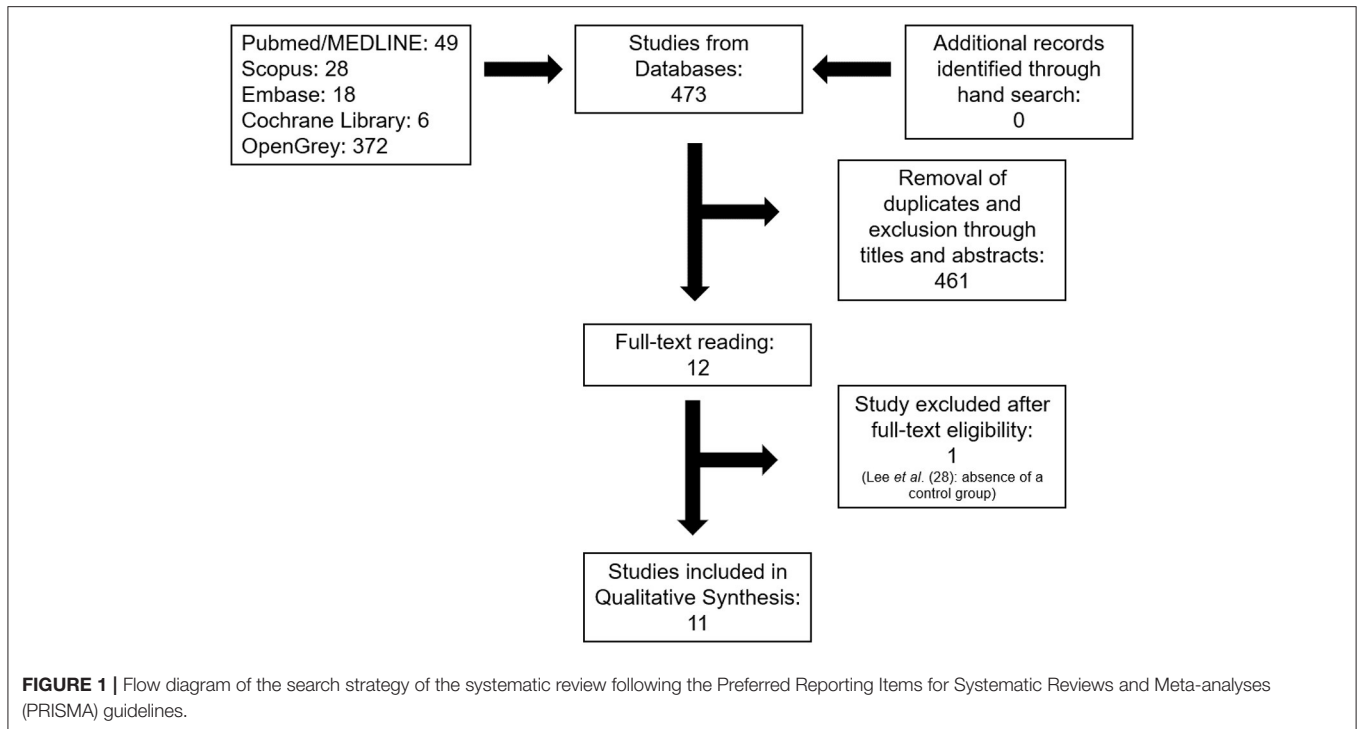
METHODS

The present study was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (25, 26). This systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD42020213767.

Eligibility Criteria

The inclusion criteria were as follows: (1) studies that evaluated the effects of the bleaching gel on the process of mineralization of dental pulp cells and proteins involved in this process; (2) studies that present a non-bleached control group/without bleaching gel; and (3) *in vitro* studies. The exclusion criteria were studies that analyzed pulp cells after co-treatment with bleach or another agent rather than HP and/or carbamide peroxide.

The population-intervention-comparison-outcome (PICO) approach was used to address the following question: “Can the bleaching agent influence the mineralization process of dental pulp cells?” In this process, the population (P) was dental pulp cells. The intervention (I) was pulp cells after exposure to the bleaching agent. The comparison (C) was pulp cells being not exposed to the bleaching agent. The primary outcomes (O) evaluated were alkaline phosphatase (ALP) activity and mineralized nodule deposition. The mineralization markers



analysis in dental pulp cells and the cell viability were considered secondary outcomes.

Search Strategy and Information Sources

Electronic searches were conducted in PubMed/MEDLINE, Scopus, Embase, and Cochrane Library until January 2021. The gray literature was also consulted through OpenGrey. The search strategy was firstly defined for the MEDLINE database *via* Pubmed using a controlled vocabulary (MeSH terms) and free keywords. The MEDLINE search was also adapted to the other databases as shown in **Supplementary Table 1**. Manual searches were also performed in the reference lists of the included articles to find additional studies. No restrictions to publication date or language were considered.

Study Selection

The articles retrieved by the literature search were selected by two independent authors (IGR and AHRP) in a two-step procedure. In Step 1, the two authors appraised titles and abstracts of studies that met the eligibility criteria. The studies were arranged alphabetically by title, and duplicates were identified and removed manually. In Step 2, the two authors assessed the full texts of each study. Only studies in which the full text fulfilled the proposed eligibility criteria were included in this review. Any disagreements between the two authors were resolved through discussion, and when necessary, a third author (LFSAM) was consulted.

Data Collection and Analyses

One author collected data (IGR) from the included studies and tabulated it to analyze the results. The following data

were retrieved: author and year, experimental model, groups and bleaching protocol, period of analysis, and outcomes of evaluating the ALP activity, Alizarin red or von Kossa staining, mineralization markers, and cell viability. The second author (AHRP) revised the collected data.

Modified Risk of Bias Assessment

Two investigators (AHRP and HGSC) independently assessed the methodological quality of the selected studies according to their levels of evidence as proposed by a modified version as described previously (26) in Joanna Briggs Institute critical appraisal checklist for experimental studies (27). To further characterize bleaching reporting in the selected studies, the authors also assessed if a clear bleaching protocol was present. The following were the other items in the checklist: clearly stated aim, justification of sample size, sample randomization, the possibility of comparison between controls and treatment groups, baseline equivalence of control and treatment groups, measurement method, measurement standardization, and adequate statistical analysis. Each item was scored using a 2-point scale: 0, not reported or reported inadequately; 1, reported and adequate. Doubts and discrepancies between both investigators were discussed, and if not solved, a third examiner (CBA) was consulted.

RESULTS

Selected Studies

The selection process of the articles is presented in **Figure 1**. A total of 473 articles were found on databases search. After the first screening and removal of duplicates, 12 studies were

selected. These studies were then subjected to full-text evaluation that resulted in the exclusion of one study that had no control group (28). Finally, 11 studies were included in this review (3, 20–23, 29–34).

The assessed Cohen kappa coefficient value for the inter-investigator agreement was equal to 0.959 for PubMed, 0.837 for Scopus, 0.889 for Embase, 1.000 for the Cochrane Library, and 1.000 for OpenGrey. These values indicated an almost perfect agreement among reviewers according to the scale of Landis and Koch (35).

Characteristics of the Included Studies

The characteristics of the studies are depicted in **Table 1**. Most studies (a total of seven) used tooth discs for the application of bleaching treatments with artificial pulp chambers and different cell types, such as dental pulp stem cells (DPSCs), human dental pulp cells (HDPCs), and odontoblast-like MDPC-23 (22, 23, 29–33). Of these, six studies used tooth discs from bovine incisors. Four studies performed the analyses exposing only some cell cultures to the different concentrations of extracts of bleaching gel (3, 20, 21, 23).

The bleaching agent with 35% HP concentration was used in five studies (22, 30–33), with different application times. The bleaching agent with 10% HP concentration was used in only one study (33), as well as bleaching agents with 17.5% HP concentration (23), 16% carbamide peroxide (CP) (29), and 10% CP (22). Other studies used extracts that were previously prepared with lower HP concentrations (3, 20, 21).

A total of nine studies evaluated ALP activity. The period of analysis was immediately after the treatments (22, 29), after 24 h (3, 30, 31), after 4 days (21), after 7 days (23, 32), after 14 days (23, 33), and after 21 days (23). Regarding mineralized nodule deposition, six studies performed this analysis after 7 (23, 32), 14 (23), 15 (21), 21 (23), and 28 days (20). All studies used Alizarin red staining for this analysis, but one study used von Kossa staining (20).

Regarding the mineralization markers, three studies evaluated the mRNA gene expression of these markers by using reverse transcriptase-PCR (3, 20, 23). The markers evaluated were dentin matrix acidic phosphoprotein-1 (DMP-1) and dentin sialophosphoprotein (DSPP) in two studies (3, 23), OCN and OPN in another study (20), and matrix extracellular phosphoglycoprotein (MEPE), osteonectin (ON), and bone sialoprotein (BSP) in one last study (3).

Each of the included studies was analyzed in terms of similarities to determine whether a meta-analysis could be applied for these records; however, despite a favorable number of studies in this review, considerable heterogeneity was found between the studied groups. There were several bleaching gel concentrations or protocols, types of analysis performed, experimental periods, besides different cell types; therefore, a meta-analysis was not performed. The data regarding studies outcomes are summarized in **Table 1** and are described below.

ALP Activity

Nine studies that evaluated ALP activity (21–23, 23, 29–33) found a decrease in this enzyme for approximately all bleached groups

using different protocols and HP concentrations for the majority of the assessed period of analysis; however, two of these studies also reported an increased APL activity with 0.1 mM and 0.2 mM HP after 4 days (21) and after one session of 17.5% HP for 5 min or 15 min and after 21 days (23), using MDPC-23 cells and HDPCs, respectively.

Mineralized Nodule Deposition

From the selected studies, only six performed the mineralized nodule deposition analysis. These studies reported that bleached groups reduced the mineralized nodule deposition by using 35% HP in MDPC-23 cells (32), 0.1 and 0.3 $\mu\text{g}/\text{mL}$ HP (23), or 17.5% HP (23) in HDPCs after 7 days. At 14 or 15 days, a reduction of mineralized nodule deposition with 17.5% HP in HPDCs (23) and with 0.2- and 0.3-mM HP in MC3T3-E1 (21) was observed. In addition to these data, a decrease in the mineralized nodule formation was also observed in 3 sessions of 10% HP (33) or 35% HP (23, 33), after 21 days. On the contrary, two studies reported a significant induction of mineralized nodules in 0.2- or 0.3-mM (21) and 100 $\mu\text{mol}/\text{L}$ HP (20) for MDPC-23 cells and HDPCs after 15 and 28 days, respectively.

Mineralization Markers

From the three articles that evaluated the mineralization markers in cells after bleaching, DMP-1 and DSPP markers were investigated in two of these studies (3, 23). One study reported a reduction in the expression of DMP-1 after 7, 14, and 21 days on bleached groups using 17.5% HP (23), while the other study revealed that up to 0.5 mmol/L HP stimulates DMP-1 expression (3). Regarding the expression of DSPP, the application of 17.5% HP for 45 min was associated with a reduction in its expression in one study (23), while up to 0.5 mmol/L HP increased DSPP values in another study (3).

Only one study evaluated OPN and OCN expressions (20). The study showed that 100 $\mu\text{mol}/\text{L}$ HP for 5 or 10 min promoted a greater expression of these mineralization markers in HDPCs after 6 (OPN), 9 (OCN), 12 (OPN), or 18 (OCN) days compared with control (20). One study (3) that investigated more mineralization markers demonstrated a reduction in MEPE at 0.5 mmol/L HP, a significant induction of HO-1 with 0.2 mmol/L after 24 h, and no significant differences in the production of ON and BSP among the groups.

Risk of Bias

Figure 2 and **Table 2** summarize the results of the critical appraisal of the eligible studies. Only one study (31) reported the presence of sample randomization. All the included articles showed a possibility of comparison between control and treatment groups in the beginning and a reliable measurement method. Low risk of bias was also observed in the clearly stated aim, baseline equivalence of control and treatment groups, clear bleaching protocol, measurement standardization, and appropriate statistical approach. Conversely, a high risk of bias was noticed for some specific items, such as sample randomization and justification of sample size.

TABLE 1 | Characteristics of the studies included in the review and synthesis of outcomes.

Authors	Experimental model	Groups and bleaching protocol	Analysis period	Alkaline phosphatase activity	Mineralized nodule deposition (AR or VK staining) staining	Mineralization markers	Cell viability
De Oliveira Duque et al. (33)	Enamel/dentin discs (human ICs or PMs), APCs, DPSCs	ICs. G1: control; G2: 35% HP (3 × 15 min); G3: 10% HP (3 × 15 min); G4: 10% HP (1 × 15 min); G5: 10% HP (1 × 5 min). PMs. G6: control; G7: 35% HP (3 × 15 min); G8: 10% HP (3 × 15 min); G9: 10% HP (1 × 15 min); G10: 10% HP (1 × 5 min)	ALP activity: 14 days. MND: 21 days. Cell viability: 0 and 72 h	All bleached groups (10% and 35% HP) decreased the ALP activity in DPSCs compared to control at 14 days	The 1 × 15 min and 1 × 5 min 10% HP showed the same MND as control. The 3 × 15 min 35 or 10% HP decreased MND at 21 days	n.a.	ICs: G2, G3, G7, and G8: lower viability compared to controls (G1 and G6) at 0 h; at 72 h, G2, G3, and G4 decreased cell viability, and G5 significantly increased viability compared to G1. PMs: G9 increased viability compared to control (G6) at 0 h, while G7 and G8 decreased viability at 72 h. Regarding the disc thickness, the cells of G3 and G4 at 0 and 72 h featured lower cell viability than the PMs (G8 and G9)
Soares et al. (32)	Enamel/dentin discs (bovine incisors), APCs, MDPC-23 odontoblast-like cells	G1: control; G2: 35% HP (3 × 15 min); G3: 35% HP (3 × 15 min) + RMGIC; G4: 35% HP (3 × 15 min) + RMGIC + HD; G5: 35% HP (3 × 15 min)+RC for 24 h; G6: 35% HP (3 × 15 min) + RC + HD	ALP activity and MND: 7 days. Cell Viability: Immedia-tely	All bleached groups (35% HP) decreased the ALP activity in MDPC-23 cells compared to control at 7 days, mainly with RMGIC	All bleached groups decreased MND compared to control at 7 days	n.a.	All bleached groups had a significant reduction in cell viability for the G2 (38.7%), G3 (38.5%), G4 (61.7%), G5 (39.4%), and G6 (31.5%), respectively. The greatest decrease in viability was observed in G4
Soares et al. (23)	HDPCs	G1: no exposure; G2: 0.1 μg/ml HP, 30 min; G3: 0.3 μg/ml HP, 30 min	ALP and MND: 7 days. MTT assay: Immedia-tely	All bleached groups (0.1 or 0.3 μg/mL HP) decreased the ALP activity in HDPCs compared to control at 7 days	All bleached groups decreased MND compared to control at 7 days	n.a.	A significant reduction in cell viability was observed for G2 and G3. The greatest decrease was noticed in G3 (63%)
Soares et al. (23)	Enamel-dentin discs (bovine incisors), HDPCs	G1: 45-min 17.5% HP (3 × 15 min); G2: 15-min 17.5% HP (1 × 15 min); G3: 5-min 17.5% HP (1 × 5 min); G4: control group (no treatment)	ALP, MND, and markers: 7, 14, and 21 days. Cell viability and death: immedia-tely	Only 3 × 15 min 17.5% HP decreased ALP activity in HDPCs compared to control at 7 days. All bleached groups (17.5% HP) decreased ALP activity at 14 days. At 21 days, 1 × 15 and 1 × 5 min 17.5% HP increased ALP activity compared to control	All bleached groups decreased MND compared to control at 7 and 14 days. At 21 days, only 3 × 15 min HP decreased MND compared to control	DMP-1: significantly reduced at 7, 14, and 21 d on bleached groups, except on the 5-min at 7 d. DSPP: 45-min group showed the lowest significant DSPP values; the 15 and 5-min featured no significant difference from the control at 21 d	Reductions of around 80, 75, and 64% of HDPC viability were observed for the 45-, 15-, and 5-min groups, respectively. Regarding cell death assay, 65, 58, and 35% of Eth-1-positive cells were observed for the 45-, 15-, and 5-min groups, respectively. All of these values were significantly different from those observed in the control group
Lima et al. (31)	Enamel/dentin discs (bovine incisors), APCs, MDPC-23 odontoblast-like cells	G1: control; G2: 15 min 35% HP; G3: laser 4 J cm ⁻² ; G4: laser 10 J cm ⁻² ; G5: laser 15 J cm ⁻² ; G6: 15 min 35% HP + laser 4 J cm ⁻² ; G7: 15 min 35% HP + laser 10 J cm ⁻² ; G8: 15 min 35% HP + laser 15 J cm ⁻²	24 h	All bleached groups (35% HP) decreased the ALP activity or expression in MDPC-23 cells compared to controls, at 24 h, regardless of the laser application	n.a.	n.a.	A significant reduction in MDPC-23 viability (40–60%) was observed in all bleached groups. A higher reduction in viability occurred in G8 compared with the other groups exposed to bleaching

(Continued)

TABLE 1 | Continued

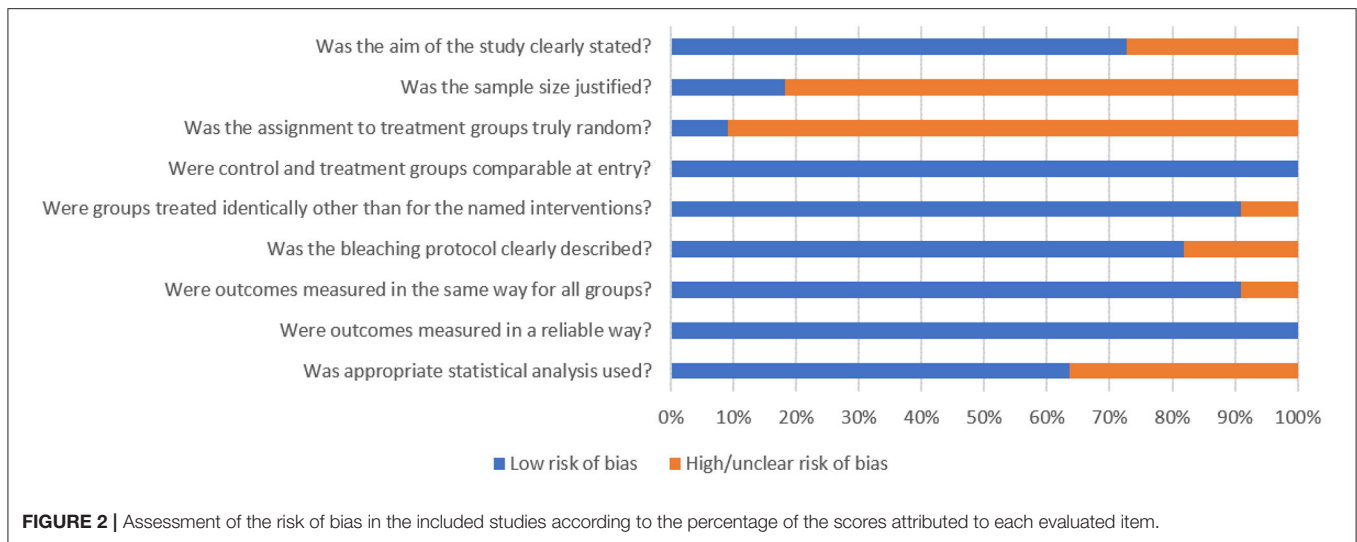
Authors	Experimental model	Groups and bleaching protocol	Analysis period	Alkaline phosphatase activity	Mineralized nodule deposition (AR or VK staining) staining	Mineralization markers	Cell viability
Soares et al. (29)	Tooth discs (bovine incisors), APCs, MDPC-23 odontoblast-like cells	G1, Control: AS; G2: 16% CP (8 h/day/1 day); G3: 16% CP (8 h/day/7 days); G4: 16% CP (8 h/day/14 days); G5: 16% CP (8 h/day/7 days) + 0.05% F (1 min); G6: 16% CP (8 h/day/14 days) + 0.05% F (1 min); G7: 16% CP (8 h/day/7 days) + 0.2% F (1 min); G8: 16% CP (8 h/day/14 days) + 0.2% F (1 min)	Immediately after the treatments	All bleached groups (16% CP) decreased the ALP activity in MDPC-23 cells compared to control, regardless of the time application (24 h, 7, or 14 days)	n.a.	n.a.	The metabolic activity of the MDPC-23 cells decreased by 47, 41, 47, 37, 35, 36, and 40% for G2, G3, G4, G5, G6, G7, and G8, respectively. Significant cell metabolism reduction was observed in G2, G3, G4, and G5 compared to the control (G1). No significant difference in cell metabolism was observed when the bleached and non-fluoridated groups were compared with the bleached and fluoridated groups
Lima et al. (22)	Tooth discs (bovine incisors), APCs, HP diffused through the discs, MDPC-23 odontoblast-like cells	G1: control; G2: 10% CP (8 h/day/5 days); G3: 10% CP (1 × 8 h); and G4: 35% HP (3 × 15 min)	Immediately after the treatments	Only 10% CP for 5 days (against 8h of 10% CP and 45 min 35% HP) decreased ALP activity in MDPC-23 cells compared to control immediately after the treatments	n.a.	n.a.	One application of 10% CP did not cause a reduction in cell metabolism. Application of 10% CP on 5 days, a significant reduction in cell metabolism was observed (75%) with the other groups. With 35% HP, a reduction in cell viability (45%) was significant compared with the control and with one application of 10% CP. The daily application of 10% CP caused a high intensity of disruption of the plasma membrane and cell necrosis (67%)
Soares et al. (30)	Enamel/dentin discs (bovine incisors), HP diffused through the discs, MDPC-23 odontoblast-like cells	G1: control; G2: 3 × 15 min 35% HP; G3: 1 × 15 min 35% HP; G4: 3 × 5 min 35% HP; G5: 1 × 5 min 35% HP	ALP activity: 24 h. Cell viability: immediately	All bleached groups (35% HP) decreased the ALP activity in MDPC-23 cells compared to control at 24 h, except 1 × 5 min 35% HP	n.a.	n.a.	There was a significant decrease in cell metabolism between 3 × 15-min and control. The HP resulted in alterations in cell morphology. In addition, 3 × 15-min had cell-free areas (death of cells). The groups with 1 × 15-min and 3 × 5-min had a slight decrease in the number of cells, while 1 × 5 min no decreased the number of cells but caused a reduction of their size

(Continued)

TABLE 1 | Continued

Authors	Experimental model	Groups and bleaching protocol	Analysis period	Alkaline phosphatase activity	Mineralized nodule deposition (AR or VK staining) staining	Mineralization markers	Cell viability
Matsui et al. (20)	HDPCs	100 μ mol/L HP; groups: 5 and 10 min	MND: 28 days. OPN released: 6–15 days. OCN released: 12–24 days	n.a.	All bleached groups (100 μ mol/L HP) increased MND compared to control at 28 days, regardless of the test performed	OCN/OPN expression. Significant increase in expression in the 10-min group at 6 (OPN) and 9 days (OCN). OPN production in the 5, 10-min, and control groups increased time-dependent and peaked at 12 days; production was significantly greater in the 10-min group than in control. OCN production in the 10, 5-min, and control groups increased time-dependent and peaked at 18 days; the production was significantly greater in the 10-min than control	n.a.
Min et al. (3)	HDPCs	HP application: 0, 0.05, 0.1, 0.2, 0.3, and 0.5 mmol/L	6, 12, 24 and 48 h	n.a.	n.a.	DSPP and DMP-1: up-regulated by HP up to 0.5 mmol/L; maximal DSPP at 24 h with 0.2 mmol/L HP. MEPE: HP decreased the MEPE, with a maximal decrease at 0.5 mmol/L. ON and BSP: no changes at 24 h with HP. HO-1: maximal induction at 24 h with 0.2 mmol/L HP	Pulp cells exposed to different HP concentrations for various times showed concentration- and time-dependent reductions in cell viability. In addition, HP treatment (0.2 mmol/L) for 24 h increased the percentage of early apoptotic cells in pulp cells from 17.40% in control untreated cells to 31.96% in treated cells
Lee et al. (21)	MC3T3-E1 osteoblasts and MDPC-23 odontoblasts	HP application: 0 (control), 0.025, 0.05, 0.1, 0.2, 0.3, and 0.5 mM	ALP activity: 4 days. MND: 15 days. Cell Viability: 1, 4, and 15 days	MC3T3-E1: 0.2 and 0.3 mM HP decreased ALP activity compared to control, at 4 days. MDPC-23: 0.1 and 0.2 mM increased ALP activity compared to control, at 4 days	MC3T3-E1: 0.2 and 0.3 mM HP decreased MND compared to control, at 15 days. MDPC-23: 0.2 and 0.3 mM HP increased MND compared to control, at 15 days	n.a.	For low doses (0.1 or 0.2 mmol/L) of HP, the cell viability of MDPC-23 and MC3T3-E1 was not significantly affected compared to the controls over the course of 14 days

AR, Alizarin red; VK, Von Kossa; ICs, Incisors; PMs, Premolars; APCs, Artificial pulp chambers; DPSCs, Dental pulp stem cells; HP, Hydrogen peroxide; ALP, Alkaline phosphatase; MND, mineralized nodule deposition; RMGIC, Resin-modified glass ionomer cement; HD, Hydrolytic degradation; RC, Resin composite; HDPCs, Human dental pulp cells; MTT, Methyl tetrazolium; DMP-1, Dentin matrix acidic phosphoprotein-1; DSPP, Dentin sialophosphoprotein; AS, Artificial saliva; CP, Carbamide peroxide; F, Fluoride; OPN, Osteopontin; OCN, Osteocalcin; MEPE, Matrix extracellular phosphoglycoprotein; ON, Osteonectin; BSP, Bone sialoprotein; HO-1, Heme oxygenase-1.



DISCUSSION

This systematic review investigated if the bleaching gel can influence the mineralization process of dental pulp cells. A total of 11 *in vitro* studies were included in this review. Overall, the evaluated data showed that the bleaching gel decreases the ALP activity and mineralized nodules deposition in dental pulp cells. In addition, it was possible to understand that, particularly, the high concentrations of HP after different times of exposure are capable of reducing the potential of mineralization in pulp cells. Meta-analysis was not performed due to heterogeneity among the bleaching protocols or analysis periods of the included studies, besides different cell types.

It is known that in the presence of an aggressor, such as the HP of bleaching gel, the pulp tissue responds with the production of tertiary dentin (36, 37). When there is mild aggression, the odontoblastic cells themselves are responsible for producing the reactionary dentin. If intense damage occurs, the odontoblasts are lost, and the pulp tissue stem cells generate new odontoblastic-like cells, which will produce reparative dentin (19, 36, 37). In this way, both differentiated and non-differentiated cells are useful for the mechanisms of tertiary dentin production (38).

Thus, it was expected that HP could induce the mineralizing potential of pulp cells; however, this review shows that in most studies, HP reduced ALP activity and deposition of mineralized nodules, which could indicate a loss in the mineralization potential of pulp cells after dental bleaching. These data disagree with those observed in *in vivo* studies, where a large formation of tertiary dentin is observed in bleached teeth (11, 12, 17, 21). This may be due to the differences of the models in *in vitro* and *in vivo* evaluations. In *in vitro* studies, an absence of cytoplasm extensions of odontoblast and dentin fluid that act as a physical barrier to the penetration of HP is found (10), besides the lack of HP-degrading enzymes (39). Thus, when damage occurs to the cell monolayer, it is expected to increase cell death, which can

reduce the potential for remaining cells to respond against the aggressor.

In addition, HP causes damage mainly to the occlusal third of the coronary pulp in *in vivo* studies, meaning that the underlying pulp tissue receives the lowest concentration of HP, and it is induced to proliferate and replenish the lost tissue, depositing tertiary dentin (18). Then, bleaching agents containing a low HP concentration and the underlying pulp tissue can be both associated with the expression of proteins related to the mineralization process, which occurs later in animal models (19). Thus, the three-dimensional (3D) system can be more appropriated than the traditional two-dimensional system for *in vitro* analysis because it can better represent *in vivo* cellular conditions using scaffold supports of cell growth and cell-to-cell interactions (40); however, the 3D model was not used by any of the selected studies in this review.

The production of tertiary dentin in *in vivo* studies after dental bleaching was observed from the 7th day (6, 19); however, most studies in this review found a reduction in ALP activity in this period (23, 23, 30, 32). ALP is an enzyme expressed by odontoblasts with an important role in the repair and regeneration of pulp tissue (29). Only one study in this review showed that despite the reduction of ALP activity at 7 and 14 days, induced by 17.5% HP, the ALP activity increased after 21 days when compared to the control group (23). In addition, this was the only study that assessed ALP activity over a prolonged period (21 days), in contrast with other studies that performed this analysis for up to 15 days. Thus, further analysis of ALP activity after a prolonged period is still necessary to fully understand this process.

Another study that evaluated a single application of 8 h of 10% CP or 45 min of 35% HP found no significant difference with the control regarding ALP activity immediately after bleaching (22). These results are in contrast with the study of Soares et al. (29), who observed a reduction in ALP activity after 8 h of 16% CP. These were the only studies that used CP and may indicate that the increase in concentration from 10 to 16% was enough

TABLE 2 | Risk of bias of included studies.

Quality criteria	Was the aim of the study clearly stated?	Was the sample size justified?	Was the assignment to treatment groups truly random?	Were control and treatment groups comparable at entry?	Were groups treated identically other than for the named interventions?	Was the bleaching protocol clearly described?	Were outcomes measured in the same way for all groups?	Were outcomes measured in a reliable way?	Was appropriate statistical analysis used?	Total score
De Oliveira Duque et al. (33)	1	0	0	1	1	1	1	1	0	6
Soares et al. (32)	1	0	0	1	1	1	1	1	1	7
Soares et al. (23)	1	1	0	1	1	1	1	1	0	7
Soares et al. (23)	0	0	0	1	1	1	1	1	1	6
Lima et al. (31)	1	0	1	1	1	1	1	1	1	8
Soares et al. (29)	0	1	0	1	1	1	0	1	1	6
Lima et al. (22)	1	0	0	1	0	1	1	1	0	5
Soares et al. (30)	0	0	0	1	1	1	1	1	1	6
Matsui et al. (20)	1	0	0	1	1	1	1	1	0	6
Min et al. (3)	1	0	0	1	1	0	1	1	1	6
Lee et al. (21)	1	0	0	1	1	0	1	1	1	6

0, not reported or reported but inadequate; 1, reported and adequate.

to influence the results of ALP activity. In addition, regarding 45 min of 35% HP, the other three studies that evaluated this concentration performed ALP activity analysis after 24 h or more, and all showed a reduction in ALP activity (30, 32, 33).

The decrease in ALP activity can be related to the cytotoxicity observed in the studies since high concentrations of HP are used in most of them. It is observed, for example, in two studies (3, 20) of this review, that the low concentration of HP led to an increase in the expression of markers of mineralization. But these results were not correlated with the analysis of the ALP. Matsui et al. (20) explained that the oxidative stress induced by HP to the cells results in the formation of calcium phosphate in them, which induces the expression of mineralization markers.

On the contrary, Soares et al. (23) found a reduction in the expression of mineralization markers when a high concentration of HP was used. A previous study in molars of bleached rats showed that a high concentration of HP can cause severe damage to the dental pulp, while low concentrations of HP can induce proliferation in the pulp tissue, which could explain these results. In addition, two studies showed an increase in OCN in the pulp tissue after dental bleaching (19, 41), which demonstrates that despite the importance of *in vitro* studies, different results can be observed from those that occur in *in vivo* models. In addition, the ROS can accumulate during oxidative stress, but due to their reactivity, this accumulation occurs in a transitory way; however, it can damage essential biomolecules, which can be challenging to recover in cell culture, but reversible in living tissue (42).

Regarding mineralized nodules deposition, the results followed those of the ALP activity; however, one study showed that minimal time of the application of 10% HP did not change the mineralized nodules deposition in dental pulp cells compared with control (33), despite reducing ALP activity. Further, these results were obtained in different analysis periods, which may indicate that in more prolonged periods, the production of mineralization nodules may occur. In addition, only one study evaluated the presence of mineralized nodules at 28 days after bleaching and revealed an increased number of mineralized nodules compared with the control group (20). Thus, studies with long periods of analysis for ALP activity and deposition of mineralized nodules would be important to clarify the results.

It should be noted that most of the studies located in this systematic review were carried out by the same group of authors (23, 23, 29, 30, 32). This can be considered a limitation of the present study due to a strong influence of methodological characteristics of a single research group on the results found in this review. Still, only *in vitro* studies were selected, requiring further systematic analysis of *in vivo* studies; however, this systematic review presents studies with a low risk of bias (Figure 2), where most of them revealed that high concentrations of HP could impair the mineralization potential of dental pulp cells. Some methodological limitations, such as the absence of randomization and no justification of sample size, were noticed. Future research should address these issues, including well-described sample randomization and suitable calculation of the sample size. In addition, although few studies have evaluated low HP concentrations or longer periods

of exposure, there seems to be a tendency for induction of mineralization to occur under these conditions, which needs to be further investigated.

It is also important to investigate adequate protocols and methods of evaluation that analyze the effect of this intervention in the mineralization of pulp tissue in clinical trials. Additional randomized and longitudinal investigations considering the different age ranges of participants, bleaching protocols, and anatomical variations should be considered to confirm these results; however, caution is highlighted when using high concentrations of HP, mainly used in in-office bleaching, since cytotoxic effects can be observed in pulp cells. This is in accordance with the European Union Council Directive 2011/84/EU (amending EU Council Directive 76/768/EEC) that stated that bleaching gel used for dental clinical practice might only contain up to 6% HP (43). Based on these results, we enhance the importance of dentist supervision under both home and in-office bleaching, mainly, when high concentrations of bleaching gel are used. Accelerated aging of the pulp tissue, in addition to inflammation, may be a consequence of the use of a high concentration of HP (15, 19).

CONCLUSION

Within the limitations of this review of *in vitro* studies, it can be concluded that mostly high concentrations of bleaching

gels could reduce the potential of mineralization in pulp cells; however, different HP concentrations, bleaching protocols, and analysis periods can influence this outcome.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

FB, CA, and LM: conceptualization. IG, AR-P, and LM: study selection. IG and AR-P: data collection. AR-P, HC, and CA: quality assessment. FB, AR-P, HC, and CA: methodology. FB, AB, and LC: project administration. LC and FB: supervision. AB, LM, and FB: validation. CA, AB, and LC: visualization. IG, AR-P, and HC: writing—original draft. LM, AB, LC, FB, and CA: writing—review and editing. All authors read and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fdmed.2021.689537/full#supplementary-material>

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