RESEARCH PAPER

Comparative Spectrophotometric Evaluation of Enamel After Treatment with Resin Infiltrate, Casein Phosphopeptide-Amorphous Calcium Phosphate, Bioactive Glass, Tricalcium Phosphate as A Remineralizing Agent in Human Permanent Teeth – An In-Vitro study

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ABSTRACT

Introduction

White Spot Lesions (WSLS) is defined as the early demineralization of the enamel at surface and subsurface, they are reversible and present on the smooth surfaces of teeth as a milky white opacity. This opacity is very unaesthetic and treated non-invasively using remineralizing/infiltration agents.

Aims and Objectives

The aim of the study is to evaluate the color change produced by treatment with Resin Infiltrate, Casein Phosphopeptide - Amorphous Calcium Phosphate, Bio-Active Glass and Tricalcium Phosphate on artificial caries like lesions on human enamel.

Methodology

A total number of 160 undamaged maxillary central incisors with whole apices were included in the study, which were placed in 0.1% thymol solution till the time to perform the study. A square piece of modelling wax of dimension 4 mm x 4 mm, was used to create the artificial white spot lesion, using a demineralizing solution. The samples were divided into four groups of 40 specimens each, depending upon method used to treat the samples: Group I: ICON, Group II: CPP-ACP, Group III: NovaMin, Group IV: Functionalized Tricalcium Phosphate. The specimens in all groups after treatment were stored in artificial saliva. Color measurements using a spectrophotometer were recorded at baseline, 4th and 8th weeks after treatment by using spectrophotometer.

Results

The statistical analysis was performed using the one-way Anova test. Post hoc comparison was used to compare the significant data differences between the groups. Among the four groups tested, resin infiltration had the most significant color change (ΔE) between demineralized lesion and after treatment at 4th week that were infiltrated as compared to other groups. ($P < 0.05$).

Conclusion

Within limitation of our study, the results of the current investigation together with the aforementioned literature showed that ICON resin infiltration was the better remineralizing/infiltration agents in comparison to CPP-ACP, functionalized tricalcium phosphate and NovaMin based dentifrices.

Clinical Significance

Resin infiltration as a remineralizing/infiltration agent, brings about a fast change in the color of white spot lesions, giving a satisfactory result to both clinician and patient. Treatment with ICON® is also long lasting and causes structural changes in enamel even in the subsurface lesion of white spot lesions, thus improving structural integrity of enamel over lesion area.

Keywords: ICON® resin infiltration, functionalized tricalcium phosphate, Caseinphosphopeptide-amorphouscalciumphosphate, bioactive glass, white spot lesions.

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INTRODUCTION:

Since the advent of fire and cooked food, man has been plagued by the disease known as Dental Caries. This term Dental Caries, also known as Tooth Decay in layman terms, is a latin word which basically means "to rot" or "to decay".[1] Dental Caries is defined as the reversible microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth, which often leads to cavitation.[1] In the early 1980s, there was a decline in the rates of coronal caries after the introduction of fluorides in drinking water and toothpastes.[2] Carious process is usually described as a dynamic sequence of biofilm-tooth interaction that can occur over time on and within the tooth surface.[3]

White Spot Lesions (WSLS) is defined as the early demineralization of the enamel at surface and subsurface, they are reversible and present on the smooth surfaces of teeth as a milky white opacity. Implementation of the philosophy of minimal intervention dentistry, keeping in mind the inherent possibility of remineralization, is better illustrated by the approach to managingincipient carious lesions.[4] Remineralizing agents such as, Casein phosphopeptide-amorphous calcium phosphate, NovaMin (Calcium sodium Phosphosilicate), ICON® resin infiltration and functional tricalcium phosphate (fTCP) come in the form of creams, pastes and topical remineralization treatments.[5] Spectrophotometers provide a quantitative measurement of the parameter to measure color. Thus, this in-vitro study was designed to evaluate the color change before and after treatment of human teeth with resin infiltrate, CPP-ACP, bio-active glass and tricalcium phosphate using a spectrophotometer.

METHODOLOGY:

A total number of 160 undamaged maxillary central incisors with whole apices were included in the study.

Formation of white spot lesions (demineralization procedure)

The collected teeth, 160 in number, were placed in 0.1% thymol solution till the time to perform the study. The teeth samples were then removed from the solution on the day of the study, washed and dried for use. A square piece of modelling wax of dimension 4 mm x 4 mm, was placed on the tooth structure and the remaining area of crown was coated with acid resistant varnish. A solution to demineralize the teeth samples were prepared after which all samples was immersed in this solution for 4 days at 37 °C to create artificial WSL's. After the creation of artificial white spot lesion, the nail varnish was removed using varnish remover.

Color measurements using a spectrophotometer were recorded before (Baseline) and after formation of artificial WSL's. It is stored in artificial saliva after the treatment period is over.

Preparation of artificial saliva

A solution of artificial saliva was prepared using the formulation of which was used for storage of specimens throughout the study.

The pH will be maintained at 7.4 to 7.8.

Grouping and treatment interventions

The 160 samples were divided into four groups of 40 specimens each, depending upon method used to treat the samples:

- Group I: ICON smooth surface caries Resin Infiltrate (Icon, DMG, Hamburg, Germany). Resin infiltration procedures were carried out as per the instructions provided by the manufacturer. Samples are kept in artificial saliva for 8 weeks.
- Group II: Casein Phospho Peptide-Amorphous Calcium Phosphate (CPP-ACP), applied as slurry for 7 days without any mechanical agitation. The solution is changed every 24 hours after treatment.
- Group III: NovaMin – NovaMin, applied as slurry for 7 days without any mechanical agitation. The solution is changed every 24 hours after treatment.
- Group IV: Tricalcium Phosphate, applied as slurry for 7 days without any mechanical agitation. The solution is changed every 24 hours after treatment.

The specimens in all groups after treatment were stored in artificial saliva. Color measurements using a spectrophotometer were recorded at 4 and 8 weeks after remineralization by using spectrophotometer.

Spectrophtometric evaluation of color change

Color measurement was done by Spectrophotometer (CHN Spec, CS-600, Wavelength Range: 400-700nm, China), at baseline, WSL formation and after treatment (4th

week and 8th week). The specific location on the specimens, having underlying dentin (middle to cervical area) was placed near the light source. The tooth was placed flush and perpendicular to the light source. With the WSLs facing down towards the light source and kept in contact with the machine, while taking measurement, until the measurement was taken the specimen was kept steady and in place.

Optical results were analyzed and calculated. Color change values were calculated by the CIE L*a*b* software using the following formula: ΔE=[(ΔL*)2+(Δa*)2+(Δb*)2]1/2 Where, ΔE represents the color change; L* represents the lightness scale; a* = green-red axis; b* = blue-yellow axis. All measurements of spectrophotometer were performed under standardized ambient conditions to ensure accuracy and reproducibility.

Statistical analysis

SPSS version 21.0. (Amonk, IBM Corp., NY) was used for statistical analysis. Descriptive statistics were computed with mean and standard deviation and one-way analysis of variance. Post hoc analysis with the Tukeys honest significant difference test was used to compare the data between the groups. Statistical significance was set at $P < 0.05$.

RESULTS

The descriptive table (ANOVA Test) suggests that the Mean of the treatment 1 (4th Week) to Baseline has a significant change in color in groups I, II, III, and IV. This shows that there is a significant change in color from Baseline (Control) to Treatment 1 (4 weeks after Treatment). The color change produced by treatment with Resin Infiltrate, Casein Phosphopeptide - Amorphous Calcium Phosphate, Bio-Active Glass and Functionalized Tricalcium Phosphate on artificial caries like lesion was compared using one-way Anova test (Table 1).

The ΔE for treatment 1 (4th week) - baseline, treatment 2 (8th week) – demineralized lesion, and treatment 1 (4th week) – treatment 2 (8th week) was found to be statistically significant (Table 2). On post hoc comparison, significant differences were observed between group I and group II, group I and group III, and group I and group IV only (Table 3).

The mean differences in color change between Groups I to IV in between Treatment 1 (4th week) and Baseline suggest significant changes in color in the following order (Table 2). There is the least mean color change between Treatment (4th week) to Baseline for Group I followed by Group II, Group IV and Group II respectively. This clearly indicates a better performance from Group I among other Groups (Graph 1).

The mean differences in color change between Groups I to IV in between Treatment 2 (8th week) and Baseline suggest no statistically significant changes in color. Although there was no statistically significant difference between Treatment 2 (8th week) and Baseline. There was a statistically significant change observed for the Treatment 1 (4th week and Baseline). The values regarding Treatment 1 (4th week) to Baseline comparison shows the following order of least mean color difference to highest mean color difference (Graph 2).

TABLES

Table 1: Comparison of color change produced by treatment with Resin Infiltrate, Casein Phosphopeptide - Amorphous Calcium Phosphate, Bio-Active Glass and Tricalcium Phosphate on artificial caries like lesions:

		Sum of Squares	df	Mean Square	F	Sig.
TREATMENT 1(4th WEEK)-BASELINE	Between Groups	189.268	3	63.089	7.038	.0001*, sig
	Within Groups	1398.354	1568.964			
	Total	1587.622	159			

Table 2: Descriptives of color change produced by treatment with Resin Infiltrate, Casein Phosphopeptide - Amorphous Calcium Phosphate, Bio-Active Glass and Tricalcium Phosphate on artificial caries like lesions:

ΔE		r	Mean	Std. Deviation	Std. Error
TREATMENT 1 (AT 4TH WEEK)-BASELINE	GROUP I	4 (	3.5847	1.7631	0.2788
	GROUP II	4 (	6.0469	2.5797	0.4079
	GROUP III	4 (	6.1733	3.9829	0.6298
	GROUP IV	4 (	6.0617	3.1981	0.5057

Table 3: Post HOC Comparison for Treatment 4th Week - Baseline Color Change (Tukey HSD):

		Mean Difference (I-J)	Std. Error	Sig.
GROUP I ICON	GROUP II	2.462*	0.669	0.002, sig
	GROUP III	2.588*	0.669	0.001, sig
	GROUP IV	2.476*	0.669	0.002, sig

FIGURES

Graph 1: Comparison of 4th week treatment to Baseline:

There is the least mean color change between Treatment (4 weeks) to Baseline for Group I followed by Group II, Group IV and Group II respectively. This clearly indicates a better performance from Group I among other Groups.

Graph 2: Comparison of 4th week treatment to 8th week treatment:

The above graph depicts no statistically significant difference between groups II and III, when comparing mean color difference between Treatment 1 (at 4 weeks) and Treatment 2 (at 8 weeks). Similarly group I and IV show no statistically significant difference.

DISCUSSION

This study is to investigate in vitro the capability of remineralizing agents to restore the optical properties of human enamel having white spot lesions to its most natural color of enamel. Dental caries is the most common and curable disease facing the human race. If an incipient lesion occurs on the surface of enamel, the demineralized lesion will have an outer surface and an inner body of the lesion. It is in this body of the lesion, where porosities occur due to the demineralization, in these porosities pockets of air (RI = 1.00) and water (RI = 1.33), having refractive index lower than that of sound healthy enamel (RI = 1.62), this causes refraction and splitting of light when it enters into these lesions; giving the desiccated lesion a white chalky appearance.[6] Although it is also found that the remineralization achieved by fluorides is superficial. This being a critical disadvantage, as the inner part of the lesion is most susceptible to demineralization due the gradients in enamel solubility.[7]

To remineralize enamel subsurface lesions, the best treatment that can bring about a masking of the white spot lesion, which is determined by measuring the color using the CIE L*a*b* parameters, by using a spectrophotometer. More is the remineralizing potential, better will be the replenishment of mineral content, better is the masking effect of remineralizing agents on WSL.

ICON Resin Infiltrant (DMG, Germany) is a resin infiltrant, unlike remineralizing agents it is an Infiltration Concept (ICON®), it is a relatively new resin product developed in Germany and used in the treatment of incipient lesions.[8] The commercial ICON® kit contains 15% hydrochloric acid as etchant, ethanol for drying and the infiltrate resin material.[9] The ICON® (infiltration concept) were developed using a microinvasive technique. ICON® is an abbreviation for Infiltration Concept, introduced into the field of dentistry in 2008 for treating white spot lesions.

In this study the color change (colorimetric distance = ΔE) of four novel remineralizing / infiltration agents based on four different mechanisms of action has been used. GC Tooth Mousse™ , based on recaldent technology, cheese and milk consumption can cause remineralization and as a result this was developed.[10] Trypsin digested milk protein complexed with calcium and inorganic phosphate ions produced CPP-ACP.[11] CPP-ACFP otherwise known by the trade name GC Tooth mousse Plus contains roughly 900 ppm of fluoride in the form of sodium fluoride (2.2% w/w). Although GC Tooth mousse (CPP-ACP) does not have any fluoride.[12]

NovaMin® is the trade name given to calcium sodium phosphosilicate (CSP) NovaMin (BAG) that has been developed for use in oral health care.[13] The most important feature of BAG is the ability to act as a biomimetic mineralizer which matches the body's own mineralizing traits.[14] The NovaMin toothpaste used in this study has 1450 ppm Fluoride content. FTCP tooth crème (3M ESPE) is another calcium phosphate-based compound that is known chemically as functionalised tricalcium phosphate (fTCP), commercially developed as a toothpaste (FTCP Tooth Crème; 3M ESPE, USA). The FTCP Tooth Crème contains 950 ppm of Fluoride.[15]

The spectrophotometer is a very accurate device for measuring color. In this study color difference were measured using a digital spectrophotometer. The CIE system has been used (CIE L*a*b). This in-vitro study was done to evaluate the color change capability of remineralizing agents/infiltration agent, to blend color of white spot lesions to surrounding healthy sound enamel, using a spectrophotometer.

From the present study, we concluded that the minimum mean difference in color change from baseline to Treatment 1 (at 4 weeks) was seen with Group I (ICON Resin Infiltration) followed by Group II (CPP-ACP based Toothpaste, GC Tooth Mousse™) followed by Group IV (Functionalized Tricalcium Based Toothpaste, 3M ESPE FTCP®) followed by Group III (NovaMin based toothpaste), which displayed the least color change.

Although the use of remineralizing agents to mask color change is a slow process and cannot be completed in 7 days of application. The composition of remineralizing agents may have a significant role in the amount of lesion depth penetrated during treatment, as the surface treatment of white spot lesions is easier compared to the sub-surface lesion underneath.

CPPACP based remineralizing agents have a low penetration depth compared to other methods used in this study, a study conducted by Lata S et al.[16] states that CPPACP is less effective against sub-surface lesions. NovaMin is readily available to provide calcium and phosphate ions for remineralization, although the remineralization potential is not directly related to its ability to mask optical discrepancies in the sub-surface lesions. NovaMin mechanism of action is also a very pH dependent process that cannot be regulated by artificial saliva, which are the conditions of the in-vitro study here.[11]

In contrast, The Casein phosphopeptide-amorphouscalciumphosphate is an acting vehicle for calcium and phosphate localizing in plaque and the salivary pellicle.CPP-ACP acts as a reservoir to maintain saturation levels of minerals, especially calcium phosphate. This has been shown in studies conducted by Job TV et al. (2018)[17].

f-TCP although has shown to be very effective in in-vivo conditions, due to fact that in in-vitro conditions, the pH of the solution cannot be regulated by natural saliva and artificial saliva is not able to provide buffering action. Varma V et al (2019)[18] conducted a study to compare the remineralization efficacy of CPP-ACP and fTCP. It was concluded from the study that CPP-ACP was able to perform better than fTCP as a remineralizing agent. This result was contradicted by a study performed by Sreekumar P et al. (2019)[19].

Artificial saliva was found to cause adequate remineralization, which may have inadvertently caused the modification of optical properties. This resulted in a statistically non-significant decrease in ΔE values when comparing Treatment 2 (8th week) to baseline

Refractive Index (RI) of resin infiltrate is 1.46 which converts the RI of WSLs (RI = 1.33 to 1.00) close to that of sound healthy enamel (RI = 1.62). After infiltration the refractive index is raised to a RI = 1.52. Thereby better masking capability is achieved using ICON.[20] KL Prasada et al. (2018)[6] conducted a study to evaluate spectrophotometrically the efficacy of ICON resin infiltrate as a treatment modality. The study concluded that ICON is an efficacious treatment modality for subsurface lesions

The novel Resin infiltration method does have a

better penetration depth as it employs pretreatment with Hydrofluoric acid as an etchant, that allows the resin to infiltrate deeper into the subsurface lesion to depth of maximum 100 µm.[21] ICON resin infiltration always shows immediate improvement right after treatment, but as this modifies the crystalline structure of the enamel lesions, the effectiveness of the treatment does not depreciate even after 8 weeks after treatment. As demonstrated in studies like Giudice RL et al (2020)[22] who showed that there was no decrease in the effect of resin infiltration even after a 1-year follow-up and Borges AB et al (2014)[23] who showed in his study that resin infiltrants maintained color stability especially after polishing (after treatment) and repolishing (after 8 days of treatment). It has been shown that resin infiltration can improve the structural stability of porous sub-surface lesions, thus improving its ability to prevent cavitation. It makes use of both preventive and restorative actions in the treatment of noncavitated carious lesions It is a promising therapeutic technique. The penetration of the low-viscosity resin (i.e., an infiltrant) into the lesion depth is driven by capillary forces which occurs after the removal of the hypermineralized surface layer.[24] This shows that ICON resin infiltration is an efficient treatment for WSLs clinically, as there effect is immediate and long lasting.

CONCLUSION

Dental caries is not a straightforward process of demineralization of enamel and dentin, but a complex yet sophisticated process of demineralization and consequent remineralization changing refractive index of sound enamel to a lower number due to the presence of porosities in the subsurface lesions that may contain water or air. Resin infiltration can improve the color of WSLs and restore natural appearance of enamel, being stable for relatively long periods; however, long-term evaluation of its stability in clinical practice is needed.

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