RANDOMIZED CONTROL TRIAL

Comparison of Remineralizing Agents in the Management of White Spot Lesions in Three- to Five-Year-Old Children: A Clinical Trial

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Abstract: ***Purpose:*** *The purpose of this study was to compare the effectiveness of P_{11-4} self-assembling peptide (Curodont™ Repair [CR]) and fluoride varnish with xylitol-coated calcium phosphate (Embrace™ Varnish [EV]) on enamel permeability and in the management of white spot lesions (WSLs) in primary teeth.* ***Methods:*** *A clinical trial was conducted among 30 children aged three to five years with WSLs in 60 anterior teeth. They were randomly assigned to receive CR or EV. Preintervention and postintervention evaluation was done by International Caries Detection and Assessment System (ICDAS) and morphometric analysis. The secondary outcome was to assess the enamel permeability using scanning electron microscopy (SEM) of polyvinyl siloxane impressions.* ***Results:*** *A statistically significant reduction in the ICDAS scores (P=0.05) and percentage area of WSLs in morphometric analysis (P=0.008) was seen in the CR group after six months. No statistically significant difference was observed in the EV group after six months. The SEM evaluation did not show a significant reduction in the percentage area of droplets in both the CR and EV groups (P=0.06 and P=0.21, respectively). No significant difference was seen between EV and CR in the three parameters assessed.* ***Conclusion:*** *Curodont™ Repair is effective at remineralizing white spot lesions in primary teeth and can be considered as a remineralizing agent.* *(Pediatr Dent 2023;45(2):99-104.E16-E17) Received March 31, 2022 | Last Revision December 13, 2022 | Accepted December 14, 2022*

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White spot lesions (**WSLs**) are defined as subsurface enamel porosity from carious demineralization that presents themselves as "a milky white opacity located on smooth surfaces."[1] Development of WSLs along the gingival margin of maxillary primary incisors is an early manifestation in the development of early childhood caries (**ECC**).[2] The overall prevalence of ECC in India has been reported to be 49.6 percent.[3] The enamel of primary teeth is less mineralized, organic, porous, elastic, and softer than permanent teeth.[4] The formation of caries is not a continuous unidirectional process of demineralization. Instead, it is a cycle of demineralization and remineralization with various stages being either reversible or irreversible.[5] Once this dynamic equilibrium tips toward demineralization, the first sign of reversible WSLs occurs.[6] Early diagnosis of caries before cavitation can avoid extensive restorative procedures.[7] If left untreated, it can lead to dentinal involvement, cavitation, and crown destruction[6] and ultimately can result in serious general health problems, significant pain, interference with eating, and loss of school time.[8]

Traditionally, topical fluorides have been used to promote the remineralization of carious lesions. Materials like xylitol were found to have anti-cariogenic and cariostatic effects.[9] A combination of these two agents has been used for better effectiveness. Embrace™ Varnish ([**EV**]; Pulpdent Corporation, Watertown, Mass., USA) contains five percent sodium fluoride and xylitol-coated Calcium and Phosphate (**CXP™**) for unsurpassed fluoride release.[10] The addition of xylitol provides a continuous reaction of calcium and phosphate ions, helping to form fluorapatite crystals.[11] Development of peptide treatment in caries management led to the introduction of the P_{11-4} self-assembling peptide, which mimics the action of enamel matrix proteins during tooth development.[12] Curodont™ Repair ([**CR**]; Credentis AG, Windisch, Switzerland) is a non-fluoridated biomimetic material that diffuses deeply into the subsurface carious lesion and forms a three-dimensional scaffold matrix. The remineralizing ions like calcium and phosphate from saliva and enamel diffuse into the matrix and induce the de-novo formation of hydroxyapatite crystals.[13] Alkilzy et al. reported a superior remineralization efficacy of CR compared to fluoride varnish in early carious lesions of permanent teeth.[14]

Lucchese et al. linked the permeability of enamel to the development of caries.[15] The structure and composition of enamel are altered by the cariogenic attack, and this ultimately affects the permeability of enamel.[16] Healthy barriers can reduce the enamel permeability and, therefore, the development of caries. Chersoni et al. reported a reduction in enamel permeability following topical fluoride application.[17]

There are no clinical studies on the effectiveness of CR in primary teeth. Hence the purpose of this study was to compare the effectiveness of Curodont Repair and Embrace Varnish in managing white spot lesions and changing the enamel permeability of primary teeth. The null hypothesis of this trial was that there was no difference between CR or EV on enamel permeability changes and in the management of WSLs in primary teeth.

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Methods

A parallel, double-blinded, clinical equivalence trial was designed from December 2020 to July 2021. The study protocol was analyzed and approved by the Institutional Review Board and Institutional Ethics Committee of KSR Institute of Dental Science and Research, Tiruchengode, Tamil Nadu, India. Informed consent from the parents and assent from their children were obtained before the commencement of the study. Clinical trial registration was done (CTRI/2021/02/031288). The reporting of the study has been done according to the CONSORT (Consolidated Standards of Reporting Trials) 2010 guidelines.[18]

Sample size estimation. The sample size estimation was done using G power 3.1 software based on the study results by Alkilzy et al.[19] The *P*-value, power, and effect size were set at 0.05, 80 percent, and 0.76, respectively. The estimated sample size was 26 teeth in each group. Anticipating an attrition percentage of 15 percent in six months, the sample size was increased to 30 teeth per group.

Study population selection. The study participants were recruited from three government schools in Tamil Nadu. Prior permission was obtained from the higher officials of the institutions. A total of 300 children from three to five years were screened by the principal investigator with the help of the dental assistants and school attendees Infection control and prevention strategies were followed. Oral examination was done using a mouth mirror under natural sunlight[20]. Children suspected to have WSLs were re-examined after drying their teeth.

Selection criteria. Children with two WSLs were included. The lesions should be non-cavitated, active,[21] visible, and accessible in maxillary primary anterior teeth with codes one to three following the International Caries Detection and Assessment System (ICDAS).[22] Children who had cavitated lesions were excluded. Subjects with recent glass ionomer restorations were excluded as their fluoride release property can potentially influence the study results. The presence of developmental defects, concomitant medication affecting salivary flow, and any metabolic disorders were not included.

Baseline evaluation. Based on the selection criteria, 60 primary teeth in 30 children were selected for the study. ICDAS assessment and morphometric analysis were recorded for all the teeth. Selected teeth were cleaned using sterile cotton, and the principal investigator made preoperative photographs and impressions.

Oral hygiene instructions. Oral prophylaxis was done for all the children immediately before the intervention. Oral hygiene instructions and diet counseling were given immediately after the intervention. Children were asked to brush twice daily as their routine oral hygiene measure during the entire study period.

Intervention. Thirty children with two WSLs were randomly assigned to either the CR group or EV group (15 children per group). Simple randomization with a one-to-one allocation ratio was done using a computer-generated random allocation sequence by the secondary investigator. Children who met the inclusion criteria were asked to withdraw a sealed envelope to determine the assigned intervention. The children and the principal investigator were blinded to the treatment provided. The intervention was provided by another investigator who was not aware of the study protocol. Group one children received CR, and group two children received EV. In group one, after proper isolation, three percent sodium hypochlorite was used to wipe the tooth with WSL for the elimination of organic debris. Etching was done with 37 percent phosphoric acid to remove organic materials and create microporosities for the effective subsurface penetration of the material.[23] CR was applied on the caries lesion for three to five minutes to allow diffusion of the material.[19] In group two, EV was applied on teeth with WSLs in a horizontal swipe motion. The study

Table 1. DESCRIPTIVE STATISTICS OF STUDY POPULATION*

		Groups: Curodont™ Repair N (%)	Groups: Embrace Varnish™ N (%)	*P*-value**
Gender	Boys	7 (46.6)	6 (40)	0.11
	Girls	8 (53.4)	9 (60)	
Tooth brushing frequency	Once	9 (60)	8 (53.4)	0.20
	Twice	6 (40)	7 (46.6)	
Primary maxillary anterior teeth with WSL	Central incisor	16 (53.3)	13 (43.3)	0.06
	Lateral incisor	7 (23.3)	7 (23.3)	
	Canines	7 (23.4)	10 (33.4)	
ICDAS codes†	Code 1	11 (32.1)	11 (36.7)	0.08
	Code 2	18 (64.3)	17 (56.7)	
	Code 3	1 (3.6)	2 (6.6)	

* Abbreviations used in this table: WSL=white spot lesion; ICDAS=International Caries Detection and Assessment System[22].

** *P*-value using chi-square test.

† Code 1: first visual change in enamel; Code 2: distinct visual change in enamel; Code 3: localized enamel breakdown.[22]

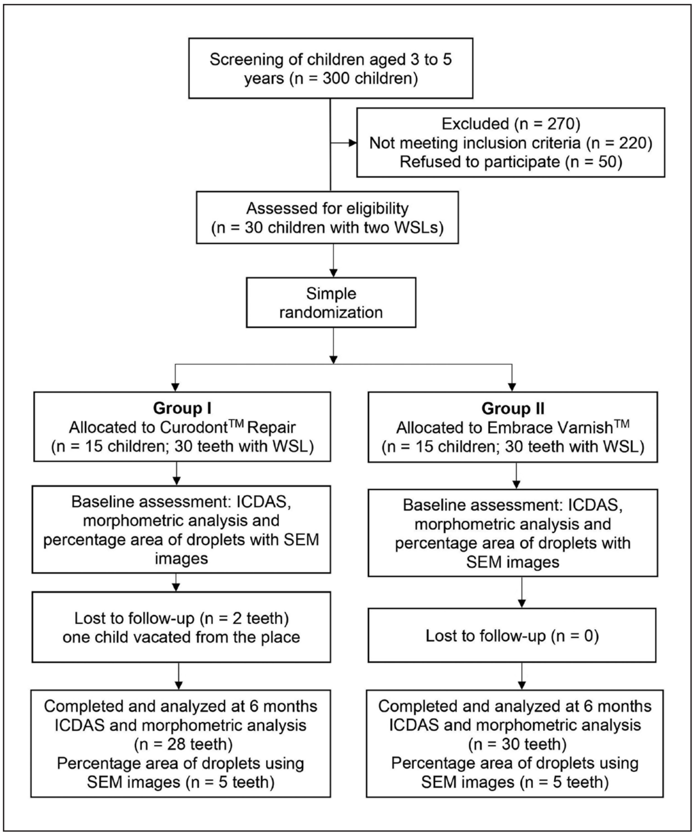

Figure. CONSORT flow diagram.

materials were applied only once during the study period. All children were instructed to avoid eating and drinking water for the next 20 minutes to prolong the duration of action of the intervention. They were also instructed to refrain from tooth brushing on the day of application. All the treated teeth were examined at six months, and postoperative photographs and impressions were taken by the principal investigator.

Morphometric analysis. All the photographs were taken using a Nikon Dx camera with a 25-mm wide-angle G lens (AF-P NIKKOR 18-55mm 1:3.5 to 5.6 G) with 10X optical zoom. The camera was adjusted manually such that the focal length was 24 mm, the exposure time was one-sixtieth of a second, the aperture was F/3.8, and the ISO sensitivity was 400. Standardized photographic procedure and positioning of children for the photographs were followed throughout the study in accordance with Hassan and Allam 2017.[24] The surfaces of teeth were wiped with cotton and dried with a chip blower (Model: Chip blower, TDS Excellence Defined, Jalandhar city, Punjab, India). All photographs were taken under natural light. The captured images were analyzed using Image J 1.47 analysis software (National Institutes of Health, Bethesda, Maryland, USAto calculate the area of the user-defined selections (see **Supplemental Electronic Data—sFigures 1 and 2**). To standardize, all the images were magnified to 75 percent and converted to eight-bit grayscale.[25] The percentage area of WSL was calculated from the overall area of the tooth surface.

Calibration for ICDAS scoring and morphometric assessment. Over a month period prior to the commencement of the study, two investigators were trained and calibrated by a clinical expert. A series of photographs were given to both investigators for ICDAS scoring.[26] Children with WSL were also examined clinically during this training period. For the morphometric assessment, software images were analyzed by the investigators to assess the percentage area of WSLs. In case of any disagreement between the two investigators, the clinical expert was consulted to resolve the disagreement. During the study period, they were blinded to each other's scoring. Inter- and intra-examiner reliability was checked.

Enamel permeability assessment. The permeability changes in primary teeth enamel with WSLs after the application of the respective remineralizing agents were assessed using scanning electron microscopy (**SEM**). *Preintervention and postintervention* impressions of 10 teeth (five teeth in each group) with WSLs were made, with a polyvinyl siloxane impression material of putty and light body consistency, in a two-stage procedure using a spacer. The labial surface of the impression in each study tooth was cut into individual samples of approximately 20×20 µm in three different representative areas at the cervical, middle, and incisal thirds. The impression was manipulated and analyzed under SEM for the presence of droplets based on the protocol given by Narrenthran et al.[4] Stepwise procedure to assess the area of droplets in SEM images is described in **Supplemental Electronic Data—sFigure 3**. The representative area with the highest distribution of droplets was considered for analysis. The area of droplets was quantified using Image J software by the principal investigator. The color threshold was changed to mark every droplet on the image after clearing the background. The area of all marked droplets was calculated by the software, and the average mean values were taken as the final value. The percentage area covered by the droplets in the cervical, middle, and incisal third were calculated manually. The average of the three regions was recorded as the final percentage area of droplets for the individual tooth.

Statistical analysis. The data obtained were statistically analyzed using SPSS® 17.0 software (IBM Corp., Armonk, N.Y., USA). An intra-group comparison of the categorical variable was done using McNemar's test, whereas an intergroup comparison was done using the chi-square test. An intra-group comparison of normally distributed quantitative data was done using a paired test; for non-normally distributed data, the Wilcoxon signed-rank test was used. Inter-examiner reliability for the continuous variable was assessed using the intraclass correlation coefficient (**ICC**); for the categorical variable, Cohen's Kappa was calculated. The internal consistency was calculated using Cronbach's alpha. Intention-to-treat (**ITT**) analysis was used.

Results

At baseline, 30 three- to five-year-olds with WSLs in 60 maxillary anterior teeth were recruited for

Table 2. INTRAGROUP COMPARISON OF ICDAS CODES, MORPHOMETRIC ANALYSIS, AND PERCENTAGE AREA OF DROPLETS UNDER SEM*

Groups	ICDAS codes** N (%)						*P*-value
	Baseline			At 6 months			
	1	2	3	1	2	3	
Curodont™ Repair	11 (36.6)	18 (60)	1 (3.4)	15 (50)	12 (40)	3 (10)	**0.05†**
Embrace Varnish™	11 (36.6)	17 (56.7)	2 (6.7)	9 (30.0)	16 (53.3)	5 (16.7)	0.08†
Percentage area of WSLs in morphometric analysis (Mean±SD)|							
	Baseline			At 6 months			
Curodont™ Repair	12.03±8.80			11.39±9.30			**0.008‡**
Embrace Varnish™	10.18±7.15			10.00±5.13			0.304 ‡
Percentage area of droplets under SEM (mean±SD)||							
	Baseline			At 6 months			
Curodont™ Repair	37.16±14.38			24.24±10.44			0.06§
Embrace Varnish™	37.33±12.64			28.83±16.33			0.21§

* Abbbreviations used in this table: WSL=white spot lesion; ICDAS=International Caries Detection and Assessment System[22]; SEM=scanning electron microscopy; SD=standard deviation; intention-to-treat (ITT) analysis was followed.

** ICDAS Code 1: first visual change in enamel; Code 2: distinct visual change in enamel; Code 3: localized enamel breakdown.[22]

† *P*-value using McNemar test. ‡ *P*-value using Wilcoxon signed-rank test.

§ *P*-value using paired *t*-test.

| **Morphometric analysis**: Area was calculated in square millimeters using Image J 1.47 analysis software. The percentage area of white spot lesion was calculated from the overall area of the tooth surface (see Supplemental Electronic Data sFigures 1 and 2).

|| **Enamel permeability assessment**: The area of the droplets was calculated in square millimeters using Image J 1.47 analysis software. The percentage area covered by the droplets in the cervical, middle, and incisal third of the tooth with white spot lesions was calculated manually. The average of the three regions was recorded as the final percentage area of droplets for individual teeth (see Supplemental Electronic Data sFigure 3).

Table 3. INTERGROUP COMPARISON OF ICDAS CODES, MORPHOMETRIC ANALYSIS, AND PERCENTAGE AREA OF DROPLETS UNDER SEM*

ICDAS codes**		Interventions N (%)		P-value
		Curodont™ Repair	Embrace Varnish™	
At baseline	Code 1	11 (36.6)	11 (36.6)	0.67†
	Code 2	18 (60)	17 (56.7)	
	Code 3	1 (3.4)	2 (6.7)	
At 6th month	Code 1	15 (50)	9 (30)	0.51†
	Code 2	12 (40)	16 (53.3)	
	Code 3	3 (10)	5 (16.7)	
Percentage area of WSLs in morphometric analysis (Mean±SD)|				
At baseline (T0)		12.03±8.80	10.18±7.15	0.94‡
At 6th month (T1)		11.39±9.30	10.00±5.13	0.77‡
Difference (T0 – T1)		-0.64±1.52	-0.12±3.38	0.88‡
Percentage area of droplets under SEM (Mean±SD)||				
At baseline (T0)		37.16±14.38	37.33±12.64	0.98†
At 6th month (T1)		24.24±10.44	28.83±16.33	0.61†
Difference (T0 – T1)		12.91±11.16	8.49±12.62	0.57†

* Abbbreviations used in this table: WSL=white spot lesion; ICDAS= International Caries Detection and Assessment System[22]; SEM= scanning electron microscopy; SD=standard deviation; intention-to-treat (ITT) analysis was followed.

** ICDAS Code 1: first visual change in enamel; Code 2: distinct visual change in enamel; Code 3: localized enamel breakdown.[22]

† P-value using chi-square test.

‡ P-value using Mann Whitney U test.

| **Morphometric analysis**: Area was calculated in square millimeters using Image J 1.47 analysis software. The percentage area of white spot lesion was calculated from the overall area of the tooth surface (see Supplemental Electronic Data sFigures 1 and 2).

|| **Enamel permeability assessment**: The area of the droplets was calculated in square millimeters using Image J 1.47 analysis software. The percentage area covered by the droplets in the cervical, middle, and incisal third of the tooth with white spot lesions was calculated manually. The average of the three regions was recorded as the final percentage area of droplets for individual teeth (see Supplemental Electronic Data sFigure 3).

the study. There were 13 (43.4 percent) boys and 17 (56.6 percent) girls with a mean age of 3.86±0.6 years in both groups. All the observations from the start of the study were analyzed using an ITT analysis. The descriptive statistics of the study population are shown in Table 1. Postoperative evaluation at six months had one child with two teeth lost to follow-up (Figure) in the CR group because of the change in residence. Missing data were imputed using the last observation carried forward method. There were no changes in the study protocol during the study.

The intragroup comparison of ICDAS scores, morphometric analysis, and percentage area of droplets under SEM for CR and EV at baseline and six months are presented in Table 2. A statistically significant reduction in the ICDAS scores (P=0.05) and percentage area of WSLs in morphometric analysis (P=0.008) were noted in the CR group after six months. SEM evaluation did not show a significant reduction in the percentage area of droplets in both the CR group and EV group (P=0.06 and P=0.21, respectively) compared to the baseline. However, the intergroup comparison showed no statistically significant difference between the two groups at baseline and six months based on the ICDAS scores, morphometric analysis, and percentage area of droplets under SEM (Table 3).

Kappa statistics result from ICDAS scoring was 0.90. ICC revealed an excellent agreement of 0.99 intra-rater and inter-rater reliability in performing morphometric analysis. Cronbach's alpha value of 0.98 showed consistent results both at baseline and at six months.

Discussion

Prevention of dental caries starts with treating the WSLs at the earliest possible time to provide long-term patient protection. Two commercially available remineralizing agents, one with fluoride and one without fluoride, were chosen for the present study to analyze their effectiveness in the management of WSLs. This study showed that postoperative ICDAS and morphometric analysis scores were significantly lesser than the baseline scores in the CR group but not in the EV group. This suggested that CR might have a superior effect on the remineralization of WSLs. However, since this study did not include a gold-standard control group, the supremacy of one group over the other could not be established. The present study failed to reject the null hypothesis.

Children aged three to five years were included in the present study for the following reasons: a full complement of primary dentition; increased incidence of WSLs; and better acceptance of dental procedures. Government school children were targeted, as they have less access to oral health care, thereby increasing their caries risk.[27] Early diagnosis and treatment reduce its pervasive effects on the developing dentition.[6] Henry et al. reported the prevalence of non-cavitated WSLs in south Indian children as 50.3 percent until three years of age.[28] The prevalence was higher in maxillary canines followed by mandibular canines, maxillary lateral incisors, maxillary central incisors, and mandibular incisors.[29] Hence, maxillary primary anterior teeth with WSLs were selected in the present study. Abirami et al. have shown that the combined effect of oral hygiene maintenance and dietary guidance reversed ECC effectively.[30] Hence in the post-intervention period, all the children were instructed to brush twice daily, as brushing frequency could influence the study results.

There was a decrease in the ICDAS scores and morphometric analysis in both groups; however, a significant reduction was noted in the CR group at six months. Alkilzy et al.[19] and Kobeissi et al.[23] reported regression of ICDAS scores by application of CR. Shahmoradi et al. reported a decrease in enamel demineralization after using EV.[31] The superiority of CR may be due to the matrix-mediated mineralization process that can regenerate enamel. This is proposed to occur by the attraction of calcium ions from saliva by anionic side chains of peptides that induce precipitation of calcium phosphate salts on subsurface carious lesions.[13] Fluoride depletion and poor substantivity of EV could be the reasons for its reduced effectiveness in the present study. Sidhu et al. found that fluoride release in EV was noted for only one month, after which it started to reduce.[32] Milburn et al. reported a higher fluoride depletion in EV over the first 24 hours, reducing its substantivity.[11]

Iijima showed that image analysis had definite advantages over DIAGNOdent and quantitative light-induced fluorescence in monitoring remineralization.[33] Manual errors like the construction of symmetrical images and angulation changes are common in photographic techniques. All photographs were taken without flash under natural light to reduce the overestimation of WSLs that occur due to the reflection of flash from the tooth surface, lighting conditions, and wetness of the tooth.[34] Calculating the percentage area of WSLs from the overall tooth surface can also minimize errors.[24] The increase or decrease in the percentage values represented the progression or regression of WSLs respectively. Any type of assessment needs to be reproducible.[34] In the present study, the reliability and internal consistency showed excellent agreement in performing the morphometric analysis. These results were consistent with Hassan and Allam,[24] Livas et al.,[25] and Cochran et al.[35]

Children with ECC had highly porous enamel.[4] By understanding the enamel permeability of teeth, methods to create barriers that reduce their permeability can be devised. An *in vivo*, non-invasive, and non-toxic technique to determine the enamel permeability was used in the present study. Liquid discharge on the surface of enamel during impression-making will be displayed as droplets under SEM that indirectly signifies the enamel permeability.[15] Narrenthran et al. assessed these droplets using a modified visual grading scale.[4] A precise quantification might not be possible as this scale can either overestimate or underestimate the results. The area of individual droplets was quantified using Image J analysis software in the present study. A decrease in the percentage area of droplets was noted in both the study groups from baseline to six months, showing the reduction in enamel permeability. A greater decrease was noted in CR. This may be due to the penetrating ability of P_{11-4} into the subsurface carious lesions. It assembles higher aggregate peptides throughout the whole volume of the lesion and supports de novo hydroxyapatite formation.[36] It does not replace the use of fluoride; instead, it enhances the uptake of calcium and phosphate into the lesion which can improve remineralization. This might have boosted the results of CR. Cardosa et al. reported that EV efficacy was limited to surface enamel remineralization and has no effect on subsurface enamel remineralization. This may be because fluoride ions in EV inhibit the diffusion capacity of xylitol into the enamel, thereby affecting its remineralizing efficacy in subsurface lesions.[37]

Parameters that quantify an outcome can be subjected to measurement bias. Intra- and inter-rater reliability were checked to overcome such bias during ICDAS and morphometric assessment. There were certain limitations in this study. The absence of a control group and a 'no treatment' group to prove that the remineralization happened because of the intervention and not by chance could be a limitation. The presence of a well-established fluoridated or non-fluoridated control group could have helped to establish the effectiveness of newer materials. The 'no treatment' group was not considered in this study as it would be unethical to include children with WSL without any treatment. A randomized control trial with larger samples, higher power, and control groups is warranted.

Conclusions

Within the limitations of this study, the following conclusions can be made:

1. Curodont™ Repair and Embrace™ Varnish can remineralize white spot lesions in primary teeth.
2. The CR group showed a better reduction in the percentage area of WSLs over six months compared to the EV group.
3. CR could be considered an effective alternative to other remineralizing agents in the management of WSLs.

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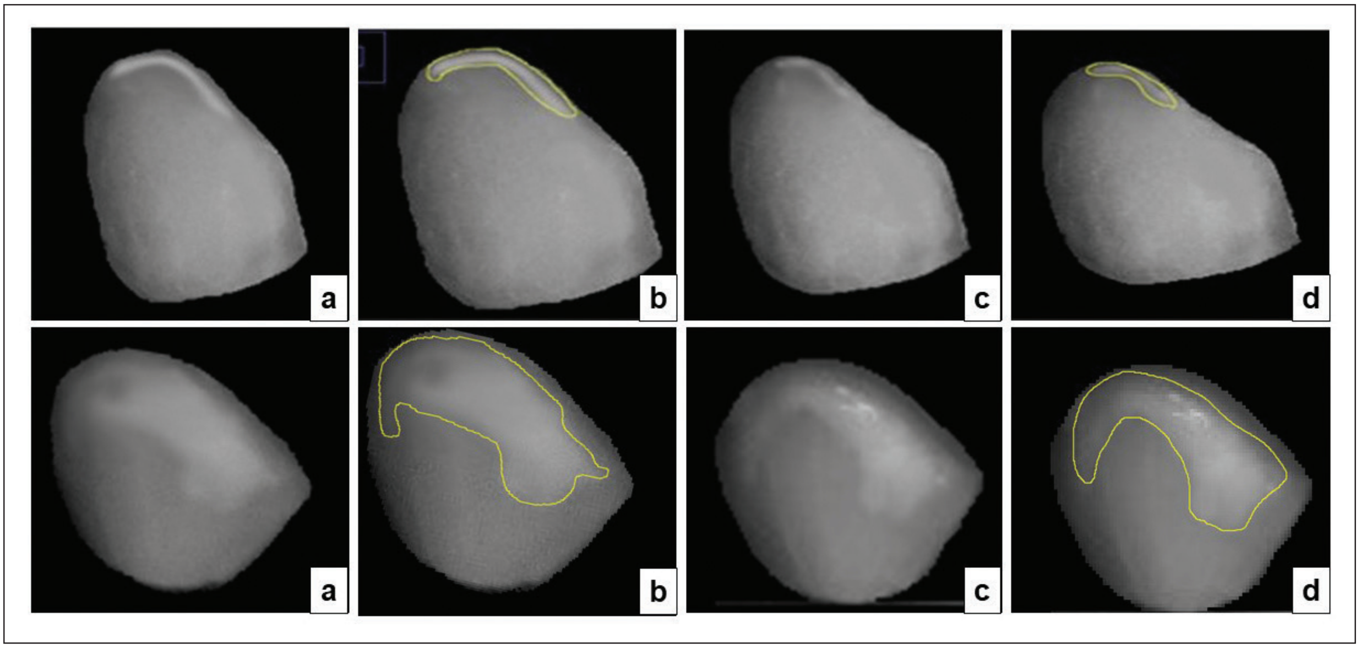
References

1. Bishara SE, Ostby AW. White spot lesions: Formation, prevention, and treatment. Semin Orthod 2008;14(3):174-82.
2. Anil S, Anand PS. Early childhood caries: Prevalence, risk factors, and prevention. Front Pediatr 2017;5:157.
3. Ganesh A, Muthu MS, Mohan A, Kirubakaran R. Prevalence of early childhood caries in India: A systematic review. Indian J Pediatr 2019;86(3):276-86.
4. Narrenthran JS, Muthu MS, Renugalakshmi A. In vivo scanning electron microscope assessment of enamel permeability in primary teeth with and without early childhood caries. Caries Res 2015;49(3):209-15.
5. Usha C, Sathyanarayanan R. Dental caries: A complete changeover (Part I). J Conserv Dent 2009;12(2):46-54.
6. Roopa KB, Pathak S, Poornima P, Neena IE. White spot lesions: A literature review. J Pediatr Dent 2015;3(1):1-7.
7. Gugnani N, Pandit IK, Srivastava N, Gupta M, Sharma M. International Caries Detection and Assessment System (ICDAS): A new concept. Int J Clin Pediatr Dent 2011;4(2):93-100.
8. Edelstein BL, Maiorini E, Casamassimo PS, Thikkurissy S. Beyond the dmft: The human and economic cost of early childhood caries. J Am Dent Assoc 2009;140(6):650-7.
9. Mickenautsch S, Yengopal V. Anticariogenic effect of xylitol versus fluoride: A quantitative systematic review of clinical trials. Int Dent J 2012;62(1):6-20.
10. Pulpdent® Corporation. Embrace™. Available at: "https://www.pulpdent.com/pulpdent-products/embrace-varnish/". Accessed March 27, 2023.
11. Milburn JL, Henrichs LE, Banfield RL, Stansell MJ, Vandewalle KS. Substantive fluoride release from a new fluoride varnish containing CXP™. Dentistry 2015;5(12):1-6.
12. Arifa MK, Ephraim R, Rajamani T. Recent advances in dental hard tissue remineralization: A review of literature. Int J Clin Pediatr Dent 2019;12(2):139-44.
13. Kirkham J, Firth A, Vernals D, et al. Self-assembling peptide scaffolds promote enamel remineralization. J Dent Res 2007;86(5):426-30.
14. Alkilzy M, Santamaria RM, Schmoeckel J, Splieth CH. Treatment of carious lesions using self-assembling peptides. Adv Dent Res 2018;29(1):42-7.
15. Lucchese A, Bertacci A, Chersoni S, Portelli M. Primary enamel permeability: A SEM evaluation in vivo. Eur J Paediatr Dent 2012;13(3):231-5.

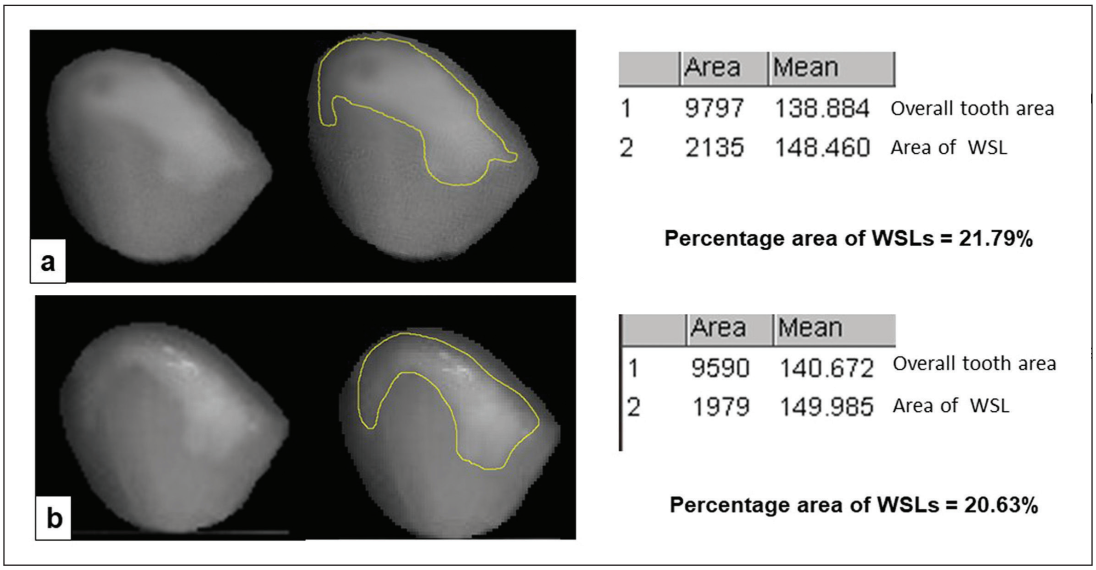
References continued on the next page.

16. Niviethitha S, Muthu MS, Kavitha S. In vitro assessment of enamel permeability in primary teeth with and without early childhood caries using laser scanning confocal microscope. J Clin Pediatr Dent 2016;40(3):215-20.
17. Chersoni S, Bertacci A, Pashley DH, Tay FR, Montebugnoli L, Prati C. In vivo effects of fluoride on enamel permeability. Clin Oral Investig 2011;15(4):443-9.
18. Schulz KF, Altman DG, Moher D, CONSORT Group. CONSORT 2010 statement: Updated guidelines for reporting parallel group randomized trials. Int J Surg 2011;9(8):672-7.
19. Alkilzy M, Tarabaih A, Santamaria RM, Splieth CH. Self-assembling peptide P11-4 and fluoride for regenerating enamel. J Dent Res 2017;97(2):148-54.
20. Peter S. Survey procedures in dentistry. In: Peter S, ed. Essentials of Public Health Dentistry (Community Dentistry). 6th ed. New Delhi, India: Arya (Medi) Publishing House; 2017:404-5.
21. Nyvad B, Machiulskiene V, Baelum V. Reliability of a new caries diagnostic system differentiating between active and inactive caries lesions. Caries Res 1999;33(4):252-60.
22. Ismail AI, Sohn W, Tellez M, et al. The International Caries Detection and Assessment System (ICDAS): An integrated system for measuring dental caries. Community Dent Oral Epidemiol 2007;35(3):170-8.
23. Kobeissi R, Badr SB, Osman E. Effectiveness of self-assembling peptide P_{11-4} compared to tricalcium phosphate fluoride varnish in remineralization of white spot lesions: A clinical randomized trial. Int J Clin Pediatr Dent 2020; 13(5):451-6.
24. Hassan TI, Allam GG. A standardized in vivo photographic technique to assess the remineralization of white spot lesions after orthodontic treatment. Egypt Dent J 2017;63(1):121-8.
25. Livas C, Kuijpers-Jagtman AM, Bronkhorst E, Derks A, Katsaros C. Quantification of white spot lesions around orthodontic brackets with image analysis. Angle Orthod 2008;78(4):585-90.
26. Christian B, Amezdroz E, Calache H, Gussy M, Sore R, Waters E. Examiner calibration in caries detection for populations and settings where in vivo calibration is not practical. Community Dent Health 2017;34(4):248-53.
27. Singh N, Gaur S, Kumar M, et al. Comparative study of dental health status and its determinants among children attending government and private schools in Kanpur City. Int J Clin Pediatr Dent 2021;14(5):666-73.
28. Henry JA, Muthu MS, Saikia A, Asaithambi B, Swaminathan K. Prevalence and pattern of early childhood caries in a rural south Indian population evaluated by ICDAS with suggestions for enhancement of ICDAS software tool. Int J Paediatr Dent 2017;27(3):191-200.
29. Sruthi MA, Gurunathan D, Ravindran V. Prevalence of white spot lesions in 3-year-old children visiting a private dental college: An observational study. World J Dent 2020;11(5):408-12.
30. Abirami S, Panchanadikar N, Muthu MS, et al. Effect of sustained interventions from infancy to toddlerhood in children with cleft lip and palate for preventing early childhood caries. Caries Res 2021;55(5):554-62.
31. Shahmoradi M, Hunter N, Swain M. Efficacy of fluoride varnishes with added calcium phosphate in the protection of the structural and mechanical properties of enamel. Biomed Res Int 2017;1(2):1-7.
32. Sidhu S, Thomas AM, Kundra R, Cherian JM. Assessment of fluoride release pattern from different fluoride varnishes: An in vitro study. J Pharm Res Int 2020;32 (33):104-15.
33. Iijima Y. Early detection of white spot lesions with digital camera and remineralization therapy. Aust Dent J 2008; 53(3):274-80.
34. Benson PE. Evaluation of white spot lesions on teeth with orthodontic brackets. Semin Orthod 2008;14(3): 200-8.
35. Cochran JA, Ketley CE, Sanches L, et al. A standardized photographic method for evaluating enamel opacities including fluorosis. Community Dent Oral Epidemiol 2004;32(1):19-27.
36. Kind L, Stevanovic S, Wuttig S, et al. Biomimetic remineralization of carious lesions by self-assembling peptide. J Dent Res 2017;96(7):790-7.
37. Cardosa C, Castilho A, Salomao P, Costa E, Magalhaes A, Buzalaf M. Effect of xylitol varnishes on remineralization of artificial enamel caries lesions in vitro. J Dent 2014; 42(11):1495-501.

Supplemental Electronic Data — Figures

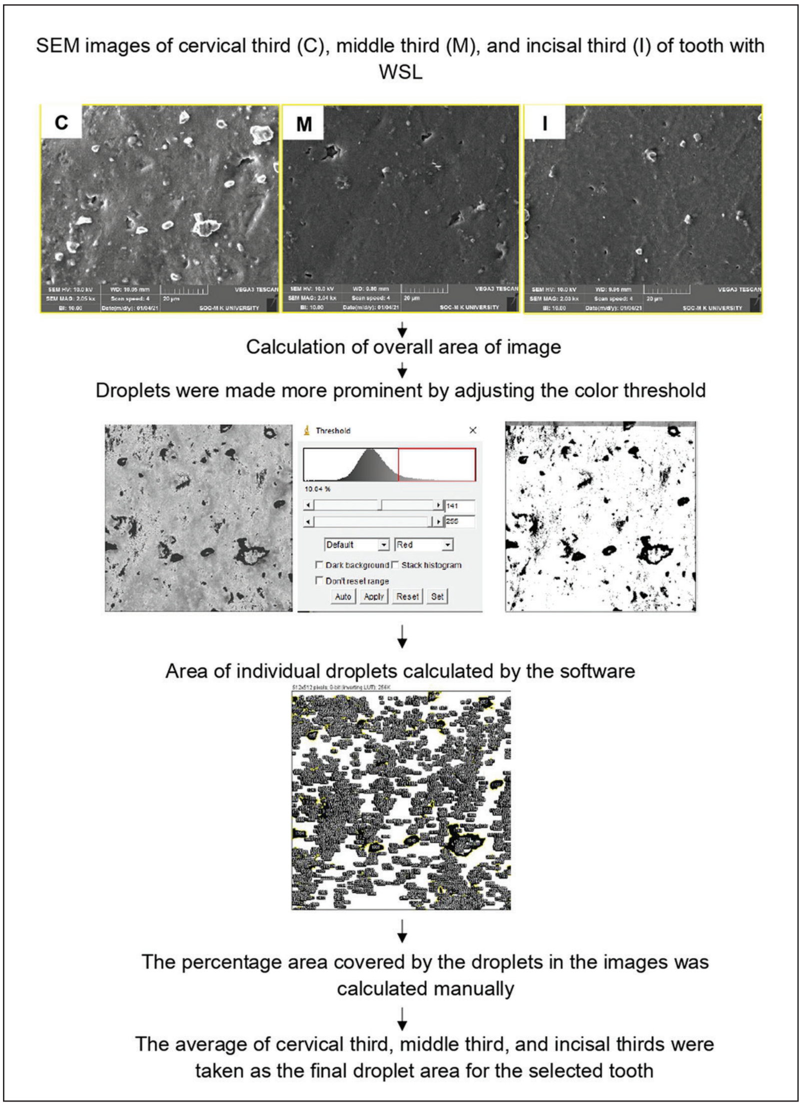


sFigure 1. Morphometric analysis of upper right lateral incisor and canine using Image J analysis software: (a-b) pre-operative white spot lesion in its image analysis; (c-d) post-operative white spot lesion and its image analysis.



sFigure 2. Morpho-metric analysis of upper right canine using Image J analysis software: (a) preoper-ative white spot lesion and its image analysis; (b) post-operative white spot lesion and its image analysis.

Supplemental figures continued on the next page.



sFigure 3. Stepwise procedure to assess the area of droplets with scanning electron microscopy images using Image J analysis software.