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Effectiveness of Hydrogen Peroxide Concentrations on Bleaching and Tooth Sensitivity - A Systematic Review

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ABSTRACT

Background In this study, the literature was systematically reviewed to investigate the effect of hydrogen peroxide concentrations on bleaching effect and tooth sensitivity.

Data Sources: Electronic databases were screened, and hand searched. Searched electronic databases are PubMed, Scopus, Cochrane Library, Ovid Medline, Elsevier science direct, Wiley online library, Grey literature, Embase.

Materials and Methods: A systematic review on controlled clinical trials MeSH terms used are (hydrogen peroxide) AND (bleaching) AND (tooth sensitivity). A trial on the bleaching effect and tooth sensitivity with various hydrogen peroxide concentrations were included. PRISMA guidelines were followed, and the PICO model was used. Cochrane risk assessment tool for randomized controlled trials was used.

Results: A total of 5 articles fulfilled the inclusion criteria. The analysis showed that high hydrogen peroxide concentration has a better bleaching effect accompanied by tooth sensitivity. Low hydrogen peroxide concentration also has a bleaching effect slightly minimal from the former, followed by less tooth sensitivity.

Conclusion: This study concludes that the hydrogen peroxide with higher concentration is effective in bleaching and with lower concentration is less likely to produce tooth sensitivity

Keywords: H_2O_2, Bleaching, Sensitivity, Tooth shade, Aesthetic dentistry

INTRODUCTION

In contemporary clinical dentistry, aesthetic dentistry is one of the most dynamic areas. Through basic and clinical research, knowledge about esthetic dentistry has expanded over the past decade, which has led to the development of a multitude of new restorative materials and clinical technique. The tooth-coloured resin-based materials gradually enhance the quality of the esthetic restoration.[1]

To have a healthier and youthful appearance, an increasing number of people

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have begun to seek a white-toothed smile, which plays an important social role. The bleaching process comprises the application of the bleaching gel over the tooth surface for a longer duration of time, which may cause some damaging effects on its structure. The detrimental effects are sensitivity due to increased enamel porosity, gingivitis, and changes in enamel microhardness, increasing its surface roughness, throat and gastric irritation. Chapple described the first dental whitening technique in 1877.[2]

Tooth discolouration is mainly caused due to intrinsic and extrinsic stains. Intrinsic stains are also called internal staining, caused due to certain factors such as genetics, antibiotics, age, antibiotics, high levels of fluoride, and developmental disorders. Extrinsic stains are also called external stains, caused due to environmental factors, including antibiotics, smoking, pigments in beverages and foods and metals such as iron or copper. The mechanism is that the coloured compounds from these sources are adsorbed into the acquired dental pellicle, causing a stain to appear.[3]

The bleaching technique is a procedure, involves the lightening of the tooth colour by application of a chemical agent to oxidize the organic pigmentation in the tooth. The most commonly used materials for bleaching are hydrogen peroxide (HP) and carbamide peroxide (CP). These oxidizing materials cause initial diffusion into enamel and dentin, then breaks down to produce unstable free radicals. "Whitening effect" is obtained when these free radicals attack organic pigmented molecules by reflecting less light. The most frequently used vital bleaching techniques are in-office (power bleaching) and at-home bleaching techniques. Other variables like concentration and type of bleaching agent are used and their application time in these two bleaching techniques. The most common side effects of bleaching treatment are "tooth sensitivity", which normally persists for up to 4-7 days after the conclusion of bleaching treatment. Tooth sensitivity during the bleaching technique is associated with microscopic surface defects and subsurface pores in the enamel. It has been proposed that tooth sensitivity occurs when these surface defects allow rapid access to the pulp's whitening agent.[4]

H_2O_2 is the molecular formula for hydrogen peroxide, a clear, colourless liquid with no odour, and its molecular weight is 34.0128. Hydrogen peroxide is quite unstable, and through slow decomposition, it releases oxygen. This decomposition is accelerated by light and heat, which is noted in all power bleaching technique. Though hydrogen peroxide in high concentrations can be toxic and with prolonged exposure, concentrated 30-35% of H_2O_2 have been used for in-office bleaching techniques. Except for post-treatment sensitivity, there are no major adverse effects on soft and hard tissues. H_2O_2 is completely soluble in water and gives an acidic solution. One per cent solution has a pH of 5.0-6.0.[5] Hence this systematic literature review evaluates the effect of hydrogen peroxide concentrations in bleaching and tooth sensitivity.

MATERIALS AND METHOD

STUDY DESIGN

This study is a systematic literature review of clinical trials using hydrogen peroxide and the effectiveness of various hydrogen peroxide concentrations in the intensity of bleaching and tooth sensitivity.

SEARCH STRATEGY

Electronic databases used to find the effectiveness of hydrogen peroxide concentration in bleaching and tooth sensitivity are PubMed,

Scopus, Cochrane library, Ovid Medline, Elsevier science direct, Wiley online library, Grey literature, Embase. MeSH terms were used in the databases to identify the articles. The MeSH terms used were hydrogen peroxide AND bleaching AND tooth sensitivity.

ELIGIBILITY CRITERIA FOR THE STUDY

INCLUSION CRITERIA

- Articles on the effectiveness of hydrogen peroxide in bleaching and tooth sensitivity.
- Clinical trial studies.
- Full-text articles.
- Publications over the years.
- In-vivo studies.
- Articles that are in the English language.

EXCLUSION CRITERIA

- Only abstracts available.
- Unrelated articles.
- In-vitro studies.
- Review articles.

SEARCHED DATABASES

- PubMed
- Cochrane library
- Ovid Medline
- Elsevier science direct
- Wiley online library
- Grey literature
- Embase

Flow chart: 1 Flow chart diagram showing the number of studies identified, screened, assessed for eligibility, excluded, and included in the systematic review

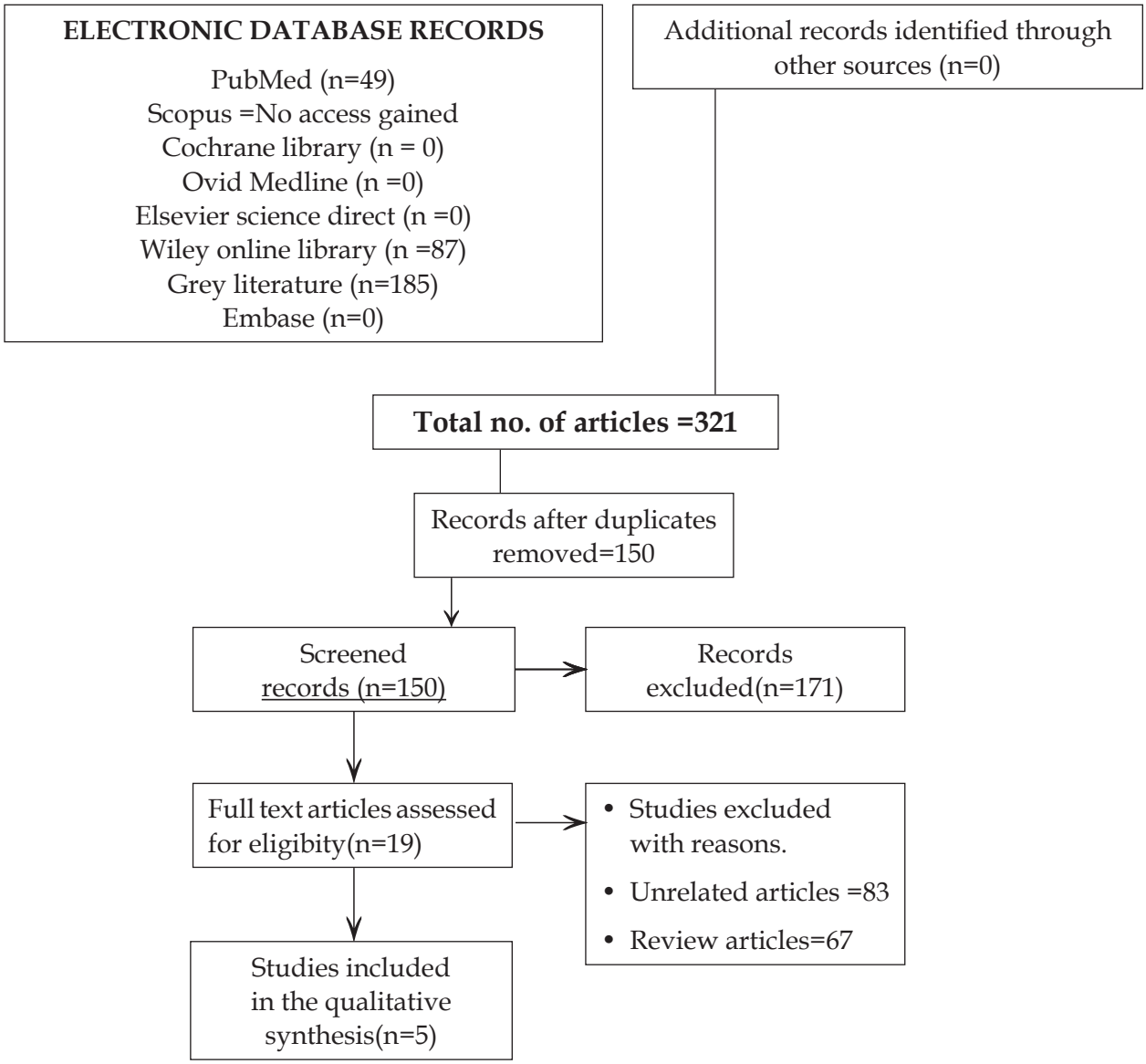

Table 1: Characteristics of Interventions Included in the Study

S.no	*Author name*	*Year*	*Patient selection*	*Duration*	*Preparations used*	*Interventions*
1	Marcela Leticia Leal Goncalves et al.[6]	2017	53 patients aged 11 to 12 years	-	Hydrogen peroxide gel	**Group 1:** Whitening hydrogen peroxide 35% **Group 2:** Whitening hydrogen peroxide blue 35% **Group 3:** Whitening hydrogen peroxide blue 20%
2	Suellen Nogueira Linares Lima et al.[7]	2017	25 volunteers aged between 18 to 40 years old	14 days	Hydrogen peroxide gel preparations	**Group 1:** Hydrogen peroxide 35% **Group 2:** Hydrogen peroxide 15%
3	J.F. Bortolatto et al.[8]	2014	40 volunteers aged between 18-25 years old	21 days	Hydrogen peroxide gel preparations	**Group 1:** Hydrogen peroxide 15 % **Group 2:** Hydrogen peroxide 35%
4	J. Martin et al.[9]	2015	31 patients over 18 years old	21 days	Hydrogen peroxide gels	**Group A:** 35% hydrogen peroxide **Group B:** 6% hydrogen peroxide with nitrogen-doped titanium dioxide light
5	A Reis et al.[10]	2013	60 volunteers with atleast 18 years old	Nine days	Hydrogen peroxide gels	**Group 1:** Hydrogen peroxide 35% **Group 2:** Hydrogen peroxide 20%

Table 2: Outcome of the Data Included in the Studies

S.no	*Author*	*Year*	*Outcome*	*Result*
1	Marcela Leticia Leal Goncalves et al[6]	2017	Visual analogue scale Group 3 reported less sensitivity in comparison to group 1 and group 2 Greater bleaching occurred in group 1 when compared to group 2 and group 3	Tooth sensitivity is lesser in group 3, in which the intervention is Whitening hydrogen peroxide blue 20%
2	Suellen Nogueira Linares Lima et al[7]	2017	Tooth sensitivity assessed using a visual analogue scale and numerical rating scale. Group 1 showed higher tooth sensitivity than group 2	Tooth sensitivity is lesser in hydrogen peroxide 15% groups
3	J.F. Bortolatto et al. [8]	2014	Tooth sensitivity assessed using a visual analogue scale (low, average, high, very high). Group 1 showed lesser sensitivity than group 2	Tooth sensitivity is lesser with hydrogen peroxide 15 %
4	J. Martin et al.[9]	2015	No significant change in colour and tooth sensitivity for both groups. Tooth sensitivity assessed using a visual analogue scale.	Tooth sensitivity is similar in both group A (35% hydrogen peroxide) and group B (6% hydrogen peroxide with nitrogen-doped titanium dioxide light)

S.no	Author	Year	Outcome	Result
5	A Reis et al.10	2013	Tooth sensitivity was recorded on a 0-4 scale and bleaching efficacy using the Vita Classical shade guide. No significant change in tooth sensitivity for both the groups and 35% hydrogen peroxide showed faster bleaching	The tooth sensitivity intensity is similar in group A (35% hydrogen peroxide) and group B (20% hydrogen peroxide), and the bleaching efficacy is greater with 35% hydrogen peroxide.

FLOW CHART 1: The total number of articles retrieved from the electronic databases are 321. Out of 321, 150 articles were assessed and screened for eligibility of the study and 171 articles were excluded. From 150 articles, 19 full-text articles were retrieved, excluding 85 unrelated articles and 67 review articles. Eventually, for qualitative synthesis, four studies were included.

Table 1: Shows the characteristics of interventions included in the study. In all the above four studies, the effect of hydrogen peroxide on tooth bleaching and tooth sensitivity was compared and reviewed.

Table 2: Shows the outcome and result of the five studies included in the systematic review

TOOTH SENSITIVITY ASSESSMENT IN THE INCLUDED STUDIES

- The Visual Analogue Scale (VAS) employs a 10-cm horizontal line with the words "no pain" at one end and "worst pain" at the opposite end.

	Score
None	0
Mild	1
Moderate	2
Considerable	3
Severe	4

- The NRS comprises the following criteria:

Table 3: Bias Analysis for the Included Studies

S. no.	Author and Year	Random Sequence Generation	Allocation Concealment	Selective Reporting	Incomplete Outcome Data	Blinding Of Outcome Assessment	Blinding Participants And Personals
1	Marcela Leticia Leal Goncalves et al[6]	-	-	-	-	?	?
2	Suellen Nogueira Linares Lima et al7	-	-	-	?	-	-
3	J.F. Bortolatto et al.[8]	-	-	?	?	-	-
4	J. Martin et al.[9]	-	-	-	-	-	-
5	A Reis et al.[10]	-	-	-	-	-	-

The bias is assigned as low risk (-), high risk (+), and unclear (?)

DISCUSSION

According to the literature, following the manufacturer's protocol, the tooth whitening procedure is safe and effective, yet the profession and the users should be aware of the risks. Increased tooth sensitivity and gingival irritation are certain risks related to tooth whitening, and the profession and public are aware of it. Current research also shows that degradation of dental restorations, tooth surface roughening and softening, increased potential for demineralization and unacceptable colour change of dental restorations. To increase the persistence of whitening and to reduce tooth sensitivity by optimizing the whitening procedure is focused on new research.[3]This present study aims to determine hydrogen peroxide concentration on tooth whitening and tooth sensitivity through a systematic review. A total of 4 articles were selected and retrieved based on the inclusion and exclusion criteria from the electronic database.

(TABLE 1) In 2017, Marcela Leticia Leal Goncalves et al., done a randomized, controlled clinical trial on In-Office bleaching using 20% and 35% hydrogen peroxide for patients aged between 11 to 24 years. (TABLE: 2) Vita Classical Shade guide and the visual analogue scale was used for the change in the tooth colour and tooth sensitivity, respectively. Reduction in tooth sensitivity was detected in lower concentrations of hydrogen peroxide 20%. Increased sensitivity was seen in 35% hydrogen peroxide. There is a significant colour change after each of the two bleaching sessions, and the study showed statistically significant with $p \leq 0.05$. These clinical trial studies not mentioned the blinding of either the participants or the observer and also unclear in the blinding of outcome assessment.[6]

(TABLE 1) In 2017, Suellen Nogueira Linares Lima et al. conducted a randomized double-blinded clinical trial using 15% and 35% hydrogen peroxide on 25 volunteers. Tooth sensitivity was scored using a visual analogue scale and numerical rating scale, and subjective and objective methods determined the bleaching efficacy. The bleaching effect was higher in hydrogen peroxide 35%, and the reduction in tooth sensitivity is seen in hydrogen peroxide 15%. (TABLE 2) This study concluded that the low concentration hydrogen peroxide, i.e. 15%, should be considered a good treatment alternative for in-office bleaching than the higher concentration for in-office bleaching because it generates greater risk and increased tooth sensitivity for patients. The p-value is not statistically significant in this study. This study has a lower risk in random sequence generation, allocation concealment, reporting, performance and detection bias. In attrition bias reason for the dropout has not mentioned; hence it is unclear.[7]

(TABLE 1) F. Bortolatto in 2014 conducted a randomized double-blinded clinical trial on 40 healthy volunteers of both male and female aged between 18 and 25 yrs to test the efficacy and tooth sensitivity of an in-office 15% H2O2 bleaching agent containing nanoparticles of TiO N photocatalyzed with LED/laser light (HP15) and a control of 35% H2O2 (HP35). Reflectance spectroscopy was used to evaluate the bleaching efficacy. A visual analogue scale was used to evaluate tooth sensitivity. (TABLE 2) He concluded that the hydrogen peroxide 15% (lower concentration) reduces tooth sensitivity and promotion in efficacy compared to the control group hydrogen peroxide 35%. This randomized clinical trial study has a lower risk of detection and performance bias.[8]

(TABLE: 1) In 2015, J. Martin et al.[9] conducted a randomized clinical, double-blinded, split-mouth study on 31 patients with 35% H_2O_2 in one upper arch and 6% H_2O_2 in the other upper arch. Tooth shade was evaluated using the VITA Classic guide, and VAS assessed tooth sensitivity. (TABLE:2) He concluded that there is no clinical difference between both groups with a p-value <0.05, which is statistically significant. This study also reported as the clinical significance that good clinical results with minimal adverse effects

might be reached with a low concentration of hydrogen peroxide bleaching agent. Like the previous study mentioned above, the tooth sensitivity was evaluated using a visual analogue scale, and the tooth colour efficacy was evaluated using the colorimeter method. The study concluded that the H_2O_2 ozone combination efficacy is greater and satisfying, whereas H_2O_2 38% increased the bleaching sensitivity.[9]

(TABLE: 1) A Reis et al.[10] in 2013 conducted a randomized, double-blind, parallel-group clinical trial on 60 volunteers who are atleast 18 years old, group 1 is hydrogen peroxide 35%, and group 2 is hydrogen peroxide 20%. Tooth sensitivity was recorded on a 0-4 scale and bleaching efficacy using the Vita Classical shade guide. (TABLE 2) The tooth sensitivity intensity is similar in both group A and group B, and the bleaching efficacy is faster in group B 35% hydrogen peroxide. [10]

In 2017 Mahmoud K. AL-Omiri conducted a randomized controlled trial on 45 participants who were randomly allocated into three groups (n=15). Group 1 participants are treated with ozone for 60 seconds and then 38% H_2O_2 for 20 minutes. Group 2 participants were treated with 38% H_2O_2 for 20 mins, followed by ozone for 60 secs, followed by air produced by the heal ozone machine for 60 secs. Group 3 participants are treated with 38% H_2O_2 for 20 mins which was considered a control group. Same as the previous study mentioned above, the tooth sensitivity was evaluated using a visual analogue scale, and the tooth colour efficacy was evaluated using the colorimeter method. The study concluded that the H_2O_2/ozone combination efficacy is greater and satisfying, whereas H_2O_2 38% increased the bleaching sensitivity.[11] A Reis et al in his study concluded that in-office bleaching gels i.e 35% and 20% hydrogen peroxide showed similar tooth sensitivity but faster bleaching is achieved by 35% hydrogen peroxide. [10]

Studies on systematic review and meta-analysis of Li-Bang He et al evaluated the influence of light on bleaching efficacy and tooth sensitivity at in-office vital tooth bleaching. Their study concluded that light-activated system produced instant superior bleaching effects when low concentrations of hydrogen peroxide is used i.e., 15-20% than the non-lightactivated system. Bleaching effect is not improved at higher concentration through light activated system. Light activated system also increases the risk of tooth sensitivity.[12]

Silveira Machado et al in 2014 said that bleaching with 10% hydrogen peroxide was effective without tooth sensitivity during applications in a short period of time.[13] Most recently in 2021 Marcelo et al. evaluated the effect of violet illumination on tooth whitening alone and with combination of 35% hydrogen peroxide on bovine teeth. The result obtained by Marcelo was Violet illumination with hydrogen peroxide gel treatment completely restored the teeth whiteness caused by coffee staining. Concluded that Violet illumination with gel treatment can be recommended for the stains caused by drinking coffee.[14]

Several studies were performed with different concentrations of hydrogen peroxide to produce better results with minimal adverse effects. However, most studies stated that the desired bleaching effect could be obtained with higher concentrations along with minimal tooth sensitivity reactions.

LIMITATIONS

This present study included only five articles related to hydrogen peroxide's effectiveness in the bleaching effect and tooth sensitivity. Based on the inclusion and exclusion criteria, various other studies have been excluded. Many other trial studies are required to establish the bleaching effect of hydrogen peroxide and its tooth sensitivity effect based on its concentrations.

CONFLICTS OF INTEREST: None.

CONCLUSION

This study concludes that hydrogen peroxide with higher concentration is effective in bleaching and with lower concentration is less

likely to produce tooth sensitivity. The quote "With bad comes good" implies that the bad is tooth sensitivity, and good is an esthetic bleaching effect. People with an esthetic mind should go through the tooth sensitivity effect, which fades out later to have white teeth.

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Systematic Literature Review on the Effect of Play Way - A Game Based Oral Health Education on Oral Hygiene of School-Going Children

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ABSTRACT

Objective: In this study, the literature was systematically reviewed to evaluate the effect of play way a game based oral health education on oral hygiene of school children.

Data Sources: Electronic databases were screened and hand searched. Searched electronic databases are PubMed, Cochrane library, Ovid Medline, Elsevier science direct, Wiley online library, Grey literature, Embase.

Materials and Methods: Systematic literature review of clinical trials evaluating the effect of play way a game based oral health education on oral hygiene of school children by following PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines. PICO model was used for population, intervention, comparison and outcome assessment. Cochrane risk assessment tool was used for bias analysis.

Results: A total of 5 articles fulfilled the inclusion criteria. The p-value is less than 0.05 showing that all the studies were statistically significant. Therefore, game-based oral health education is superior to conventional oral health education.

Conclusion: This systematic review concludes that game-based oral health education effectively improves the oral hygiene of school children than conventional oral health education.

Keywords: Oral hygiene, Health education, School children, Gaming method.

INTRODUCTION

Oral cavity problems are one of the most prevalent conditions globally, which can be largely preventable. School children are affected by 60-90% of dental caries. Dental caries is increasingly prevalent in developing countries and highly prevalent in some Asian and Latin American countries. Prevalence of a periodontal disease is present globally, with 5-15% of severe periodontitis in most of the populations. In some countries, edentulism is high among older patients with age 65 and

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above. Universally, oral cancer is in 8th place among cancers. Oral diseases limit activities at work, in school, and at home, causing millions of work and school hours to be lost every year worldwide. In addition, quality of life is shrunken by the psychosocial impact of these oral diseases.[1]

In 2003, WHO indicated that the focus of Oral Health Education actions should be on conditions and behaviours that promote oral health or reduce the risk of oral diseases. Health promotion at school should encourage toothbrushing daily, supervised toothbrushing, fluoride use and promotion of good nutrition, among other strategies.[2] OHE aims to improve the overall knowledge of the people, which may lead to the adoption and maintenance of favourable oral health behaviours that, in return, lower oral health morbidity.[3] Oral health education can be delivered to groups of people in an extensive scope through dental practices, at schools, workplaces, day-care and residential settings for older adults.[4,5]

Oral health education is an important tool in the prevention of oral diseases by educating the school children. Health education seeks to protect or improve health through voluntary changes in behaviour due to learning opportunities. It includes personal education and development, mass media information and education. Personal education and development provides improved knowledge about health, offers health risk advice, promotes self-esteem and self-empowerment. Mass media information and education turns to be non-personal and raises public awareness, creates a climate of opinion and offers health risk information and advice. It includes public relations, marketing, advertising, news information and distance learning projects through radio, television, newspapers and other publications.[6] Playing games benefits the children by developing visual alertness, increasing attention span, and assisting with memory strategy and reasoning, thus making learning an enjoyable one.[7] Game-based teaching has a dual effect of facilitating and reinforcing a child's learning in a thought-provoking and self-motivating format.[8] Conventional health education, if conducted by professionals or disseminated through pamphlets, posters and media campaigns, may not be enough to reach favourable behaviours and positive attitudes.[9] Game-based teaching with its dynamic effect and benefiting features to the children. This systematic review of the literature evaluates the effect of oral health education through the gaming method on the oral hygiene of school children.

MATERIALS AND METHOD

STUDY DESIGN

Systematic literature review of clinical trials evaluating the effect of play way a game based oral health education on oral hygiene of school-going children following Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.

Population: School children.

Intervention: Game-based oral health education.

Comparison: Conventional teaching method.

Outcome: Oral hygiene

SEARCH STRATEGY

Electronic databases used to find the effect of play way a game based oral health education on oral hygiene of school children are PubMed, Cochrane library, Ovid Medline, Elsevier science direct, Wiley online library, Grey literature, Embase. MeSH terms were used in the databases to identify the articles. The MeSH terms used were "game based oral health education" AND "game based oral health education on oral hygiene" AND "game based oral health education on school children".

INCLUSION CRITERIA

- Articles on the effect of play way a game based oral health education on oral hygiene of school children.
- Articles with full text were included.
- *In vitro* studies were included.

EXCLUSION CRITERIA

- Review articles were excluded.
- Articles other than the English language.

RESULTS

Flow chart 1: Flow chart diagram showing the number of studies identified, screened, assessed for eligibility, excluded, and included in the systematic review.

TABLE 1: represents the population, duration of the study, methods used for oral health education and intervening groups for all the five studies included in this systematic review.

DISCUSSION

In general, traditional oral health education effectively reduced plaque accumulation over a short period of small magnitude.[2] OHE is also effective in improving the knowledge attitude and practice regarding oral health.[1]

Nyandindi et al., in their study on the impact of oral health education on primary school children before and after teacher's training in Tanzania, concluded that the group with conventional oral health education had somewhat better oral health knowledge. Still, their practices were no better than the referents.[16]

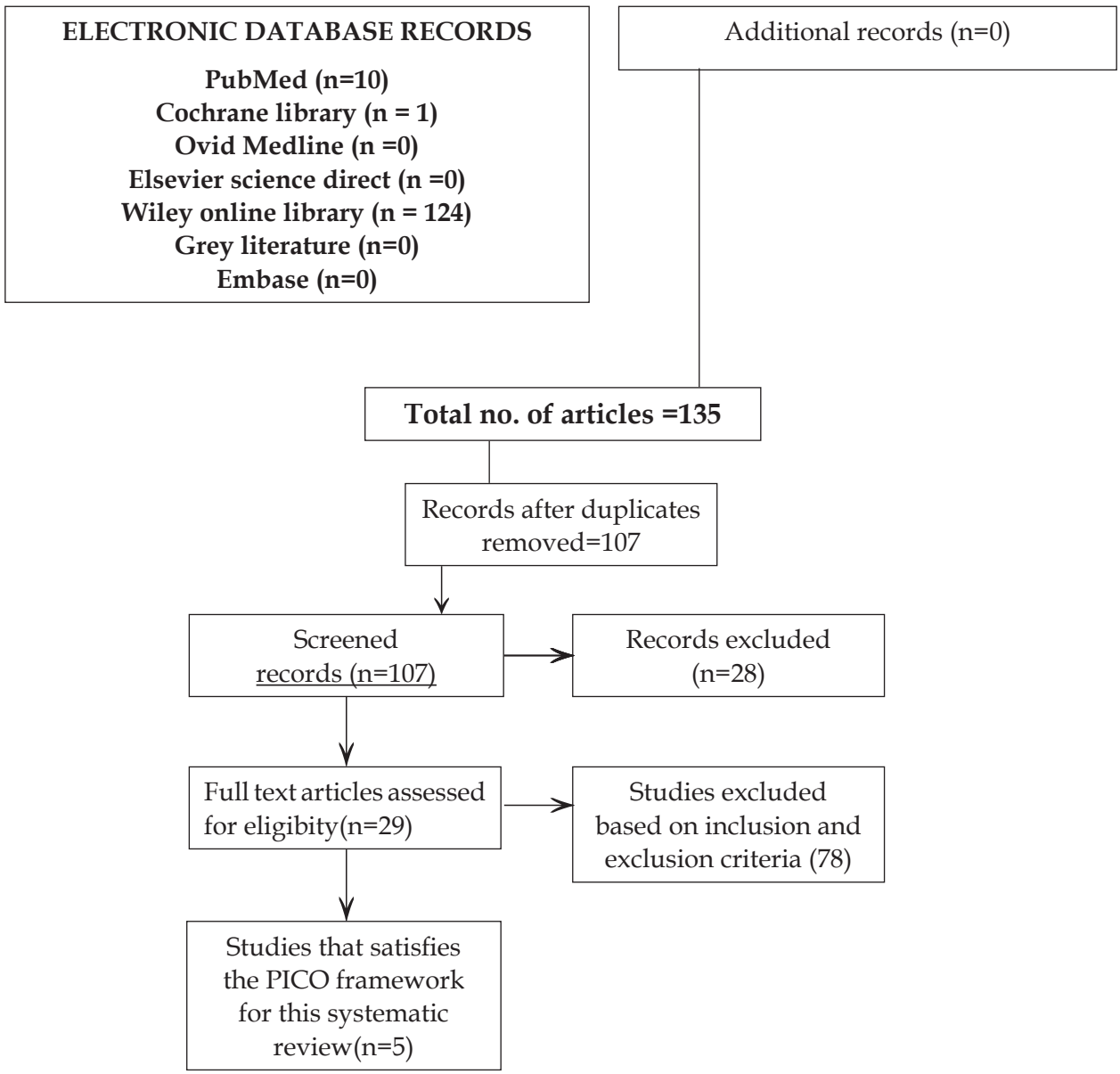

Table 1: Characteristics of Interventions Included in tise Study

S.no	*Author name*	*Year*	*Patient selection*	*Duration*	*Methods used for oral health education*	*Interventions*
1	Maheswari UN et al.[10]	2014	120 children aged between 5 to 10 years old	Three months	Conventional teaching method Game-based teaching method	**Group A:** 60 children received oral health instructions through flashcards once a day for seven days. **Group B:** 60 children received oral health instructions through snakes and ladders combined with the flashcard method.
2	Kumar Y et al. 1.[11]	2015	Sixty children aged between 7 to 10 years old	Three months	Conventional teaching method Game-based teaching method	**Group A:** 30 children educated with the flashcard method. **Group B:** 30 children educated with both flashcards and connect the dots game method.
3	Malik A et al.[12]	2017	150 children aged between 8 to 12 years	Three months	Conventional teaching method Game-based teaching method	**Group I:** 75 children received health education through PowerPoint presentation once daily for seven days. **Group II:** 75 children received health education through crosswords and quizzes along with PowerPoint presentations.
4	Geetha Priya PR et al.13	2020	360 children aged between 8 to 9 years old	Three months	Conventional teaching method Game-based teaching method	**Group I:** drama method. **Group II:** play way method using the snake and ladder game. **Group III:** flashcard method.
5	Nomair AM et al.14	2020	174 children aged between 4 to 6 years old	Six months	Conventional teaching method Game-based teaching method	**Group I:** Motivational interviewing **Group II:** Motivational interviewing + Gaming method **Group III:** Conventional dental health education

Kumar Y et al.[11] in 2015 compared the effect of conventional and game-based teaching on knowledge and practice regarding oral hygiene among 7 to 10 years school children. A sample of 60 children aged between 8 to 10 years were randomly divided into two groups: group A (oral health education through flashcards once daily for seven days) and group B (play-way method, i.e. connect the dots game combined with

flashcards). The intervention was started after the pretest evaluation of their knowledge regarding oral health and estimation of Debris Indexsimplified (DI-S). Oral hygiene and DI-S were recorded on the 8th day after intervention. A follow-up score was also recorded after 1 and 3 months. There was a significant increase in oral hygiene scores and a decrease in debris scores when compared to baseline in both groups at one week and one

Table 2: Outcome of the Studies included in the Systematic Review.

S. No	*Author name*	*Methods used in game-based health education*	*Methods used for assessing oral hygiene*	*P-value*	*Outcome*
1	Maheswari UN et al. [10]	**Group A:** Oral hygiene instructions through flash. Group B: snakes and ladders combined with a flashcard. Snakes and ladders game consisted of a checkered board with numbers 1 to 100. The coin moves with the corresponding dice numbers. If the player lands on the space at the bottom of the ladder (do's of the oral hygiene). If the children land on the head of the snake (don't's of the oral hygiene)	Debris Index-Simplified (DI-S) was used for assessing oral hygiene (post-intervention)	< 0.05	In the game-based oral health education group, there is a significant increase in good oral hygiene scores and a significant decrease in fair and poor debris scores post-intervention day one and at three months follow up.
2	Kumar Y et al.[11]	Group A: Children were shown the flashcards with a picture in front and oral hygiene instructions behind. Group B: (Anagram and connect the dots game). The anagram used was 'Bright Smile', where each alphabet represented a specific oral hygiene instruction. Connect the dots game was developed in tooth structure with 11 alphabets as in the anagram and oral hygiene instruction.	Debris Index-Simplified (DI-S) was used for assessing oral hygiene (post-intervention)	p<0.05	There was a significant increase in the Debris index score from 0 to 15 children with good oral hygiene in group B (game-based method) when compared to group A(conventional method).

S. No	Author name	Methods used in game-based health education	Methods used for assessing oral hygiene	P-value	Outcome
3	Malik A et al.[12]	Group I: PowerPoint presentation. Group II: crosswords and quizzes along with PowerPoint presentation.	Plaque index	p <0.05	In both the groups, there was a significant increase in good oral hygiene scores and a significant decrease in plaque scores on postintervention 1 and 3 months follow-up, but much better scores were seen in group II compared to group I at both the follow-ups
4	Geetha Priya PR et al. [13]	Group I: drama method (dialogues and the scenes in the drama were framed to educate children about oral health) Group II: Play-way method using the snake and ladder game. (The square with the snake's head provided information about oral diseases and poor oral health practices, and the square with the snake's tail showed the consequences or the risk of practising harmful oral hygiene habits)	dft/DMFT indices, oral hygiene (OHI-S) and oral health-related quality of life (OHRQoL)	p=0.03	The game mode group had the highest impact, followed by drama and flashcard modes.
5	Nomar AM et al. [14]	Group II: Gaming method (Android tablet games "Happy teeth, Healthy kids") + Motivational interviewing	Oral Hygiene Index-Simplified (OHI-S)	p<0.001	MI with game-based learning method was effective in promoting preschool children's oral hygiene.

month. At three months interval, both groups showed a decrease in oral hygiene scores from baseline, with group B showing highly significant reduction and concluded that the connect the dots game, including good dental hygiene and dietary habits, can be an effective intervention aid for teaching the basic oral health concepts among school-going children.

Malik A et al.[12] in 2017 conducted a study to find the effectiveness of game-based oral health education over conventional on the oral health-related knowledge and oral hygiene status among 8- to 12-year-old schoolchildren.

Table:3: Bias Analysis For The Included Studies

S. No	*Author*	*Random Sequence Generation*	*Allocation Concealment*	*Selective Reporting*	*Incomplete Outcome Data*	*Blinding of Outcome Assessment*	*Blinding Participants And Personals*
1	Maheswari UN et al.[10]	-	-	-	-	+	+
2	Kumar Y et al.[11]	-	-	-	+	-	-
3	Malik A et al.[12]	-	-	+	+	-	-
4	Geetha Priya PR et al.[13]	-	-	-	-	-	-
5	Nomar AM et al.[14]	-	-	-	-	-	-

The bias is assigned as low risk (-), high risk (+), and unclear (?)

A sample of 150 children aged between 8 to 12 years were divided into two groups, group I (oral health education through PowerPoint presentation once daily for seven days) and group II (play method, i.e., crosswords and quiz with a PowerPoint presentation. A pretest evaluation of their knowledge regarding oral health and the estimation of plaque index was carried out. The evaluations regarding oral health-related knowledge and plaque scores were recorded on postintervention 1 and 3 months. The result obtained in both the groups had a significant increase in good oral hygiene scores and a significant decrease in plaque scores on postintervention 1- and 3-months follow-up. Still, much better scores were seen in group II when compared to group I at both the follow-ups.

Geetha Priya PR et al.[13] in 2020 compared the effect of 3 modes of school dental health education and the two frequencies of reinforcements on the oral health status of children. Three hundred sixty school children aged between 8 to 9 years participated in this study. For dental caries (DFT/DMFT) indices, oral hygiene (OHI-S) and oral health-related quality of life (OHRQoL) scores were recorded. Post-intervention data were collected after two years. The result shows that there was a significant reduction in the dft scores [drama mode ($p = 0.006$), game mode ($p = 0.001$) and flashcards ($p = 0.002$)] and OHI-S scores [drama and game mode ($p < 0.001$) and flashcards method ($p = 0.01$)] and significant improvement in the total OHRQoL scores [drama mode ($p = 0.001$), game mode ($p = 0.016$) and flashcards method ($p = 0.023$)] in all the three modes. Every three months, children who received game mode school dental health education had a significantly higher number of filled primary teeth. When compared to children who received the reinforcement every six months, she concluded that all three modes effectively improved the oral health status of school children. The game mode had the highest impact when compared to the other two modes. Health educators should focus on child-friendly modes to make the health information more retentive

Nomar AM et al.[14] evaluated the effect of motivational interviewing (MI) and games in changing oral health behaviours among preschool children in Egypt. The participating children were recruited from the 12 governmental preschools available within the Edko administration of Beheira governorate, Egypt. Experimental group I (MI), experimental group II (MI+ gaming) or the control group (conventional dental health education (CE). The outcomes of oral hygiene index simplified (OHI-S) for children and knowledge, attitude and practice of children's mothers were measured at baseline and after six months follow-up. The result after six months follow-up, compared with the control group (1.41 ± 0.77), preschool children in experimental group I and II had significantly lower mean ± SD of OHI-S (0.69 ± 0.61 and 0.70 ± 0.72), $P < 0.05$. He concluded that using MI (motivational interviewing) as an oral

health education tool was significantly more effective in promoting preschool children's oral hygiene and improving mothers' knowledge, attitude and oral health-related practices than CE (conventional health education) alone.

LIMITATION OF THE STUDY

This systematic literature review concluded based on the game-based health education on oral hygiene of school-going children only. Further research is needed for game-based education on oral hygiene of diseased dental patients for medically compromised children and visual impairment children. Therefore, generalizability with this study cannot be finalized.

CONCLUSION

This systematic review concludes that game-based oral health education effectively improves the oral hygiene of school children than conventional oral health education.

CONFLICT OF INTEREST: NIL

SOURCE OF FUNDING: NIL

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Systematic Review on the Efficacy of Icon Resin Infiltration on White Spot Lesions

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ABSTRACT

Background: Resin infiltration is a new therapeutic technique for WSLs (white spot lesions) that may bridge nonoperative and operative treatment options. It's a minimally invasive procedure for filling, reinforcing, and stabilizing demineralized enamel without drilling or compromising good tooth structure. It has also been found to slow the progression of caries in lesions that are too advanced to be treated with fluoride. The current investigation of this systematic review is to evaluate the efficacy of ICON® resin infiltration on WSLs. This literature review was conducted and reported according to the PRISMA (Preferred Reporting Items for Systematic Review and Metaanalysis) statement. Seven databases (PubMed, Cochrane Library, CINAHL, OVID MEDLINE, EMBASE, Grey Literature, Wiley Online Library) were used. The MeSH terms used were 'Icon resin infiltration, ' white spot lesions, and 'clinical trials. PICOS analysis of Population- Participants with white spot lesions; Intervention- Icon Resin Infiltration; Comparison- Comparing with other different therapies; Outcome- Regression or disappearance of lesions; Study design- Randomized controlled trials. Five cross-sectional studies were included in this systematic review for the quality assessment of the efficacy of Icon resin infiltration on white spot lesions. All five studies reported a statistically significant difference between the Icon resin infiltration, lesion loss ratio, and color change. This systematic review concludes that the Icon resin infiltration on white spot lesion is effective during the initial period, i.e. first three months. Over time there is a color change in its masking. However, it is effective in reducing the lesion loss ratio.

Keywords: White spot lesions, Icon resin infiltration, Remineralization, enamel opacities, remineralizing agents.

INTRODUCTION

Enamel opacities form due to dental follicle injury during the eruption, enamel development abnormalities, or cariogenic activity in the event of poor oral hygiene.[1] Initial enamel demineralization commonly appears clinically as a "white spot lesion," as enamel translucency is directly related to the degree of mineralization (WSL). The WSL has been defined as "subsurface enamel porosity from carious demineralization" that presents as "a milky white opacity

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when located on smooth surfaces.[2]The treatment of the white spot lesions is topical fluoride application and minimally invasive procedures like resin infiltration, laser irradiation, erosion-infiltration, bleaching, microabrasion, remineralizing agents like casein phosphopeptides remineralization process.[3]Resin infiltration is a new therapeutic technique for WSLs that may bridge nonoperative and operative treatment options. It's a minimally invasive procedure for filling, reinforcing, and stabilizing demineralized enamel without drilling or compromising good tooth structure.[4]The acid etchant is 15 % hydrochloric acid—in Icon® resin infiltration eliminates the surface layer of the decalcified region due to its penetration depth of 58±37µm.[5][6][7][8]Absence of tooth structure loss, allowing stability for white spot lesions, preventing caries progression, plugging of the micropore forms in the body of the lesion, delaying the need for a restoration, decreasing recurrent decay, absence of pulp inflammation and postoperative sensitivity, lowering the risk of periodontitis and gingivitis, and better aesthetic outcomes in covering demineralized enamel are just a few of the advantages of resin infiltration. [9] As a result, the current investigation of this systematic review is ICON® resin infiltration on white spot lesions.

MATERIALS AND METHODS

This review was conducted and reported according to the PRISMA (Preferred Reporting Items for Systematic Review and Metaanalysis) statement. The Population, intervention, comparison and outcome model defined the inclusion and exclusion criteria. Thus, the present review investigated the efficacy of Icon resin infiltration on white spot lesions.

Search Strategy

The following databases were searched to find reports of relevant studies. Seven databases (PubMed, Cochrane Library, CINAHL, OVID MEDLINE, EMBASE, Grey Literature, Wiley Online Library) were used. The MeSH terms used were 'Icon resin infiltration, ' white spot lesions, and 'clinical trials.

The inclusion criteria are

- Articles that evaluated the effect of Icon resin infiltration on white spot lesions.
- Articles with the study design of randomized controlled trials.
- Invivo studies.

PICOS

Population: Participants with white spot lesions

Intervention: Icon Resin Infiltration

Comparison: Compared with other different therapies.

Outcome: Regression or disappearance of lesions

Study design: Randomized controlled trials

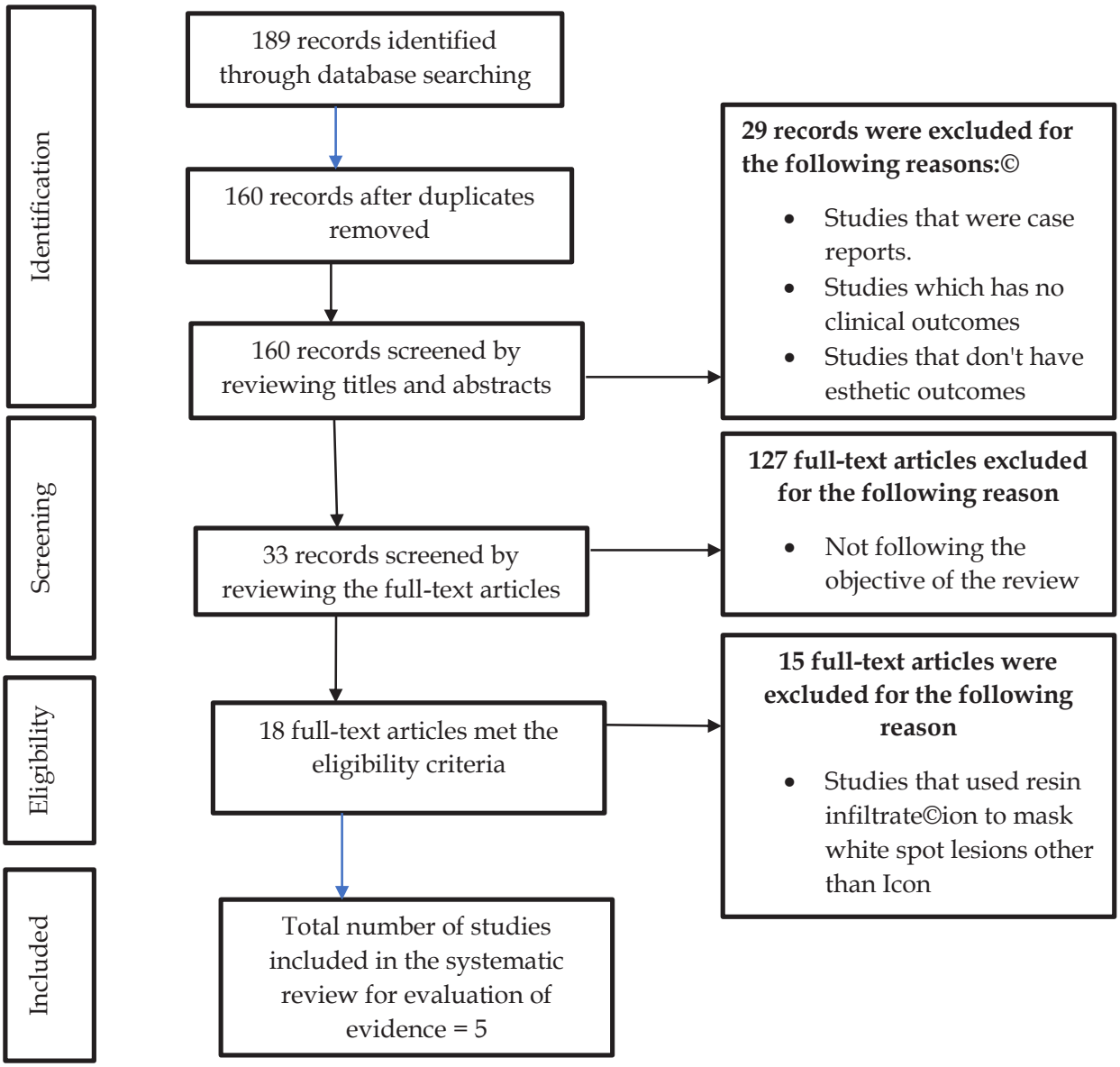
RESULT

Table 1 to 3 in next page.

DISCUSSION

Despite the fact that numerous new advanced materials and technologies have been developed to detect and cure dental caries, the problem still exists.[15]

Michael Knoselet al.[15]conducted a study by assessing the durability of resin infiltration in white spot lesions and sound enamel achieved over six months. Twenty-three consecutive subjects with 231 noncavitated, unrestored white spot lesions after multibracket treatment were recruited for lesion infiltration at the Department of Orthodontics, University of Gottingen (Germany). The treatment and control groups were assigned using a simple randomized, split-mouth, controlled design. After enamel conditioning with a 15% HCl gel, white spot lesion infiltration of the anterior teeth was conducted using low-viscosity light-cured resin in the treatment group. The colour and lightness of the white spot lesions and the



Flowchart 1

Table 1: Population and Interventions of the selected studies in this systematic review

S.No	*Author name*	*Year*	*Patient selection*	*Type of study*	*Interventions*
1	Michael Knosel et al.[10]	2013	23 subjects with 231 non cavitated, unrestored white spot lesions after multibracket treatment were recruited	Split mouth randomized controlled trial	**Group 1:** White spot lesion infiltration (Icon; DMG) low-viscosity light-cured resin after enamel conditioning with a 15% HCl gel. **Group 2:** Control
2	Annapurna Kannan et al.[11]	2019	Two hundred forty WSLs were detected in 193 teeth of 12 patients.	A randomized controlled trial	**Group 1:** Icon® resin infiltration. **Group 2:** Clinpro™ XT varnish

S.No	Author name	Year	Patient selection	Type of study	Interventions
3	Ahmed Youssef et al.[12]	2020	Two hundred forty WSLs were detected in 193 teeth of 12 patients.	Short term split-mouth randomized controlled trial	**Group 1:** Lesions were resin infiltrated with Icon (RI DMG) **Group 2:** ReminPro (R.P.;VOCO) **Group3 (control):** affected teeth were brushed with Complete Care toothpaste (CC; Himalaya).
4	Seth V. Senestraro et al.[13]	2013	20 patients	Randomized controlled trial	**Group A:** (Icon Infiltrant, DMG America, Englewood, N.J.) **Group B:** Placebo
5	Xi Gu et al.[14]	2019	Twenty patients with 128 teeth with post orthodontic WSLs were recruited.	Split mouth randomized controlled trial	**Group 1: (**Icon, DMG, Hamburg, Germany **Group 2:** microabrasion

Table 2: Outcome of the data included in the studies

S. No	Author	Year	Outcome	Result
1	Michael Knosel et al.[10]	2013	Resin infiltration improves the esthetic appearance of demineralized teeth. Esthetic	The results obtained were adequate durability over six months.
2	Annapurna Kannan et al.[11]	2019	Immediately after the intervention, Icon® resin infiltration showed statistically significant better improvement than Clinpro™ XT varnish in restoring the colour	Except at three months, the fluorescence loss sequentially reduced more for Icon® resin infiltration (4.48 ± 1.42 at T0 to 1.48 ± 0.81 at T3) and was not statistically significant
3	Ahmed Youssef et al.[12]	2020	ComparedtoR.P. and CC, RI showed prompt and subjectively satisfactory.	Statistical analysis of the objective colour differences (Δ*E**)between the three groups revealed significant differences for R.I. vs R.P. (*P*.029), R.I. vs CC (*P*.001), and R.P. vs CC (*P* .001).
4	Seth V. Senestraro et al.[13]	2013	Resin infiltration significantly improved the clinical appearance of WSLs, with stable results seen eight weeks after treatment.	The mean VAS ratings for treated teeth demonstrated marked improvement relative to that for control teeth immediately after treatment (67.7 versus 5.2, P < .001) and eight weeks later (65.9 versus 9.2, P < .001).
5	Xi Gu et al.[14]	2019	Resin infiltration showed a better esthetic improvement effect when compared with microabrasion at 12 months.	In the infiltration group, the R-value and D.E. had no significant changes from T1 to T12. In the microabrasion group, the R-value and D.E. decreased significantly from T1 to T6. The R-value of resin infiltration was lower when compared with microabrasion at every recall point (P, .001)

Table: 3 Bias analysis for the included studies

S. No	*Author and Year*	*Random Sequence Generation*	*Allocation Concealment*	*Selective Reporting*	*Incomplete Outcome Data*	*Blinding of Outcome Assessment*	*Blinding Participants and Personals*
1	Michael Knosel et al.[10]	-	-	-	-	?	?
2	Annapurna Kannan et al.[11]	-	-	-	?	-	-
3	Ahmed Youssef et al.[12]	-	-	?	?	-	-
4	Seth V. Senestraro et al.[13]	?	?	-	-	-	-
5	Xi Gu et al.[14]	-	-	-	-	-	-

The bias is assigned as low risk (-), high risk (+), and unclear (?)

neighbouring sound enamel were measured using a spectrophotometer before infiltration and after one day, one week, four weeks, three months, and six months using the Commission Internationale de l'Eclairage system. At a level of 5% and a power of 80%, multifactorial analysis of variance with repeated measures and pair-wise comparisons was employed to investigate the effects of infiltration and time elapsed on colour differences. The results of a study including 20 patients and 39 quadrants in each group (108 teeth in the control group; 111 teeth in the treatment group) revealed that both treatment settings and time duration had a generally significant impact on the colour difference values. After infiltration, colour assimilation of white spot lesions to surrounding enamel was steady over six months, with no significant alterations; the mean colour difference of white spot lesions vs adjacent sound enamel (D.E. baseline vs six months) was 2.55. (95 percent confidence interval [CI], 1.431-3.678). The untreated control teeth showed no significant changes over six months compared with the baseline: mean (D.E.), 0.29 (95% CI, 0.335-0.928). No important adverse events or side effects were observed.Resin infiltration improves the esthetic appearance of demineralized teeth. The results showed sufficient durability over six months.

Annapurna Kannanet al.[11] aimed to comparatively evaluate Icon® resin infiltration and Clinpro™ XT varnish in restoring aesthetics of white spot lesions (WSLs) present post-orthodontic treatment. Two hundred forty WSLs were detected in 193 teeth of 12 patients. The participants were analyzed—before intervention (T0), immediately after intervention (T1), three months later (T2), and six months later (T3), with a 1:1 allocation ratio for the application of Icon® resin infiltration and Clinpro™ XT varnish. Using a computer-generated allocation sequence, block randomization was done. A spectrophotometer was used to assess the colour of WSLs and the adjacent enamel, while a DIAGNOdent® was used to assess the fluorescence loss. Immediately after the intervention, Icon® resin infiltration showed statistically significant better improvement than Clinpro™ XT varnish in restoring the colour ($p = 0.000$); however, at 3 ($p = 0.001$) and 6 months ($p = 0.000$), this was reversed. Except at three months, the fluorescence loss sequentially reduced more for Icon® resin infiltration (4.48 ± 1.42 at T0 to 1.48 ± 0.81 at T3) and was not statistically significant. Clinpro™ XT varnish showed significantly better improvement than Icon® resin infiltration in restoring the colour and lightness of the WSLs at 3 and 6 months. The fluorescence loss

significantly recovered with both intervention methods between immediate application and six months. However, Clinpro™ XT varnish-treated WSLs showed a statistically significant difference compared to the adjacent sound enamel at six months.

Seth V. Senestraroet al.[13] conducted a randomized, single-masked clinical trial involving patients who had completed orthodontic treatment to assess changes in the appearance of white spot lesions (WSLs) treated with resin infiltration. The authors divided affected teeth into control and treatment groups. The treatment group restored teeth with WSLs by using resin infiltration. They evaluated changes in WSLs photographically by using a visual analogue scale (VAS) (0 = no change, 100 = complete disappearance) and area measurements (in square millimetres). The authors analyzed the data by using a two-way analysis of variance. Results. The mean VAS ratings for treated teeth demonstrated marked improvement relative to that for control teeth immediately after treatment (67.7 versus 5.2, P < .001) and eight weeks later (65.9 versus 9.2, P < .001). The results for treated teeth showed a mean reduction in the WSL area of 61.8 per cent immediately after treatment and 60.9 per cent eight weeks later, compared with a -3.3 per cent change for control teeth immediately after treatment and a 1.0 per cent centcent reduction eight weeks later. The study concluded that the resin infiltration significantly improved the clinical appearance of WSLs, with stable results seen eight weeks after treatment. Practical Implications. Resin infiltration, a minimally invasive treatment, was effective for WSLs that formed during orthodontic treatment.

Xi Gu et al.[14] compared the esthetic improvement between post orthodontic white-spot lesions (WSLs) treated by resin infiltration and microabrasion for 12 months. Twenty patients with 128 teeth with post orthodontic WSLs were recruited. A simple randomized, split-mouth, positive controlled design allocated patients to resin infiltration or microabrasion groups. The lesion area ratio (R-value) was calculated between a WSL and the labial surface of the corresponding tooth based on standardized clinical photographs. Each tooth's colour change (D.E.) was measured with a Crystaleye spectrophotometer (Olympus, Tokyo, Japan). Every measurement was taken before treatment (T0) and at different time points after treatment: 1 week (T1), six months (T6), and 12 months (T12). A total of 16 patients with 108 trial teeth were available at T12. Each group had 54 trial teeth. In both groups, there was a significant decrease in R-value and D.E. between T1 and T0 (P, .0001). In the infiltration group, the R-value and D.E. had no significant changes from T1 to T12. In the microabrasion group, the R-value and D.E. decreased significantly from T1 to T6. The R-value of resin infiltration was lower when compared with microabrasion at every recall point (P, .001). The DE had no significant differences between the two groups at any time point. Resin infiltration and microabrasion improved the esthetic appearance of WSLs and showed sufficient durability for 12 months. Resin infiltration showed a better esthetic improvement effect when compared with microabrasion at 12 months. The limitation is that the comparison group with the Icon resin infiltration is different, so the efficacy of Icon resin infiltration may vary.In addition to the lesion loss and esthetic appearance of WSLs, Icon resin infiltration had shown highest microhardness value of the demineralized enamel immediately, but after 8 weeks due to the water softening of TEGDMA polymer matrix and partial solubility of the remaining mineral in the lesion body, Icon shows decreased microhardness.[16]Icon resin infiltration incorporated with nanoparticles of amorphous calcium phosphate shows promising effect of inhibiting enamel White spot lesions, shield the enamel and increase its hardness.[17]

CONCLUSION

This systematic review concludes that the Icon resin infiltration on white spot lesion is effective during the initial period, i.e. first three months. Over time there is a colour change in its masking. However, it is effective in reducing the lesion loss ratio.

CONFLICTS OF INTEREST: None.

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Stem Cells: Impact on Regenerative Dentistry: A Review

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ABSTRACT

The best possible treatment options for patients are now available thanks to the recent revolution in regenerative dentistry using stem cell therapy. The stem cells, which are unspecialized, highly proliferative, clonogenic, and capable of self-renewal, have opened the door to new methods of regenerating missing tissues, repairing cleft palates, regenerating periodontal and jaw bones, and, most importantly, remaking the entire tooth structure. The interest in clinical dentistry is growing daily thanks to recent advancements in stem cell therapy. Stem cell therapy therefore has a bright future in tissue regeneration dentistry. This review provides a concise summary of the background, current uses, and potential future of stem cells.

Keywords: Dental pulp tissue; Stem cells; Regenerative dentistry; Revascularization.

INTRODUCTION

Stem cells are undifferentiated cells of biological type, capable of differentiating into indefinite specialized cells which divide (through mitosis) to produce more cells of a similar type. It is regarded as the predecessor of the genealogical tree of related cell types.[1]

Based on their origin and capacity for differentiation, stem cells are divided into two groups: adult stem cells and embryonic stem cells. Mesenchymal stem cells, also known as MSCs, can be extracted from various tissues including the liver, synovial membrane, bone marrow, adipose tissue, amniotic fluid, umbilical cord, placenta, and teeth. The dental pulp of both deciduous and permanent teeth contains stem cells. Dental pulp stem cells (DPSCs) and stem cells from exfoliated deciduous teeth are the two types of stem cells found inside the tooth (SHED). The dental follicle, periodontal ligament (PDL), and apical papilla have all been found to be additional sources of dental stem cells. These cells have the ability to differentiate into a variety of cell types, including adipocytes, chondrocytes, osteoblasts, bone, new dental tissue, cartilage, muscle, and even nerve regeneration.[2,3]

Dr. Songtao Shi gave the acronym SHED after isolating stem cells from his 6-year-old daughter's deciduous teeth. These cells are of the mesenchymal type, which is highly proliferative and multipotent. Consequently, it is thought to be one of the candidate cell types for tissue regeneration.[1]

History of Stem Cells Research[4]

Early in the 1960s, James Till, Ernest McCulloch, and their University of Toronto

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colleagues learned about mouse reservoirs of cells that have characteristics similar to those of stem cells.

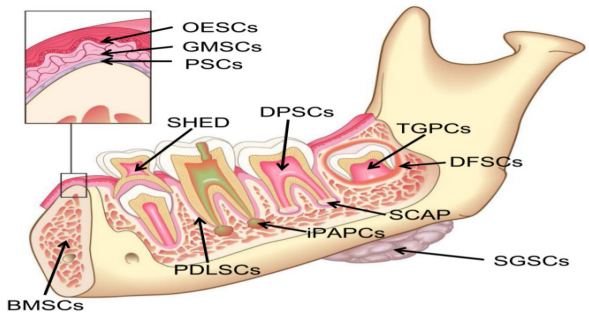
Mooney et al. (1996) performed the first in-vitro study to describe the procedure for regenerating new pulp-like tissues from cultured human pulpal fibroblasts. King et al and King and Hughes' periodontal studies suggest that the healthy PDL may be able to receive stimulation from a distance and move toward the injured, immature root apices, supporting the existence of stem cells in both the PDL and alveolar bone marrow. Following root canal procedures, Thibodeau et al. (2006) confirmed the existence of vital tissue within the root canal space. Gomez Flores et al. made an innovative attempt at in-vivo PDL regeneration using a multilayer human PDL cell sheets technique, which encouraged the formation of PDL and immature cementum-like tissue (2008).

Types of Dental Stem Cells

Diagrammatic representation of possible oral environments for post-natal stem cells. Dental pulp stem cells (DPSCs), bone marrow stem cells (BMSCs), stem cells of the apical papilla (SCAP), tooth germ progenitor cells (TGPCs), dental follicle stem cells (DFSCs), salivary gland stem cells (SGSCs), stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), gingival-derived mesenchymal stem cells (GMSCs (OESCs).[5,6]

SHED collection, separation, and preservation

The technique involves the collection, isolation, and storage of SHED and is straight forward, non-invasive, and easy to use.[1]



Step 1: Collection of tooth

A call is placed to the tooth bank or a dentist present at the bank after the exfoliated primary teeth are placed in the sterile saline solution.[7] In contrast to the grey colour of the pulp, which suggests that the pulpal blood flow has been compromised, necrotic, and is no longer viable for recovery, the pulp of an exfoliated tooth should be red in colour, indicative of cell viability.[8] Tumors or cysts that frequently have a severed blood supply, mobile teeth from trauma, teeth with apical abscesses, or teeth that are mobile due to trauma are not candidates for stem cell recovery. As a result, primary teeth are preferred over mobile teeth that are "hanging on by a thread" after extraction.[9] The tooth is placed into a vial filled with hypotonic phosphate buffered saline solution as it recovers to help prevent tissue desiccation while being transported (up to four teeth in one vial). The vial is then meticulously sealed, put into a thermite-based temperature phase change carrier, and finally placed into an insulated metal transport vessel that is kept hypothermic throughout transportation. This process is known as "Sustentation."[8] The time between harvest and arrival at the processing storage shouldn't be longer than 40 hours.

Step 2: Stem cell isolation[1]

When the tooth bank receives the vial, the following protocol is followed:

With Dulbecco's Phosphate Buffered Saline (PBSA), the tooth surface is cleaned by washing it three times without Ca^{2+} and Mg^{2+}, disinfecting it with povidone-iodine, and then washing it again with PBSA. Using a small, sterile excavator or by directly flushing pulp-rich stem cells from the tooth's centre with salt water, the pulp tissue from the pulp chamber is isolated.[10] A sterile petri dish is used to contain the contaminated pulp tissue after it has been at least three times PBSA-washed. The tissue is then digested for an hour at 37 °C using Dispase and collagenase Type I. We can also use trypsin- EDTA.

Isolated cells are put through a 70 m filter to create single-cell suspension. Mesenchymal Stem Cell Medium (MSC), which contains alpha-modified essential medium with 2 mM glutamine, 15% foetal bovine serum (FBS), 0.1mM L-ascorbic acid phosphate, 100U/ml penicillin, and 100 g/ml streptomycin, is used to cultivate cells. MSC is maintained at 37 ^{0}C and 5% CO_2 in the air. Typically, isolated colonies become visible after 24 hours. The MSC medium can be changed to produce various cell lines.

If cultures are obtained using an unselected preparation, colonies of cells with a morphology similar to epithelial cells or endothelial cells can be established. Cells vanish as they move through successive cell passages. If contamination is severe, one of three techniques can be used:

1. Because epithelial or endothelial-like cells are more strongly attached to the culture flask or dish, culture is reserpinized for a brief period of time to ensure that only stromal cells are detached.
2. Because stromal cells adhere to the culture surface earlier than contaminating cells, the medium is changed 4-6 hours after subculture.
3. Use Fluorescence Activated Cell Sorting (FACS), in which STRO-1 OR CD146 can be used, to separate stem cells. This is thought to be the most trustworthy. The donor's parents receive confirmation of the current state of their child's health and cell viability.

Step 3: Stem cell storage

Stem cell storage can be done in two different approaches:

1. Cryopreservation

It is the process of cooling cells or entire tissues to below-freezing temperatures in order to preserve them. The biological activity or any cellular processes cease at this temperature, causing cell death. Cryopreservation allows for the storage of SHED for a longer period of time while maintaining its usability. Cells that are harvested near the end of log phase growth (roughly 80–90% confluent) are the best candidates for cryopreservation. In order to ensure that another sample will be available for use even in the unlikely event that one of the storage units experiences a problem, the sample is divided into four cryotubes and each part is stored in a separate location in the cryogenic system. The cells are kept alive in liquid nitrogen vapour at a temperature of less than -150 ^{0}C. This keeps the cells viable and maintains both their latency and potency. The ideal cell density in a vial is 1-2 x 10 ^{6} cells in 1.5 ml of freezing medium. If the cell count is too low or too high, the recovery rate might be affected.[1]

Third molar pulp tissue may be a suitable source of multipotent stem cells even after cryopreservation for upcoming tissue engineering techniques and cell-based therapies, according to Zhang et al. (2006) who examined the differential potential of stem cells from the cryopreserved pulp of human third molars.[12]

2. Magnetic Freezing

This technology, known as CAS (Cell Alive System), takes advantage of the fact that when water or cell tissue is exposed to a weak magnetic field, the freezing point of those substances can drop by up to 6–7 ^{0}C. With CAS, an object is completely chilled below freezing without actually freezing. This prevents cell wall damage from ice expansion and nutrient loss from capillary action, both of which are common side effects of traditional freezing techniques. Once the object is uniformly chilled, the magnetic field is turned off, and the object snap-freezes.[1]

APPLICATIONS IN DENTISTRY

Pulp implantation:

In an open apex procedure, postnatal stem cells (obtained from skin, buccal mucosa, fat, and bone) are directly injected into clean root canal systems. This makes it easier to collect and administer autologous stem cells by syringe and increases their potential to

promote new pulp regeneration. However, the drawbacks include the cells' low rate of survival and their migration to various parts of the body. To increase the likelihood that pulp regeneration will be successful, all three components (cells, growth factors, and scaffold) must be taken into account.[1]

Apexogenesis or Apexification

In younger patients with immature roots, a conservative approach is needed to retain some vital pulp tissue and allow continued root formation. In an immature permanent tooth, the regeneration of tissue into the apex may derive from stem cells already present in the vital pulp tissue, SCAP, PDL, or alveolar bone. Stem cells are used to promote pulpal regeneration so as to treat immature permanent teeth in a conservative approach. Regenerative endodontic increases root length and thickness leading to complete root formation.[4]

Pulp Revascularization

An immature tooth with pulpal necrosis (due to caries or trauma) may stop further root development, resulting in thin root canal walls susceptible to fracture and blunderbuss apices making it difficult to obtain a hermetic seal with conventional obturation techniques. Pulpal tissue regeneration of an infected immature tooth will take place only in the absence of intra-pulpal infection which favours the environment for repopulation of mesenchymal cells arising from dental papilla or apical periodontium.[4]

Whole Tooth Regeneration

By seeding various cell types on biodegradable scaffolds, tooth-like tissues have been regenerated. Cells can be harvested, grown, and differentiated in vitro before being seeded onto scaffolds and implanted in vivo. In certain circumstances, the scaffolds are re-implanted into the jaws or an extracted tooth socket.[4]

Dental or Tooth Regeneration, Why??

The ultimate goal of tooth regeneration is to have completely functioning bioengineered teeth that can replace the lost natural teeth. Recent advances like titanium dental implants do not function as identical as natural teeth, because they lack an intervening PDL after osseointegration. Hence, the regeneration of the tooth root using dental stem cells seem to be a more realistic and clinically approachable treatment modality.[4]

Hurdles for Regenerative Procedures

A major problem routinely encountered in the clinical application of tooth regeneration technology is the identification of the most appropriate and suitable autologous stem cell source in humans, but there are numerous hurdles with in-vitro regenerative procedures: Stem cells are avascular in nature, need to be grown and expanded before being implanted into the root canal, should have proper adherence to the disinfected root canal walls and the technical hurdle in replanting the regenerated pulp without harming the cells.[4]

Current Trends and Future Perspectives of Stem Cells Regeneration

Recently, it was developed to recreate a murine "Bioengineered tooth unit" in vivo using the same cell source as the bioengineered tooth. It's interesting to note that the unit also includes the alveolar bone in addition to a fully developed tooth and periodontal ligament. In a mouse model with a vertical alveolar bone defect, the unit promoted the regeneration of a fully functional tooth along with vertical bone growth. These findings gave rise to a novel idea for tooth regeneration therapy: The transplantation of a bioengineered tooth has unparalleled ability to both fully regenerate the tooth and to correct a severe alveolar bone defect.

In the future, it may be possible to regenerate dental tissue using genetically

modified cells that deliver physiologically specific growth factors locally. Dental professionals have realised that the present is the crucial time to take action as the opportunities to bank patient stem cells will have the greatest future impact. Additional research must be done because the field of study is still in its infancy.[13]

CONCLUSION

Stem cell therapy is popular today as a treatment option for illnesses and injuries, and it has numerous health and dental advantages. The use of dental stem cells to treat diabetes mellitus, neurodegenerative diseases (Parkinson's disease, Alzheimer's disease), muscular dystrophy, lupus erythematosus, impaired vision, extensive burns, and cardiomyopathies is the focus of current research in regenerative medicine. Although there is still much research to be done, it is clear from the available evidence that primary teeth are a better source of stem cells and that dental stem cells have a huge potential for use in stem cell therapies.

CONFLICT OF INTEREST: Nil

SOURCE OF FUNDING: Self

ETHICAL CLEARANCE: Not applicable

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Silver Diamine Fluoride: A Literature Review

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ABSTRACT

Dental caries is one of the most common oral diseases. It is always better to prevent the disease before it advances which may results to pain, unesthetic look and difficulty in mastication and function. Silver diamine fluoride is one of the preventive measures performed by the dentist to prevent and arrest initial dental caries. It is very effective in all age groups especially in paediatric patients. The knowledge, the exact mechanism and its method of use is a primary requirement of the dental professional. The present article emphasizes a detailed review on silver diamine fluoride.

Keywords: silver, fluoride, silver, silver diamine fluoride.

INTRODUCTION

International data on caries epidemiology confirm that dental caries remains a significant disease of childhood that is found in a subset of at-risk children in both developing and developed countries.[1] According to WHO, the global average of DMFT is 2.4.[2]

Fluoride is primarily anti-caries effects are topical and its presence in plaque and saliva inhibits demineralization. Remineralization with fluoride leads to a crystalline structure more resistant to bacterial acid and thus caries inhibition and progression.[3]Silver diamine fluoride (SDF) $Ag(NH_3)_2F$, is used to arrest caries since 1969.[4] SDF has a unique anticariogenic ability to be a "silver-fluoride bullet."

SDF has been used to deal with high caries prevalence by arresting or slowing down the rate of caries progression, used in management of dental caries in young children, to arrest root caries, to prevent pit and fissure caries, to prevent secondary caries, to desensitize sensitive teeth, to treat infected root canals and to prevent the fracture of endodontically treated teeth.[3-8]

MECHANISM OF ACTION:

1. Action of Silver Diamine Fluoride on Bacteria

Multiple modes of action have been proposed for silver and can be explained by the multiple biological organisms like bacterial, protozoan, fungal, and viral in origin, subcellular targets like cell membranes, cell organelles, nuclei and mechanisms such as metabolism, replication of the cell have been examined. Studies have indicated that, when silver interacts with sulfhydryl groups of proteins and DNA it alters the hydrogen bond and

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results in inhibiting the respiratory processes, DNA unwinding, cell- wall synthesis, and cell division.[9] At the macro level, these interactions lead to bacterial killing and inhibit biofilm formation.[10] The central mechanism for these effects are due to the interaction of silver with thiol groups by the following mechanism.[11]

$$A/N\text{-}SH + AgX \rightarrow A/N\text{-}S\text{-}AgX + HX$$

Where A/N is amino (A) or nucleic (N) acids (respectively), SH is a thiol group, Ag represents silver, and X represents an anion (diamine fluoride). This interaction indicates how silver diamine fluoride, when applied to caries lesions, might interact with bacteria and results in caries arrest through bacterial killing and inhibit caries progress through the inhibition of biofilm formation.[3]

It is noted that the nature of silver in silver compounds is not clearly stated in the literature. Silver ions (Ag+) are expected to have antibacterial effects, but metallic silver (Ag or Ag0) is relatively inert. However, metallic silver can interact with moisture in the oral environment and releases silver ions which is a crucial point for the antibacterial effects on pathogenic organisms.[12]

There are three main antibacterial effects of silver ions:

1. Destruction of cell wall structure;
2. Denaturation of cytoplasmic enzyme and
3. Inhibition of microbic DNA replication.

Firstly, silver ions can bind with disulphide in membrane proteins; thereby allowing easy penetration through membranes. It is also reported that silver ions can electrostatically bind negatively-charged peptidoglycans in the bacterial cell wall which is negatively-charged and disturb the membrane transport functions leading to cellular distortion and loss of viability.[13]

Secondly, silver ions can bind to sulphydryl groups (-SH, the thiol group of cystine) which is essential for enzymes activities. Such interactions with cystine could inhibit enzyme activities, disrupt metabolic processes and eventually cause death of the microbe.[11]

Thirdly, it was reported that silver ions can also attach to guanine, a major component of DNA, thereby disabling the replication ability of bacteria. Microorganisms in biofilms are more resistant to antibacterials than planktonic pathogens and it is reported that biofilms may require more than 100 times the concentration required for planktonic bacteria.[14]

2. Action of Silver Diamine Fluoride onTeeth

Investigators found that the 2 compounds have complex mechanisms.[7] The most commonly recognized interaction is sodium fluoride with calcium phosphate to form fluorapatite and sodium hydroxide (and a basic environment).

$$Ca_{10}(PO_4)_6(OH)_2 + NaF \rightarrow Ca_{10}(PO_4)_6F_2 + NaOH$$

The less commonly seen interaction is the combination of tooth calcium to form calcium fluoride and a basic environment.

$$Ca_{10}(PO_4)_6(OH)_2 + NaF \rightarrow CaF_2 + Na_3O_4 + NaOH$$

When hydroxyapatite of the enamel reacts with silver nitrate it results in the formation of calcium nitrate, silver phosphate, and silver oxide.

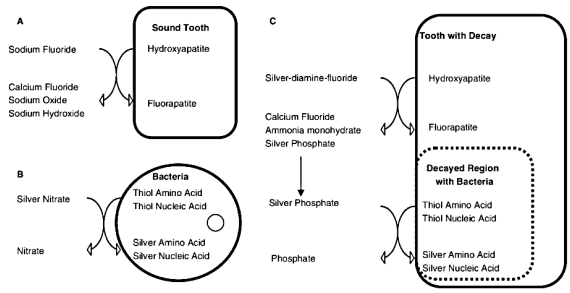
$$Ca_{10}(PO_4)_6(OH)_2 + Ag(NO_3) \rightarrow Ca(NO_3)_2 + Ag_3PO_4 + Ag_2 + H_2O$$

When fluoride and silver interact, it leads to the formation of fluorapatite. At first there is formation of calcium fluoride and silver phosphate in a basic environment.

$$Ca_{10}(PO_4)_6(OH)_2 + Ag(NH_3)_2F \rightarrow\rightarrow\rightarrow CaF_2 + Ag_3PO_4 + NH_4OH$$

Later, there is subsequent dissociation of calcium and fluoride.

$$CaF_2 \rightarrow Ca + 2F^-$$



Courtesy: Rosenblatt A et al

Effect of Silver Diamine Fluoride on teeth and bacteria

The last step is the formation of fluorapatite. The net result of these interactions is as follows.

$$Ca_{10}(PO_4)_6(OH)_2 + 2F^{-} \rightarrow\rightarrow Ca_{10}(PO_4)_6F_2 + 2OH$$

(A) In a sound tooth, when fluoride reacts with hydroxyapatite to form fluorapatite. Fluorapatite is less acid-soluble than hydroxyapatite resulting in inhibition of the caries process.

(B) In bacteria, when silver reacts with thiol groups of amino and nucleic acids, the bacteria is unable to carry out metolic and reproductive functions, resulting in bacterial lysis.

(C) In teeth with decay, silver diamine fluoride reacts with hydroxyapatite to form fluorapatite, and the by-product is silver phosphate. Silver phosphate later reacts with bacterial amino and nucleic acid thiol groups to form silver amino and nucleic acids.

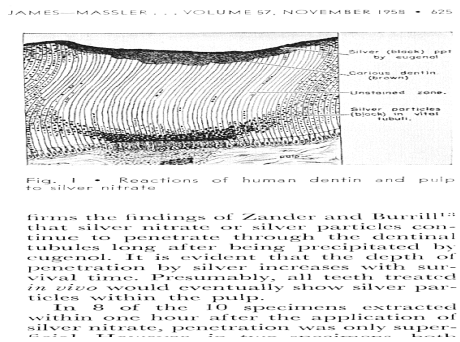
REACTIONS OF CARIOUS AND NON-CARIOUS DENTIN TO SILVER NITRATE:

The following four layers could be distinguished in dentin treated with silver nitrate:[16]

1. A thin, superficial layer of black precipitate seen on the surface of the dentin. This layer consisted of free silver precipitated by the eugenol. The precipitate was on the dentin which indicated that the eugenol acted on the surface of the dentin only or penetrated the dentin only superficially.
2. A layer of carious dentin is stained brown. This was the result of the reduction of the silver nitrate to brown free colloidal silver or a combination of the silver nitrate with the altered available protein by the carious matrix. The deepest border of this brown staining mass was irregular, that outlines the advancing carious lesion into the underlying dentinal tubules and matrix. Carious dentin which was not treated by silver nitrate stained a deep purple with hematoxylin and eosin. Carious dentin treated with silver nitrate stained a deep brown and did not take the purple stain of the hematoxylin and eosin. Hence it was concluded that the brown staining was a specific result of the silver nitrate.
3. An intermediate zone was seen relatively free of silver staining. This zone may represent a zone of reduced dentinal vitality (metamorphosed or sclerotic

UNIVERSITY OF CALIFONIA, SAN FRANCISCO (UCSF) PROTOCOL FOR ARRESTING DENTAL CARIES LESION

Indications	*Contraindications*
Extreme caries risk (Xerostomia or S-ECC)	Absolute: silver allergy
Difficult to treat dental carious lesion	Relative: ulcerative gingivitis, stomatitis
Patients without access to dental care	SSKI contraindication: pregnancy, breast-feeding
Patients with carious lesion that may not all be treated in one visit.	
Treatment challenged by behavioural or medical management.	



JAMES—MASSLER . . . VOLUME 57, NOVEMBER 1958 • 625

Fig. 1. • Reactions of human dentin and pulp to silver nitrate.

firms the findings of Zander and Burrill[13] that silver nitrate or silver particles continue to penetrate through the dentinal tubules long after being precipitated by eugenol. It is evident that the depth of penetration by silver increases with survival time. Presumably, all teeth treated *in vivo* would eventually show silver particles within the pulp.

In 8 of the 10 specimens extracted within one hour after the application of silver nitrate, penetration was only super-

Courtesy: Englander HR

Reactions of human dentin and pulp to silver nitrate.

dentin). This zone coincides with the region which has tubular contents that are degenerated in advance of the carious lesion.

4. A deeper zone containing large black globules of reduced silver particles. These were observed deep within the vital dentinal tubules and within the pulp cells.[16]

Conventional-thickness sections (6-7 Fm) showed silver granules extending for variable distances through primary dentine and in some areas of reparative dentine. The amount of reparative dentine formed was substantial and was mainly of the irregular tubular variety. The predentine layer was wider than normal (in some cases up to 5 times the normal width). Regardless of its increased width, the predentine showed a comparatively small degree of mineralization.

The coronal pulp was normal except for the presence of diffusely distributed lymphocytes in some instances. Lymphocytes and plasma cells were observed in greater numbers in areas of the root canal pulp where resorption was seen. Under conventional light microscopy, silver particles were detected within the odontoblastic and subodontoblastic layers, and in the central region of the coronal pulp, but not in the root canal pulp. Examination using confocal laser scanning microscopy revealed a much greater density of silver in the coronal pulp. Particles were observed lying within odontoblasts, fibroblasts and endothelial cells lining capillaries.[16]

CLINICAL APPLICATIONS OF SILVER DIAMINE FLUORIDE:

- To arrest Initial dental caries: *In-vitro* studies by Yamaga et al 1972, Gotjamanos and Orton 1998, klein et al 1999, and *in-vivo* studies by McDonald and Sheiham 1994, clinical trials in primary and permanent dentition by Nishino et al 1969, Almeida 1994, Chu et al 2002, Llodra et al 2005, Wong et al 2005, has been effective in controlling and preventive dental caries.[3]
- To prevent pit and fissure caries: Nishino and Massler 1997 in their study mentioned that caries score of SDF treated teeth was significantly lower than the fissures treated with 8% SnF_2 or $Ag(NO)_3$.[19]
- To prevent secondary caries: Shimizu and Kawagoe in 1976 found no recurrent caries was seen on amalgam restoration on primary teeth which was pre-treated with SDF after 24 months.[19]
- To arrest root caries: Root caries usually increases as age increases. A study done on Hong Kong's elderly community where 38%SDF was used resulted in arresting dentin caries on exposed root surface. In this clinical study the group who received SDF and oral hygiene education had 18% more arrested active root surfaces when compared to those who received SDF alone.[20]
- To desensitize sensitive teeth: SDF has the ability to occlude dentinal tubules. Hatsuyama et al 1967, Murase et al 1969, and Kimura et al 1971 have shown that SDF was effective against abrasion, erosion, hypersensitive dentine and also it was suggested 4 times application was most appropriate.[19]
- To treat infected root canals: Mathewson et al showed that SDF is as effective as 2% chlorhexidine in removing E. faecalis from infected root canals.[21]

- As an indirect pulp capping agent: The main objective of SDF treatment is to maintain pulp vitality by arresting dental caries, promoting dentin sclerosis which results in decreased permeability and stimulating tertiary dentine formation.[22] Yamaga et al 1972 described that SDF will arrest the progression of caries when applied in presence of softened caries or when removal of softened caries can cause pulpal exposure.[7] Chu and Lo proposed SDF can be applied in IPC and ART procedures. An in-vitro study by Gupta et al 2011 concluded that highest zone of bacterial inhibition was seen when SDF was used. In-vivo part of the same study by Sinha et al found SDF was a re-hardening, remineralizing and antibacterial and therefore can be used as an IPC material.[19]

PROTOCOL FOR APPLICATION BY UCSF[17]

Maximum dose: 25µL (1 DROP)/ 10 kg per treatment visit

Considerations

- Decayed dentin will darken as the caries lesions arrest. Most will be dark brown or black
- SDF can stain the skin, which will clear in 2-3 weeks without treatment.
- SDF can permanently stain operatory surfaces and clothes.
- A control restoration may be considered after SDF treatment.
- Saturated Solution of Potassium Iodide (SSKI, Lugol's Solution, various sources) can be used after SDF to decrease color changes.
- Re-application is usually recommended, biannually until the cavity is restored or arrested or the tooth exfoliates.

PROCEDURE

1. Standard Personal Protective Equipment (PPE) for provider and patient.
2. 1 drop of SDF into the deep end of a plastic dappen dish. Obtain 1 drop of SSKI in a separate dappen dish if selected)
3. Remove bulk saliva with saliva ejector.
4. Isolate tongue and cheek from affected teeth with "2x2" gauze or cotton rolls.
5. If near the gingiva, consider applying petroleum jelly with a cotton applicator for safety.
6. Dry affected tooth surfaces with triple syringe, or if not feasible dry with cotton.
7. Remove excess on side of dappen dish.
8. Apply directly onto the affected tooth surface/s with microsponge.
9. Allow SDF to absorb for up to 1 minute if reasonable, then remove excess with gauze or cotton roll. (If using SSKI, apply with a different microsponge. Repeat 1-3 times until no further white precipitates are observed. Wait 5-10 seconds between applications. Remove excess with cotton.)
10. Rinse with water.
11. Place gloves, cotton, and microbrushes into plastic waste bags.

ADVERSE EFFECTS

- Argyria: Irreversible pigmentation of the skin due to prolonged exposure to silver
- Temporary tattoo on skin or submucosa which will resolve in 2 to 14 days by natural exfoliation of skin.[18]
- Silver allergy results in transient increase in erythema in the gingiva of the teeth which disappears 48 hours without any treatment.[23]
- Metallic taste
- Accidental ingestion of large amount of SDF, vomiting can be induced to avoid its absorption in the body. 10% calcium gluconate (10ml) solution can be administered. Calcium ions reacts with fluoride ions to form insoluble calcium fluoride which cannot be absorbed in the GIT. [18]

- Staining of teeth: Staining of SDF could be due to formation of silver phosphate. Several studies with SDF/KI or SiF were tried to reduce the staining, but none were effective and further studies are necessary to provide promising results.[23]

CONCLUSION

SDF is safe, effective treatment for dental caries. Application twice a year outperforms all minimally invasive treatment including ART and sealants. Traditional approaches often provide only temporary benefit results to the highest rates of recurrent caries are in patients with the worst disease burden. The advent of treatment for non-symptomatic caries not requiring general anaesthesia or sedation addresses long-standing concerns about expense, danger and practical complexity of these services. This is a powerful new tool against dental caries particularly suited for those with high risk.

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