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REVIEW ARTICLE

A systematic review on antibacterial activity of zinc against *Streptococcus mutans*

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KEYWORDS

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Abstract *Objectives:* The aim of this study was to systematically review the growth inhibition effectiveness of zinc against *Streptococcus mutans*. The main question was, "Does the zinc inhibit the growth of oral *Streptococcus mutans* in vitro?

Methods: Literature search on PubMed, Medline, and science direct databases was carried out for in vitro studies published in English from 1990 to 2016, and the reported outcomes of minimum inhibitory concentration (MIC), minimum bactericidal concentrations (MBC), zone of inhibition (ZOI) and bacterial count method using colony forming unit (CFU) were used to assess the antibacterial effectiveness of zinc.

Results: Seventeen studies were included in this review. Seven studies reported MIC and MBC. Four studies reported ZOI, and eight studies reported CFU. MIC values using zinc chloride and zinc oxide nanoparticles were ranged from 0.025 to 0.2 mM and 0.390 to 500 ± 306.18 μg/ml respectively. MBC values using zinc oxide nanoparticles have ranged from 3.125 to 500 μg/ml. ZOI ranged from no inhibition zone to 21 ± 1.4 mm using 23.1% zinc oxide. A considerable reduction in the bacterial count was reported after adding zinc. However, only two studies have reported no inhibitory effect of zinc.

Conclusion: This review indicated a significant growth inhibition effectiveness of zinc even at lower concentrations which indicate it's safely to be used in oral health products.

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Contents

1. Introduction

Streptococcus mutans (*S. mutans*) is the main cariogenic bacteria causing dental caries (Metwalli et al., 2013; Seki et al., 2006). The key component of *S. mutans* pathogenesis is; its ability to produce large quantities of glucans and acid that exceeding the salivary buffering capacities. *S. mutans* able to strongly bind to teeth and can survive in an acidic environment (Metwalli et al., 2013).

Zinc is used as an antimicrobial, it has been added to mouth rinses and toothpaste to control dental plaque, inhibit calculus formation and reduce halitosis (Lynch, 2011). For many years zinc was incorporated into many dental materials due to the ability of zinc ion to inhibit the growth of cariogenic bacteria (Daugela et al., 2008). It is substantive in dental plaque and saliva, and its higher concentration can persist for many hours after delivery from oral health products (Lynch, 2011). It is considered a bacteriostatic agent rather than bactericidal, where its effect is reversed when cells are washed (Phan et al., 2004).

Sufficient evidence is available in the literature to indicate the inhibitory effect of zinc against oral bacteria (Phan et al., 2004). Zinc ion has multiple inhibitory effects on the intact bacterial cells activities such as glycolysis, glucosyltransferase production and polysaccharide synthesis, transmembrane proton translocation and acid tolerance (Phan et al., 2004). It can enhance proton permeabilities of bacterial cells membranes, (Phan et al., 2004) reduce Adenosine Triphosphate (ATP) synthesis in glycolyzing cells and diminish F-Type Adenosine Triphosphate Synthases (F-ATPase) activity due to its ability to inhibit of the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenases and pyruvate kinase as well as the phosphoenolpyruvate (Koo et al., 2006).

A vast number of published studies have used zinc oxide nanoparticles (ZnO-NPs) as an antibacterial agent (Sirelkhatim et al., 2015). ZnO-NPs is a bio-safe material, non-toxic to human cells (Sirelkhatim et al., 2015). Zinc in nanoparticles form is more toxic to bacteria than their micron equivalents (W. Liu et al., 2014). The antimicrobial activity of ZnO-NPs may be due to the possible interaction between the nanoparticles and bacteria. ZnO-NPs can disturb the bacterial growth either by interacting with the bacterial surface or entering inside the bacterial cells. This leading to disrupt bacterial enzyme systems by displacing magnesium ions essential for the enzymatic activity of bacteria (Hojati et al., 2013) and subsequently, showing a significant bactericidal effect (Sirelkhatim et al., 2015).

Several assays have been used to evaluate the inhibitory bacterial growth effect of zinc such as dilution methods and diffusion assays. These methods are considered the most known and fundamental methods to assess the antimicrobial activities of any agent (Balouiri et al., 2016). Dilution method is used to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of the antimicrobial agent. MIC is the lowest concentration that an antimicrobial agent inhibits the growth of the tested organisms whereas MBC is the lowest concentration of any agent killing the majority of bacterial inoculums (Ahrari et al., 2015; CLSI, 2012). Agar disk-Diffusion method is a well-known method used for routine antimicrobial susceptibility testing, in which diameters of zone of inhibition (ZOI) are measured (Balouiri et al., 2016).

Although as indicated earlier that many bacterial inhibitory mechanisms of zinc have proposed, and different in vitro assays have shown its clinical value against oral bacteria, however, it seems there is a controversy over the growth inhibitory effectiveness of the zinc against *S. mutans*. Some studies did not find a growth inhibitory effect of zinc against *S. mutans* (Eshed et al., 2012; Magnusson et al., 2007) while other studies (Ahrari et al., 2015; Xu et al., 2010) have reported a significant growth inhibitory effect. Hence, a systematic evaluation of the antibacterial effectiveness of zinc is still lacking. Therefore, the aim of this study was to systematically review the growth inhibition effectiveness of zinc against *S. mutans*.

2. Methods

The methods of this review were set according to preferred reporting items for systematic reviews and meta-analyses: The PRISMA statements (Moher et al., 2009). Initially, an

addressed question was developed to focus on the main aim of this study. The addressed question was "Does the zinc inhibit the growth of oral *S. mutans* in vitro?"

2.1. Eligibility criteria

The eligibility criteria were: original studies; in vitro studies, studies published in English only. Also studies assessed the antibacterial effectiveness of zinc on *S. mutans* using laboratory methods with reported outcomes either of the following; minimum inhibitory concentration (MIC), minimum bactericidal concentrations (MBC), zone of inhibition (ZOI) and colony forming unit (CFU) were included. Unpublished articles, case reports, short communications and letters to the editor were excluded.

2.2. Information sources

Electronic databases (PubMed, Medline, and science direct databases) were searched from 1990 to July 2016 for studies on zinc and its growth inhibition effectiveness against *S. mutans*. It was based on the fact that oral streptococci are the major constituents of dental plaque (Hoshino et al., 2004), and *S. mutans* is considered the prime bacteria causing dental caries (Metwalli et al., 2013; Seki et al., 2006). Different combinations of keywords were chosen including; zinc; antimicrobial; antibacterial; growth inhibition; mutans streptococci; and *S. mutans*. There was no a priori protocol or a protocol registration. The assessment of the studies included in this review depends on the quality of the studies, their clear results reported and their relevance to this topic. This systematic review developed to summarize the previous and most recent research related to the growth inhibition effectiveness of zinc against *S. mutans*.

2.3. Search strategy and selection of the review studies

The full electronic search strategy for all mentioned databases was carried out. Eligibility evaluation of the recourses was undertaken independently by the first two authors. The first author evaluated the titles and abstracts of each study for the eligibility. Then the full articles of the eligible studies were hand searching and screened for additional relevant references. The first author extracted the relevant data from the included studies and checked by the second author. Consensus between the two reviewers was used to resolve any disagreement.

3. Results

3.1. Selection of the studies

A total of 642 studies were selected from the databases: 61 from Medline Complete, 126 from PubMed and 455 from Science Direct, then reduced to 116 after eliminating duplications and the non-eligible studies by scanning the articles titles and abstracts. There were eighty-nine studies chose and reviewed in full. While, seventy-two articles excluded because of the following reasons:

- They were clinical or animal studies.
- The required laboratory outcomes of MIC, MBC, ZOI, and CFU were not assessed or not clearly reported.
- Used different type of oral bacteria.
- Studies tested the antimicrobial effect of zinc that mixed with other materials such as chlorhexidine and fluoride to assess the synergetic effect without testing the zinc alone.
- Studies tested/ examined non-silver-containing inorganic antibacterial agents and zinc oxide whisker (ZnOw).

Finally, seventeen studies were included and proceeded for data extraction (Fig. 1).

3.2. Characteristics of the included studies

Seventeen studies met all inclusion criteria in this review. Different *S. mutans* strains were used in these experiments as shown (Tables 1–3). Various forms of zinc were reported including zinc sulfate, (Osinaga et al., 2003), zinc chloride (Dashper et al., 2005; Eisenberg et al., 1991), zinc citrate (Bradshaw et al., 1993), zinc gluconate (Pizzey et al., 2011) and zinc oxide (Jatania and Shivalinga, 2014; L. Liu et al., 2014; Spencer et al., 2009). Zinc in form of Zn-ions that were deposit on titanium (Ti) surface (Xu et al., 2010; Zhao et al., 2013). Furthermore, zinc oxide nanoparticles were reported the most among recent studies (Ahrari et al., 2015; Eshed et al., 2012; Hernández-Sierra et al., 2008; Hojati et al., 2013; Kasraei et al., 2014; Ramazanzadeh et al., 2015; Yu et al., 2014).

Studies have reported different ways of zinc ion introduction. Some studies used aqueous solutions to introduce the zinc ion (Zn^{2+}). (Ahrari et al., 2015; Bradshaw et al., 1993; Dashper et al., 2005; Eisenberg et al., 1991; Eshed et al., 2012; Hernández-Sierra et al., 2008; Pizzey et al., 2011). Other studies incorporated Zn^{2+} into the structure of some dental materials to investigate it's the antimicrobial effectiveness (Hojati et al., 2013; Jatania and Shivalinga, 2014; Kasraei et al., 2014; L. Liu et al., 2014; Osinaga et al., 2003;

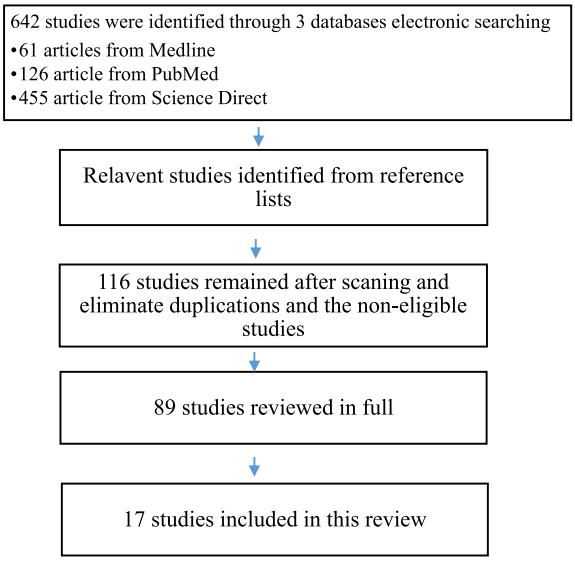

Fig. 1 Studies selection.

Table 1 Growth inhibition effectiveness of zinc using MIC and MBC.

Study	*S. mutans* strain	Tested zinc			Control material			P- value
		Type	MIC	MBC	Type	MIC	MBC	
Eisenberg et al. (1991)	*S. mutans* GS-5	Zinc chloride	0.025 mM/l (pH 5.5) 0.5 mM/l (pH 6)	NA 4 mM/l (pH 6)	Chlorhexidine	0.4 μg/ml (pH 5.5) 0.8 μg/ml (pH 6)	NA	NA
Dashper et al. (2005)	*S. mutans* Ingbritt	Zinc chloride	200 μM	NA	NA	NA	NA	NA
Hernández-Sierra et al. (2008)	*S. mutans*	ZnO-NPs	500 ± 306.18 μg/ml	500 μg/ml	NA	NA	NA	NA
Pizzey et al. (2011)	NCTC 10449	Zinc gluconate	2.8 mM	11 mM	NA	NA	NA	NA
Eshed et al. (2012)	*S. mutans* 700610 (clinical isolate)	ZnO-NPs	No growth inhibition	NA	NA	NA	NA	NA
Ahrari et al. (2015)	PTC 1683	ZnO-NPs	0.390 μg/ml	3.125 μg/ml	0.2% Chlorhexidine	62.5 μg/ml	83.33 μg/ml	$P < 0.001$
Yu et al. (2014)	ATCC 25175	ZnO-NPs	0.156 mg/ml	0.312 mg/ml	NA	NA		NA

MIC; minimum inhibitory concentration, MBC; minimum bactericidal concentration, ATCC; American Type Culture Collection, ZnO-NPs; Zinc Oxide Nanoparticles.

Ramazanzadeh et al., 2015; Spencer et al., 2009; Xu et al., 2010; Yu et al., 2014; Zhao et al., 2013). In this review, two studies have reported more than one antibacterial method to assess the antibacterial effectiveness of zinc against *S. mutans*. Hojati et al. (2013) had reported the evaluation by ZOI and CFU where Ahrari et al. (2015) reported using MIC, MBC, and CFU.

Overall, seven studies reported MIC and MBC (Ahrari et al., 2015; Dashper et al., 2005; Eisenberg et al., 1991; Eshed et al., 2012; Hernández-Sierra et al., 2008; Pizzey et al., 2011; Yu et al., 2014). Four studies have reported ZOI using agar diffusion method (Hojati et al., 2013; Jatania and Shivalinga, 2014; Osinaga et al., 2003; Spencer et al., 2009). Additionally, eight studies reported zinc antibacterial inhibition efficacy through viable CFU (Ahrari et al., 2015; Bradshaw et al., 1993; Hojati et al., 2013; Kasraei et al., 2014; L. Liu et al., 2014; Ramazanzadeh et al., 2015; Xu et al., 2010; Zhao et al., 2013). Using these outcomes, only two studies out of seventeen indicated no growth inhibition efficacy of the zinc against *S. mutans* (Bradshaw et al., 1993; Eshed et al., 2012).

3.3. Growth inhibition of zinc against S. Mutans

3.3.1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The results of the MIC and MBC evaluation indicated that zinc acts effectively against *S. mutans*. These studies were used different forms of zinc either as salts (zinc chloride, zinc gluconate) or as ZnO-NPs. Generally, low MIC and MBC values of zinc were stated. MIC values using zinc chloride were ranged from 0.025 mM to 0.2 mM. The MIC using ZnO-NPs have ranged from 0.390 to 500 ± 306.18 μg/ml. The MBC using ZnO-NPs ranged from 3.125 to 500 μg/ml. However, a study done by Eshed et al. (2012) using ZnO-NPs did not report any growth inhibition of *S. mutans* even after increasing the concentrations of ZnO-NPs to 1.0 mg/ml (Table 1).

3.3.2. Zone of inhibition (ZOI)

Table 2 provides a summarized data regarding the ZOI using agar diffusion method. In general, limited studies are using this method. In this review, all studies reported inhibition zone in millimeters expect one study which reported inhibition zone in squared millimeters^2, where the equivalent mm was calculated. All of the reviewed studies were testing the antibacterial of zinc added to orthodontic bonding agents or a resin cement. The negative controls were the same tested materials but without adding zinc. The use of zinc resulted in either no inhibition zone to a moderate inhibition zone. The inhibition zone for zinc ranged from no inhibition zone using ZnO-NPs (Hojati et al., 2013) to 21 ± 1.4 mm using 23.1% ZnO (Jatania and Shivalinga, 2014). Studies have shown that the antibacterial activity of zinc increased as the concentration of zinc increased (Jatania and Shivalinga, 2014; Spencer et al., 2009). Therefore, 23.1% of ZnO exhibited a greater ZOI than 13% ZnO. Additionally, it appears that fresh mixed zinc showed more antibacterial properties compared to old zinc mixture (Jatania and Shivalinga, 2014; Osinaga et al., 2003; Spencer et al., 2009).

3.3.3. Colony Forming Unit (CFU)

Table 3 shows the viable bacteria counts of dental materials treated with zinc as compared to the negative control (no zinc added). Only one study did not find a significant inhibition of the viable count of *S. mutans* even after 48 h of using zinc citrate (Bradshaw et al., 1993). However, all the other studies demonstrated that zinc significantly reduced the viability of *S. mutans* compared to the controls (Ahrari et al., 2015; Hojati et al., 2013; Kasraei et al., 2014; L. Liu et al., 2014; Ramazanzadeh et al., 2015; Xu et al., 2010; Zhao et al., 2013). The reviewed studies have agreed that antibacterial effectiveness of zinc was concentration-dependent. With an increase in the concentration of zinc, there was an increase in the antibacterial effectiveness (Hojati et al., 2013; Ramazanzadeh et al., 2015; Xu et al., 2010; Zhao et al., 2013). In the study by Hojati et al. (2013) both agar diffusion

Table 2 Growth inhibition effectiveness of zinc using ZOI.

Study	*S. mutans* strain	Tested zinc		Negative control		P- value
		Type	Mean ZOI	Type	Mean ZOI	
Osinaga et al. (2003)	ATCC 25175	5% and 10% zinc sulfate (ZnSO4) added to conventional glass Ionomer cement (GIC) and Resin Modified Glass Ionomer Cement (RMGIC).	After 1 hr 10 wt% provides ZOI of 2.2 mm After 15 days No inhibition zone	Conventional glass Ionomer cement (GIC) and Resin Modified Glass Ionomer Cement (RMGIC) 0% zinc GIC &RMGIC with 0% zinc	< 1.5 0	NA
Spencer et al. (2009)	NA	13% ZnO or 23.1% ZnO was added to orthodontic bonding material (Ortho Fuji LC) a resin-modified glass ionomer (RMGI)	After 48 hrs • 13% provides ZOI of 70.52 ± 17.33 mm^2 equivalent to 9.4 mm • 23.1% provides ZOI of 110.03 ± 31.00 mm^2 equivalent to 11.83 mm After 1 month • 13% provides ZOI of 16.0 ± 11.6 mm^2 equivalent to 4.5 ± 3.7 mm • 23.1% provides ZOI of 38.3 ± 13.3 mm^2 equivalent to 6.95 ± 4.0 mm	Orthodontic bonding (Ortho Fuji LC) a resin-modified glassionomer (RMGI) 0% ZnO Orthodontic bonding (Ortho Fuji LC) a resin-modified glass ionomer (RMGI) 0% ZnO	0 0	P < 0.00 P < 0.00
Hojati et al. (2013)	PTCC 1683	Five resin composites containing 1, 2, 3, 4, 5 wt% ZnO-NPs	No inhibition zone	Resin composite without ZnO-NPs	0	NA
Jatania & Shivalinga, (2014)	NA	13% and 23.1% ZnO was added to a resin modified light cure glass ionomer cement (RMGIC).	After 48 hrs • 13% provides ZOI of 12.50 ± 0.707 mm • 23.1% provides ZOI of 21 ± 1.4 mm After month • 13% provides ZOI of 10 ± 0.00 mm • 23.1% provides ZOI of 19 ± 0.00 mm	Conventional light cure composite without zinc and RMGIC without zinc Conventional light cure composite without zinc and RMGIC without zinc	0 0	P < 0.01 P < 0.01

ZOI; Zone of Inhibition, ZnO; Zinc oxide, ZnO-NPs; Zinc Oxide nanoparticles, NA; Not available.

Table 3 Growth inhibition effectiveness of zinc using CFU.

Study	*S. mutans* strain	Tested zinc		Control material		P- value
		Type	Number/mean CFU	Type	Number/mean CFU	
Bradshaw et al. (1993)	*S. mutans* R9	Zinc citrate solution (39.8 μmol/l)	Log_{10} CFU/ml post dosing of zinc citrate after 24 hrs was 4.08 and after 48 hrs was 4.36	Pre-dosing of zinc citrate	Log_{10} CFU/ml ± SD) was 4.75 ± 0.42	P > **0.05**
Xu et al. (2010)	UA159	Zn-ion were deposit on titanium (Ti) surface by time of 20 min intervals: Zn–Ti-20, Zn–Ti-40, Zn–Ti-60 and Zn–Ti-80	Zn–Ti-20 0.8 ± 0.2 Zn–Ti-40 0.6 ± 0.3 Zn–Ti-60 0.2 ± 0.1 Zn–Ti-80 0.1 ± 0.0 CFU ($\times 10^9$ mL)	cp-Ti without Zn	2.6 ± 0.3	P < 0.01
Hojati et al. (2013)	PTCC 1683	Resin composites containing 0–5 wt% ZnO-NPs	4 and 5 wt% groups entirely inhibit formation of any bacterial colony. CFU after 48 hrs was 20,000 and less No significant difference between the test and control groups after 1 week or 1 month	Resin composites with no zinc additive.	CFU of 160,000 CFU after 48 hrs was 80,000	P < 0.05 P < 0.05 P > 0.05
Zhao et al. (2013)	ATCC 25175	Micro-arc oxidation (MAO) coatings for titanium coated with different concentrations of zinc ions in electrolyte	Mean CFU of:- High Zn 0.05 mol/l was 15.25 ± 2.13 Medium Zn 0.03 mol/l was 22.10 ± 2.21 Low-Zn 0.01 mol/l was 27.45 ± 3.23	No-Zn Titanium coatings	CFU was 73.28 ± 3.67.	P < 0.05
Kasraei et al. (2014)	PTCC 1683	Composite resins containing 1% ZnO-NPs	Mean CFU was 0.93 ± 1.53	Composite resin without zinc	Mean CFU was 126.0 ± 29.47	P = 0.00
L. Liu et al. (2014)	UA 159	0.01 g and 0.05 g Calcium phosphate glass doped with ZnO	CFU/ml of 0.01 g after 2 hrs was 3.68×10^4. At 4 hrs showed 100% bactericidal activity continued for 6 hrs. 0.05 g produced a maximum bactericidal effect at 2 hrs compared to 4 hrs with 0.01 g.	Calcium phosphate glass (0.01 g CPG) without zinc doping 0.05 g CPG without zinc doping A negative control with no (CPG)	After 4 hrs CPG 0.01 g had CFU/ml of 5.48×10^4/ml 0.05 g CPG showed maximum bactericidal effect at 4 hrs CFU/ml was 1.31×10^7	**NA**
Ramazanzadeh et al. (2015)	ATCC 35668	ZnO-NPs coated brackets	Mean CFU after 0 hr 140.84 ± 65.79 2 hrs 96.78 ± 46.19 4 hrs 96.10 ± 75.64 6 hrs 55.89 ± 51.95 24 hrs 219.32 ± 304.53	Uncoated brackets	Mean CFU after 0 hr 165.91 ± 53.19 2 hrs 139.33 ± 43.71 4 hrs 135.08 ± 68.93 6 hrs 117.75 ± 66.68 24 hrs 921.25 ± 823.68	P = 0.844 P < 0.001
Ahrari et al. (2015)	PTC 1683	Colloidal solutions containing ZnO-NPs	Mean CFU after 1 min was 2900 ± 655 Mean CFU after 5 min was 1153 ± 799	Chlorhexidine (positive control) Sodium fluoride mouthrinses (negative control)	Mean CFU after 1 min and 5 min was 0 Mean CFU after 1 min and 5 min was 4666 ± 577	P < 0.001 P < 0.001

CFU; Colony Forming Unit, min; minute, cp-Ti; Commercial Titanium, ZnO; Zinc Oxide, ZnO-NPs; Zinc Oxide nanoparticles, CPG; calcium phosphate glass, hrs; hours, NA; Not available.

method and direct contact test were done to assess the zinc effectiveness against *S. mutans*. Even though no inhibition effect was reported using agar diffusion test, however, direct contact test has demonstrated greater *S. mutans* viability inhibition when zinc added to resin composite that persisted for more than 48 h. As mentioned earlier, fresh mixed zinc exhibited higher antibacterial properties than aging material. However, after one week there was no significant difference between either of the test and control groups ($P > 0.05$) (Hojati et al., 2013).

4. Discussion

This study aimed to review growth inhibitory effect of zinc against *S. mutans* and to gain further and specific conclusions about the antibacterial activity of different zinc forms.

This review has indicated that although several zinc antimicrobial inhibitory mechanisms have been proposed (Phan et al., 2004). However, we still found some laboratory studies did not support this finding (Bradshaw et al., 1993; Eshed et al., 2012).

Zinc is an environmentally friendly material and has little toxicity (Applerot et al., 2009). It is naturally present in dental plaque and saliva (Lynch, 2011). Due to its antibacterial properties, zinc effectively and commonly used for dermatological applications in creams, lotions and ointments (Applerot et al., 2009). Additionally, it has been used widely in dentistry; it incorporated in dental materials and in oral health products including toothpaste and mouth rinse without the concern of high toxicity or unfavorable side effects, where its higher concentration can persist for many hours after delivery from oral health products (Lynch, 2011).

In this review, we included studies that used zinc as antibacterial against *S. mutans* with outcomes of MIC, MBC, the ZOI and viable bacterial count method using CFU. These methods were selected as they are considered the most known assays to evaluate the antimicrobial activity of any agent (Balouiri et al., 2016).

Overall, the majority of the studies have reported antibacterial properties of zinc either in the form of powder salts or nanoparticles form against *S. mutans*, even at very lower concentrations (Ahrari et al., 2015; Dashper et al., 2005; Eisenberg et al., 1991; Hernández-Sierra et al., 2008; Hojati et al., 2013; Jatania and Shivalinga, 2014; Kasraei et al., 2014; L. Liu et al., 2014; Osinaga et al., 2003; Pizzey et al., 2011; Ramazanzadeh et al., 2015; Spencer et al., 2009; Xu et al., 2010; Yu et al., 2014; Zhao et al., 2013). Though, only two studies did not report such a finding (Bradshaw et al., 1993; Eshed et al., 2012). The first study by Bradshaw et al. (1993) that did not reported any antibacterial properties of zinc against *S. mutans*. It has indicated that using complex media components might render the tested zinc citrate ineffective. The other study by Eshed et al. (2012) did not report any MIC of ZnO-NPs against *S. mutans* even after increasing the concentrations of ZnO-NPs to 1.0 mg/ml. Although the other studies included in this review have shown even lower MIC concentrations using ZnO-NPs, thus, most likely this variation is attributed to differences in the strains of the bacteria that utilized in each study, where in the study by Eshed et al. (2012) has used clinical isolate *S. mutans* strain. Additionally, in this review we found that fresh mixed zinc exhibited higher antibacterial properties than aging material, possibly due to increasing the release of Zn^{2+} from the fresh mixture and this effect tends to be less with time (Hojati et al., 2013; Jatania and Shivalinga, 2014; Osinaga et al., 2003; Spencer et al., 2009).

A considerable body of literature has indicated that ZnO-NPs exhibits significant antibacterial activity over a broad spectrum of bacterial species (Sirelkhatim et al., 2015). In the early stage of this review, we planned to include only the powder form of zinc instead of nanoparticles. However, due to the limited number of studies, ZnO-NPs studies were included. Adequate number of the studies included in this review have tested the antibacterial effectiveness of zinc in form of nanoparticles mainly ZnO-NPs (Ahrari et al., 2015; Eshed et al., 2012; Hernández-Sierra et al., 2008; Hojati et al., 2013; Kasraei et al., 2014; Ramazanzadeh et al., 2015; Yu et al., 2014). Almost all of these studies have shown promising results indicated significant antibacterial inhibition effect and safety applications.

One of the proposed antimicrobial mechanism of ZnO-NPs could be due to the leaching of Zn^{2+} into the growth media, and the interference of Zn^{2+} ions with the enzyme systems of the bacteria by displacing with magnesium ions which is crucial for bacterial enzymatic activities (Hojati et al., 2013). The agar diffusion test is one of the most popular used methods to determine the antimicrobial activity of an agent (Balouiri et al., 2016). Agar diffusion test can be considered for materials which are soluble and capable of diffusing into the surrounding environment (Weiss et al., 1996). That may explain why the study by Hojati et al. (2013) using ZnO-NPs did not reveal any ZOI using agar diffusion method. The insolubility of the ZnO-NPs hindered diffusion of an adequate amount of Zn^{2+} to the surrounding environment to show a visible antibacterial effect (Sevinç and Hanley, 2010).

Despite the significant antibacterial activity of zinc, one of the limiting factors in the use of zinc as nanoparticles is due to the different effective antibacterial concentration against *S. mutans*, toxicological impact and potentially undesirable effects on the human body. Minimal concentration might not be effective against the *S. mutans*, and higher concentration could possibly induce cytotoxicity and genotoxicity. The presence of ZnO-NPs in dental care products such as toothpaste and mouthwash implicates a major source of exposure since people use these products 2–3 times daily. Therefore, much attention is paid to the question of whether ZnO NPs in these products are able to cross the oral mucosa barrier and results in inflammation and systemic toxicity via dermal penetration (Meyer et al., 2011). Another possible route of entry is via the oral route (Sharma et al., 2012). Even though the dental care products are not directly ingested, but there is still a risk of ingestion during the use.

Toxicity of ZnO-NPs was reported in vitro studies at different concentrations. However, it has been noticed that most of the data reported the toxicity below 100 μg/mL for a normal cell. As reported by Meyer et al. (2011), 85% reduction in cell viability was observed upon treatment of human dermal fibroblast with ZnO-NPs at concentrations ranging from 25 to 100 μg/ml and at concentration 50 μg/ml the ZnO-NPs had induced the cell apoptosis. A significant increase in DNA damage in human nasal mucosa was also observed upon repetitive (2–3 times) and short exposure (1 h per exposure) to 5 μg/ml of ZnO-NPs (Hackenberg et al., 2011). ZnO-NPs also

showed cytotoxic activity by acting on different targets in renal cells, with $IC_{50} \geq 73.05$ μg/ml (Uzar et al., 2015).

The nanoparticles when ingested into the body can be distributed to different regions because of their small size. Even though zinc is an essential trace element in the human body and ZnO is generally considered to be a compound with low toxicity, but the nanoparticle form is more reactive and responsive with higher absorptivity. Biodistribution or accumulation on ZnO-NPs was reported in many organs depending on route of administration. For experiment, treatment via the oral administration on mice (fed treatment), ZnO-NPs (2.5 g/kg) were reported to enter into blood circulation within 30 min post-dosing, and clearance in serum began 6 h after administration (Li et al., 2012). Sharma et al. (2012) reported significant accumulation of nanoparticles in the mouse liver leading to cellular injury after sub-acute oral exposure of ZnO-NPs (300 mg/kg) for 14 consecutive days. In addition, the levels of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in the treated group were higher compared to the control group and the histopathology examination of liver and kidney showed pathological lesions.

In spite of many advantages in using nanotechnology, several studies indicate that nanoparticles may cause hazardous effects because of their unique physicochemical properties (Uzar et al., 2015). The obtained toxicological data so far for ZnO-NPs are also conflicting and inconsistent. The reported doses do not directly reflect the actual concentrations of ZnO-NPs that could induce toxicity in human because no human study or clinical trial was reported. However, the toxicological data derived from in vitro and in vivo studies can be used to assess the possible human health risks from exposure to ZnO-NPs.

Lastly, although the focus of this review was on a single bacterial approach which is *S. mutans,* the prevailing cariogenic bacteria involving in initiation and progression of dental caries (Metwalli et al., 2013; Seki et al., 2006) and in vitro studies, the collected findings allowed to definitely assess the effectiveness of zinc against *S. mutans*. However, certain limitations in the present study should be highlighted. First, neither definitive conclusions about wider range of bacteria nor the antibacterial efficacy of zinc in the clinical studies can be made from this review, as in vitro studies and one type of bacteria were included. Second, language bias which might be present because we included only papers published in English.

5. Conclusions

In the light of the above facts, zinc is an effective antibacterial agent against the growth of *S. mutans* bacteria even at lower concentrations. Its effectiveness is dependent on the zinc concentrations and type. Therefore, we concluded that zinc in different forms is an antibacterial agent, potentially can be considered for developing further valuable oral health care products and to be added to dental materials. Additionally, with the evolution of nanotechnology and use of ZnO-NPs, promising steps toward more effective therapeutic approaches can be developed to enhance the antibacterial properties of zinc in future.

Conflict of interest

Authors declare that there is no conflict of interest.

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References

Ahrari, F., Eslami, N., Rajabi, O., Ghazvini, K., Barati, S., 2015. The antimicrobial sensitivity of *Streptococcus mutans* and streptococcus sangius to colloidal solutions of different nanoparticles applied as mouthwashes. Dent. Res. J. 12, 44–49.

Applerot, G., Perkas, N., Amirian, G., Girshevitz, O., Gedanken, A., 2009. Coating of glass with ZnO via ultrasonic irradiation and a study of its antibacterial properties. Appl. Surf Sci, 256.

Balouiri, M., Sadiki, M., Ibnsouda, S.K., 2016. Methods for in vitro evaluating antimicrobial activity: a review. J. Pharm. Anal. 6, 71–79.

Bradshaw, D., Marsh, P., Watson, G., Cummins, D., 1993. The effects of triclosan and zinc citrate, alone and in combination, on a community of oral bacteria grown in vitro. J. Dent. Res. 72, 25–30.

CLSI, 2012. Methods for Dilution Antimicrobial Susceptibility Tests f or Bacteria That Grow Aerobically; Approved St andard—Ninth Edition. CLSI document M07-A9. Clinical and Laboratory Standards Institute, Wayne, PA.

Dashper, S.G., O'Brien-Simpson, N.M., Cross, K.J., Paolini, R.A., Hoffmann, B., Catmull, D.V., Malkoski, M., Reynolds, E.C., 2005. Divalent metal cations increase the activity of the antimicrobial Peptide kappacin. Antimicrob. Agents Chemother. 49, 2322–2328.

Daugela, P., Oziunas, R., Zekonis, G., 2008. Antibacterial potential of contemporary dental luting cements. Stomatologija 10, 16–21.

Eisenberg, A.D., Young, D.A., Fan-Hsu, J., Spitz, L.M., 1991. Interactions of sanguinarine and zinc on oral streptococci and Actinomyces species. Caries Res. 25, 185–190.

Eshed, M., Lellouche, J., Matalon, S., Gedanken, A., Banin, E., 2012. Sonochemical coatings of ZnO and CuO nanoparticles inhibit *Streptococcus mutans* biofilm formation on teeth model. Langmuir 28, 12288–12295.

Hackenberg, S., Zimmermann, F., Scherzed, A., Friehs, G., Froelich, K., Ginzkey, C., Koehler, C., Burghartz, M., Hagen, R., Kleinsasser, N., 2011. Repetitive exposure to zinc oxide nanoparticles induces DNA damage in human nasal mucosa mini organ cultures. Environ. Mol. Mutagen. 52, 582–589.

Hernández-Sierra, J.F., Ruiz, F., Cruz Pena, D.C., Martínez-Gutiérrez, F., Martínez, A.E., de Jesús Pozos Guillén, A., Tapia-Pérez, H., Martínez Castañón, G., 2008. The antimicrobial sensitivity of Streptococcus mutans to nanoparticles of silver, zinc oxide, and gold. Nanomed. Nanotechnol. Biol. Med. 4, 237–240.

Hojati, T.S., Alaghemand, H., Hamze, F., Babaki, A.F., Rajab-Nia, R., Rezvani, M.B., Kaviani, M., Atai, M., 2013. Antibacterial, physical and mechanical properties of flowable resin composites containing zinc oxide nanoparticles. Dent. Mater. 29, 495–505.

Hoshino, T., Kawaguchi, M., Shimizu, N., Hoshino, N., Ooshima, T., Fujiwara, T., 2004. PCR detection and identification of oral streptococci in saliva samples using GTF genes. Diagn. Microbiol. Infect. Dis. 48, 195–199.

Jatania, A., Shivalinga, B.M., 2014. An in vitro study to evaluate the effects of addition of zinc oxide to an orthodontic bonding agent. Eur. J. Dent. 8, 112–117.

Kasraei, S., Sami, L., Hendi, S., Alikhani, M.-Y., Rezaei-Soufi, L., Khamverdi, Z., 2014. Antibacterial properties of composite resins incorporating silver and zinc oxide nanoparticles on *Streptococcus mutans* and Lactobacillus. Restor. Dent. Endod. 39, 109–114.

Koo, H., Sheng, J., Nguyen, P.T.M., Marquis, R.E., 2006. Co-operative inhibition by fluoride and zinc of glucosyl transferase production and polysaccharide synthesis by mutans streptococci in suspension cultures and biofilms. FEMS Microbiol. Lett. 254, 134–140.

Li, C.-H., Shen, C.-C., Cheng, Y.-W., Huang, S.-H., Wu, C.-C., Kao, C.-C., Liao, J.-W., Kang, J.-J., 2012. Organ biodistribution, clearance, and genotoxicity of orally administered zinc oxide nanoparticles in mice. Nanotoxicology 6, 746–756.

Liu, L., Pushalkar, S., Saxena, D., LeGeros, R.Z., Zhang, Y., 2014. Antibacterial property expressed by a novel calcium phosphate glass. J. Biomed. Mater. Res. 102, 423–429.

Liu, W., Su, P., Chen, S., Wang, N., Ma, Y., Liu, Y., Wang, J., Zhang, Z., Li, H., Webster, T.J., 2014. Synthesis of TiO2 nanotubes with ZnO nanoparticles to achieve antibacterial properties and stem cell compatibility. Nanoscale 6, 9050–9062.

Lynch, R.J.M., 2011. Zinc in the mouth, its interactions with dental enamel and possible effects on caries; a review of the literature. Int. Dent. J. 61, 46–54.

Magnusson, K., Petersson, L.G., Birkhed, D., 2007. Effect of dentifrices with antimicrobial agents on mutans streptococci in saliva and approximal dental plaque in orthodontic patients. Oral Health Prev. Dent. 5, 223–227.

Metwalli, K.H., Khan, S.A., Krom, B.P., Jabra-Rizk, M.A., 2013. Streptococcus mutans, *Candida albicans*, and the human mouth: a sticky situation. PLoS Pathog. 9.

Meyer, K., Rajanahalli, P., Ahamed, M., Rowe, J.J., Hong, Y., 2011. ZnO nanoparticles induce apoptosis in human dermal fibroblasts via p53 and p38 pathways. Toxicol. Vitr. 25, 1721–1726.

Moher, D., Liberati, A., Tetzlaff, Jennifer, Altman, D.G.the PRISMA group, 2009. Preferred reporting items for systematic reviews and meta-analysis : the PRISMA statement. Ann. Intern. Med. 151, 264–269.

Osinaga, P.W.R., Grande, R.H.M., Ballester, R.Y., Simionato, M.R. L., Delgado Rodrigues, C.R.M., Muench, A., 2003. Zinc sulfate addition to glass-ionomer-based cements: influence on physical and antibacterial properties, zinc and fluoride release. Dent. Mater. 19, 212–217.

Phan, T.-N., Buckner, T., Sheng, J., Baldeck, J.D., Marquis, R.E., 2004. Physiologic actions of zinc related to inhibition of acid and alkali production by oral streptococci in suspensions and biofilms. Oral Microbiol. Immunol. 19, 31–38.

Pizzey, R.L., Marquis, R.E., Bradshaw, D.J., 2011. Antimicrobial effects of o-cymen-5-ol and zinc, alone & in combination in simple solutions and toothpaste formulations. Int. Dent. J. 61, 33–40.

Ramazanzadeh, B., Jahanbin, A., Yaghoubi, M., Shahtahmassbi, N., Ghazvini, K., Shakeri, M., Shafaee, H., 2015. Comparison of antibacterial effects of ZnO and CuO nanoparticles coated brackets against *Streptococcus mutans*. J. Dent. 16, 200–205.

Seki, M., Yamashita, Y., Shibata, Y., Torigoe, H., Tsuda, H., Maeno, M., 2006. Effect of mixed mutans streptococci colonization on caries development. Oral Microbiol. Immunol. 21, 47–52.

Sevinç, B.A., Hanley, L., 2010. Antibacterial activity of dental composites containing zinc oxide nanoparticles. J. Biomed. Mater. Res. B Appl. Biomater. 94, 22–31.

Sharma, V., Singh, P., Pandey, A.K., Dhawan, A., 2012. Induction of oxidative stress, DNA damage and apoptosis in mouse liver after sub-acute oral exposure to zinc oxide nanoparticles. Mutat. Res. Toxicol. Environ. Mutagen. 745, 84–91.

Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N.H.M., Ann, L.C., Bakhori, S.K.M., Hasan, H., Mohamad, D., 2015. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-Micro Lett. 7, 219–242.

Spencer, C.G., Campbell, P.M., Buschang, P.H., Cai, J., Honeyman, A.L., 2009. Antimicrobial effects of zinc oxide in an orthodontic bonding agent. Angle Orthod. 79, 317–322.

Uzar, N., Abudayyak, M., Akcay, N., Algun, G., Özhan, G., 2015. Zinc oxide nanoparticles induced cyto- and genotoxicity in kidney epithelial cells. Toxicol. Mech. Methods 4, 334–339.

Weiss, E.I., Shalhav, M., Fuss, Z., 1996. Assessment of antibacterial activity of endodontic sealers by a direct contact test. Endod. Dent. Traumatol. 12, 179–184.

Xu, J., Ding, G., Li, J., Yang, S., Fang, B., Sun, H., Zhou, Y., 2010. Zinc-ion implanted and deposited titanium surfaces reduce adhesion of *Streptococccus mutans*. Appl. Surf. Sci. 256, 7540–7544.

Yu, J., Zhang, W., Li, Y., Wang, G., Yang, L., Jin, J., Chen, Q., Huang, M., 2014. Synthesis, characterization, antimicrobial activity and mechanism of a novel hydroxyapatite whisker/nano zinc oxide biomaterial. Biomed. Mater. 10, 15001.

Zhao, B.H., Zhang, W., Wang, D.N., Feng, W., Liu, Y., Lin, Z., Du, K.Q., Deng, C.F., 2013. Effect of Zn content on cytoactivity and bacteriostasis of micro-arc oxidation coatings on pure titanium. Surf. Coatings Technol. 228, S428–S432.