ASSESSMENT OF TREATMENT OF POST- ORTHODONTIC WHITE SPOT LESION WITH MICROABRASION AND LASER USING SPECTROPHOTOMETER: AN INVITRO STUDY

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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation titled **"ASSESSMENT OF TREATMENT OF POST- ORTHODONTIC WHITE SPOT LESION WITH MICROABRASION AND LASER USING SPECTROPHOTOMETER: AN INVITRO STUDY"** is a bonafide work done **DR.K.PREETHI,** Postgraduate student, during the course of the study for the degree of **MASTER OF DENTAL SURGERY** in the speciality of **DEPARTMENT OF ORTHODONTICS AND DENTOFACIAL ORTHOPEDICS,** Vivekanandha Dental College for Women, Tiruchengode, during the period of 2015-2018.

Date: 18. 1. 2018.

Place: Elayampalayam

S. Nagalakshmi

Dept of Orthodontics,
Vivekanandha Dental College of Women,
Elayampalayam-637 205.
Tiruchengode Tk. Namakkal Dt.

Signature of Guide & H.O.D

Prof.**Dr. S. Nagalakshmi, MDS**

Department of Orthodontics and
Dentofacial Orthopedics,
Vivekanandha Dental College
for Women.

ENDORSEMENT BY THE HEAD OF THE DEPARTMENT AND HEAD OF THE INSTITUTION

This is to certify that **DR.K.PREETHI,** Post Graduate student (2015-2018) in the **DEPARTMENT OF ORTHODONTICS AND DENTOFACIAL ORTHOPEDICS,** Vivekanandha Dental College for Women, has done this dissertation titled **"ASSESSMENT OF TREATMENT OF POST- ORTHODONTIC WHITE SPOT LESION WITH MICROABRASION AND LASER USING SPECTROPHOTOMETER: AN INVITRO STUDY"** under our guidance and supervision in partial fulfillment of the regulations laid down by the Tamilnadu Dr.M.G.R.Medical University, Chennai-600032 for M.D.S **BRANCH- V.**

S. Nagalakshmi

Dept. of Orthodontics,
Vivekanandha Dental College of Women,
Elayampalayam-637 205,
Tiruchengode Tk. Namakkal Dt.

Seal & Signature of H.O.D

Prof. **Dr.S. Nagalakshmi, MDS,**
Department of Orthodontics and
Dentofacial Orthopedics.
Vivekanandha Dental College for Women

N. Balan

PRINCIPAL
VIVEKANANDHA
DENTAL COLLEGE FOR WOMEN
Elayampalayam - 637205.
Tiruchengode-Tk. NamakkalDt. TamilNadu

Seal & Signature of Principal

Prof. **Dr. N.Balan, MDS**
Principal,
Vivekanandha Dental College for Women

DECLARATION

TITLE OF DISSERTATION	Assessment of treatment of post- orthodontic white spot lesion with microabrasion and laser using spectrophotometer: An invitro study.
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NAME OF THE GUIDE	Dr. S Nagalakshmi, MDS
HEAD OF THE DEPARTMENT	Dr.S Nagalakshmi., MDS

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S. Nagalakshmi — Head of the Department

S. Nagalakshmi — Guide

K. Preethi — Signature of the Candidate

Dept. of Orthodontics,
Vivekanandha Dental College of Women,
Elayampalayam-637 205,
Tiruchengode Tk. Namakkal Dt.

PLAGARISM CERTIFICATE

This is to certify that this dissertation work titled **"ASSESSMENT OF TREATMENT OF POST- ORTHODONTIC WHITE SPOT LESION WITH MICROABRASION AND LASER USING SPECTROPHOTOMETER: AN INVITRO STUDY"** of the candidate **DR. K.PREETHI,** with registration Number **241519502** for the award of degree **MASTER OF DENTAL SURGERY** in the branch of **ORTHODONTICS AND DENTOFACIAL ORTHOPEDICS**. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **0%** of plagiarism in the dissertation.

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Dr.K.Preethi

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LIST OF ABBREVATIONS

- WSL White Spot Lesion.
- CPP-ACP Caesin phosphopeptide amorphorous calcium phosphate.
- HCl Hydrochloric acid.
- H_2O_2 Hydrogen peroxide.
- CO_2 Laser Carbondioxide Laser.
- Nd:YAG Neodymium-doped yttrium aluminium garnet
- Er: YAG Erbium -doped yttrium, aluminium, garnett
- SEM Scanning electron microscope.
- TCP Tricalcium phosphate
- S.Mutans Streptococcus mutans.
- LB Lactobacillus.
- GIC Glass ionomer cement
- CIE Commission Internationale de l'Eclairage
- NaF Sodium fluoride.
- APF Acidulated phosphate fluoride
- L* Lightness of sample
- a* Red/green chromaticity
- b* Blue/yellow chromaticity
- T0 Baseline – prior to bonding

- T1 Pretreatment White Spot Lesion
- T2 Post microabrasion / laser treatment
- T3 After 30 days storage in artificial saliva.
- T4 After discolouration cycle
- ΔE1 Colour change between white spot lesion (T1) and post microabrasion / laser treatment (T2).
- ΔE2 Colour change between posttreatment T2 and after 30 days storage in artificial saliva (T3)
- ΔE3 Colour change after 30 days storage in artificial saliva T3 and after discolouration cycle (T4)

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INTRODUCTION

INTRODUCTION

Maintenance of good oral hygiene is mandatory during orthodontic treatment. Fixed appliances have various attachments which are prone to plaque accumulation around it.[1-3] They cause demineralization around the brackets which appear white and are known as white spot lesion. The plaque accumulations can be prevented by regular brushing, use of interdental brushes, mouth washes, proper removal of flash around the brackets etc.

White spot lesion (WSL) defined as "subsurface enamel porosity from carious demineralization" that presents itself as "a milky white opacity when located on smooth surfaces." [4,5]The term White Spot Lesion was defined as "the first sign of caries like lesion on enamel that can be detected with the naked eye." [6] White spot lesions (WSLs) are one of the most common unesthetic sequelae from fixed appliances, which is challenging to a clinician in terms of management.

White spot lesions can become noticeable around the brackets within 1 month of the bracket placement within the time frame between subsequent orthodontic appointments, although the formation of regular caries takes usually at least 6 months[7,8]. These lesions are commonly seen on the buccal surfaces of teeth around the brackets, especially in the gingival one third. Prevalence of white spot lesions among orthodontically treated patients varies widely from 2 to 96%[9].

Various preventive measures are followed to prevent the development of white spot lesion. Some of them are educating and insisting them regarding good oral hygiene

maintenance[10], application of fluoride varnish/CPP-ACP varnish[11], bonding brackets with resin modified GIC, fluoride releasing composites, fluoride rinse[12] and fluoride/CPP- ACP tooth paste[13]. Oral prophylaxis is done every 3-6months in poor compliance patients has proven to be effective.

During active treatment, there is a rapid shift in the bacterial flora of plaque with notable increase in Streptococcus mutans and Lactobacilli count.[14] These bacteria produce acid and the plaque layer act as barrier in preventing the diffusion of acid and also the limit the potential remineralization from the available exogenous calcium and phosphate ions in the saliva which results in "White Spot Lesion"[15].

White spot lesions are surface lesions and they remineralize much faster than the sub-surface ones.[16] These lesions appear whitish as light refraction of light, because the enamel is thin because of mineral loss, under light refraction the whitish appearance is directly proportional to the level of mineralization and as these lesion remineralize the opacity diminishes, it reverts back to its original colour. WSL are more susceptible to chromogens and get easily discoloured.

Willmot[17] reported that there was a significant decrease in the size of enamel surface lesions post orthodontic treatment, by the action of patient's own saliva to naturally remineralize the lesion over time, resulting in a greater repair with a less visible and more aesthetically pleasing appearance.

There is no recommended gold standard approach for treating these white spot lesion. Depending on degree and activity level of lesion these post orthodontically

developed white spot lesion were treated. For mild lesion application of Fluoride/CPP-ACP varnish[17] or prescribing CPP-ACP/Fluoride toothpaste[18] is done. Other conservative approach for treating these WSL include microabrasion,[19] bleaching,[19] and lasers irradiation and a minimally invasive approach is done by caries infiltrant.[20]

Microabrasion is well known advocated approach which eliminate small lesion and also eliminate severity of large lesion. Ardu et al[21] proved microabrasion as an effective method for colour elimination of white enamel lesions. Murphy et al[22]., reported the mean reduction in lesion size was 83% after microabrasion. On treating the entire buccal surfaces of the teeth by microabrasion, these white spot lesions become less noticeable and provide the smooth glossy enamel surface. Various microabrasion techniques like combination of acids (HCl, H2O2 and ether), hydrochloric acid with pumice slurry, hydrochloric acid with silicon carbide, phosphoric acid with pumice. The discoloured enamel was removed mechanically and it results in smooth and glossy enamel appearance and improved enamel colour.

Laser treated enamel surface has shown resistance to enamel demineralization. Various types of lasers used some of them are CO_2 laser, Argon laser, Er:YSGG laser, Er:YAG, Nd:YAG etc., Myer et al[23] used pulsed Nd:YAG laser in treated these incipient lesion, reported that laser removed the extrinsic stains and surface appear similar to the adjacent enamel surface. Yamamoto and Oaya[24] on SEM evaluation reported that the surface of enamel was smooth after irradiation and showed resistance to formation dental caries. Kim et al[25] studied effect of etching the enamel surface with

Er:YAG laser and reported that enamel irradiated with laser had resistance to demineralization.

Laser is absorbed by the water content present in the enamel, water on absorbing the strong lased energy boils abruptly leads to evaporation and micro- explosions. This ablation process occurs when the pressure exceeds the ultimate strength of the tooth. During laser ablation, there appears a successive recoil force creating craters on the surface and the irradiated surface becomes a flaky structure with an irregularly serrated and micro-fissured morphology. The temperature rise causes enamel to induce protein decomposition and formation of β-tricalcium phosphate (β-TCP) and α tricalcium phosphate (α -TCP) with the loss of carbonate. The decomposed protein and other organic matrix products melt and swell and probably lead to a blockage of the interprismatic and intraprismatic spaces, which act as ion diffusion channels, and eventually result in the decrease of calcium loss. These microfissures and microspaces in the laser-ablated region trap the free ions essential for remineralization. Hence, the laser ablation effectively induces acid resistance in enamel, by crystalline improvement and the blocking effect of the organic matrix.

Although the microabrasion and laser treatment are used to treat the incipient enamel lesion, the colour improvement using laser therapy has not yet assessed. There is no investigation regarding the colour stability from microabrasion treated and laser treated enamel when prone to discolouration. This study was to assess the colour improvement of Post orthodontic white spot lesion with microabrasion and laser and to assess the treated enamel resistance to discolouration using spectrophotometer.

AIM AND OBJECTIVES

AIM AND OBJECTIVES

Aim:

The aim of the study was to assess the colour improvement of post orthodontic white spot lesion and the colour stability of treated enamel to discolouration.

Objectives:

1) To compare the colour masking effect of microabrasion (orthophosphoric acid with pumice) and laser (Er: YAG) on post orthodontic white spot lesion using spectrophotometer and

2) To compare resistance of microabrasion and laser treated enamel surface of white spot lesion to discolouration (tea and coffee solution) using spectrophotometer

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Review of literature is presented under the following categories

1) Prevalence and factors influencing post orthodontic white spot lesion
2) Prevention and management of post-orthodontic white spot lesion
3) Microabrasion technique
4) Laser technique.

1)Prevalence and factors influencing post-orthodontic white spot lesion

Zachrisson B U[26]. (1977) evaluated the posttreatment effects of direct bonding on 46 children of 11-14 years of age. The teeth were bonded with chemically polymerized direct bonding system. A long-term evaluation of 705 bonded attachment were done and he stated that direct bonding system consumed significantly lesser time to work with and also improved gingival condition the disadvantage of direct bonding system was found to be frequent debonding of brackets and significant development of white spot lesion.

Gorelick L, Geiger A M, Gwinnet J[1]. (1982) conducted a survey on full term orthodontic treatment among patients to evaluate the incidence and severity of white spot lesion post-orthodontically by visual examination and photographic assessment. They reported that 50% patient experienced white spot lesion and maxillary lateral teeth are more commonly affected. The risk factors for

development of white spot lesion included the access of salivary flow and distance from bracket to free gingival margin, treatment duration.

Rosenbloom R, Tinanoff N.[27] (1991) studied the streptococcus mutans levels in patient before, during and after orthodontic treatment in saliva. The streptococcal mutans count was determined by culturing these bacteria in selective medium which consist of Mitis salivarius agar, 0.2U/ml bacitracin and 20% sucrose and incubated in CO2 rich atmosphere for 24 and 48 hours repectively. The results showed elevated levels of streptococcus mutans during active orthodontic treatment and the there was no significant change in S.mutans level at pretreatment and after debonding of appliance.

Shungin D, Olsson A I, Persson M (2010)[28] conducted a invivo study to quantitatively evaluate the changes in white spot during the orthodontic treatment and 12-year follow up incidence, formation and regression of white spot using photographs and quantitatively assessed by software. This prospective study included 59 orthodontically treated patients and photographs were taken before treatment and at debond, 1, 2 & 12 years after debond. In this split mouth study the teeth were bonded with composite and GIC bonded teeth showed evidently less formation of white spot lesion than acrylic material.the 12 year follow up showed significant reduction of WSL, which was more reduced in GIC than the acrylic bonding material . The results showed that GIC bonded teeth showed evidently less formation of white spot lesion than acrylic bonding material.

Al Maaitah E F., [29](2011) did a randomized control trial to determine the predictors for presence of white spot lesion and the degree of enamel demineralization the degree of enamel demineralization. 230 patients were assessed at debond for its presence, number of lesion, teeth that were affected and degree of demineralization using quantitative light induced fluorescence. They concluded that for the formation and degree of severity of white spot lesion, oral hygiene, pretreatment age, sex and clinical status of the first molar can be used as predictors.

Hess E, Campbell P M, Honeyman A L, Buschang P H[30]., (2011) determined the effects of acid etching, metal brackets, sealants and resin adhesive on enamel decalcification in a simulated oral environment. The results showed that acid etching and resin adhesives cause evident decalcification whereas, brackets do not and sealants protect them

Richter A E, Arida A O, Peters M C, Sohn W[31]. (2011) assessed the relationship of incidence of labial incipient caries with various treatment variables and patients with pretreatment and posttreatment photographs of labial surface of teeth. They concluded that incidence of new white spot lesion was 72.9% and new cavitated lesion was 2.9% lesion and the development was not associated with sex, age but they are related to improper oral hygiene and treatment duration

Olsen S H, Sandvik K, El-AlgroudiM A, Ogaard B[32] (2012) conducted study on 80 Norwegian patients, 40 in orthodontic group and 40 matched control.

40 debonded patients were instructed for following caries prophylactic regimen, which included good knowledge regarding oral hygiene maintenance, with special orthodontic toothbrush, interdental brushes, plaque disclosing tablets, fluoride toothpaste and mouth rinse. The control group not given any special prophylaxis. They stated that incidence of white spot lesion was more on orthodontic treated group. They reported that patient with good compliance had less occurrence of new WSL than that of moderate and poor compliance patient. Even after implementing comprehensive oral hygiene regimen with special prophylactic methods development of white spot lesion cannot be prevented completely

2) Prevention and management of postorthodontic white spot lesion

Gorton J and Featherstone J D B,[7] (2003) conducted an in-vivo study to assess the inhibition of enamel demineralization when bonding the brackets using fluoride releasing GIC and composite with no fluoride release. 21 patients who require premolar extractions were bonded consecutively with GIC and composite. The teeth were extracted after 4 weeks and subjected to microhardness around brackets. He concluded that the teeth bonded using GIC showed high microhardness implying presence of cariostatic effect in GIC, but there was no significant increase in salivary fluoride level.

Pascotto RC, Navarro M F L, Filho L C, Cury J A[33] (2004) carried out a in-vivo study to evaluate the effect of enamel demineralization around brackets when bonded with resin -modified glass ionomer cement and conventional composite (concise).14 patients who required therapeutic extraction of premolar

were enrolled and divided into 2 groups. After 30 days the premolar teeth were extracted, the extracted tooth was examined by cross sectional surface hardness. They reported reported that RMGIC bonded teeth showed decreased caries development.

Wilmot D R [17]., (2004) evaluated the regression of postorthodontic white spot lesion when treated with fluoridated toothpaste & rinse regimen and nonfluoridated toothpaste. These lesions were examined using polarized light and image analysis was done at the time of de-bond, 12 month and 24 month after debond. The results showed no significance between the groups, but there was a reduction in white spot lesion size to one-third in 12 weeks and half the size in 24 weeks

Brochner A et al.[34], (2010) investigated the effect of topical application of 10% CPP-ACP cream on white spot lesion. 60 patients who developed postorthodontic white spot lesion with visible grading ≥ 1 were studied. They were divided into 2 groups. In group 1 the patients were instructed to apply CPP-ACP containing agent topically on WSL once daily for period of 4 weeks and to the control group 2, to brush with fluoridated tooth paste. After 4 weeks they were evaluated using quantitative light induced laser fluorescence(QLF) and they concluded that CPP-ACP reduced WSL but it was not superior to that of regular fluoride toothpaste

Huang G J et al.[36], (2013) conducted an in vivo randomized control study on 12-20 year old patient to evaluate the effectiveness of MI paste plus and

Prevident fluoride varnish on post orthodontic white spot lesion over 8 weeks and concluded that both are not effective in reducing white spot lesion than normal homecare with non-fluoridated toothpaste, manual toothbrush and dental floss.

Gizani S et al[37] (2016) evaluated the effect of probiotic bacteria containing lozenges on WSL and S. mutans and LB counts in orthodontic patients. 85 patients were divided into 2 groups namely test group and control group. The test group was instructed to take probiotic lozenge containing 2 strains of LB reteuri and the control group was given placebo which appeared same as the probiotic lozenge but without bacteria. The patients were examined after debonding for presence of dental plaque, white spot lesion and salivary S. mutans level were recorded. There was no significance difference between groups which suggest that the intake of probiotic lozenges does not have effect in reduction of development of white spot lesion

3)Microabrasion technique:

Bishara S E, Denehy G e, Geopard S J[37] (1987) presented 3 cases, who 1) presented with stained teeth due to tetracycline, 2) post- orthodontic decalcification and 3) hypoplastic teeth. These enamel discoloured teeth were treated with microabrasion procedure using acid pumice paste containing 18% hydrochloric acid and pumice slurry. They are applied over teeth in erasing motion using wooden spatula for 5 sec followed by rinsing for 5 sec. This cycle was repeated maximum up to 10 times and the enamel surface is polished. The enamel stains reduced and the enamel gloss improved further because of remineralization

from saliva. They concluded that microabrasion as an effective and conservative method in removing superficial enamel stains and decalcification.

Croll T P and Bullock G A[38] (1988) treated 3 cases with enamel decalcification on the facial surface of teeth after orthodontic treatment with microabrasion. Microabrasion was performed with application of PREMA containing mild conc. of hydrochloric acid and fine grit of silicon carbide abrasive in a water-soluble gel. They were treated with low speed contra- angle hand piece with high torque and low rpm mounted with synthetic rubber tip. They concluded that microabrasion removes the undetectable and insignificant enamel structure and it leaves a glazed appearance.

Segura A, Donly K J, Wefel J S[39] (1997) studied resistance to demineralization of microabrasion treated enamel 1) microabrasion in conjunction with topical fluoride2) topical fluoride treatment 3) microabrasion and 4) no treatment. teeth were treated with microabrasion followed by 4 minute application of 1% neutral topical sodium fluoride exhibited significantly less enamel demineralization and their were the treatment modalities tested using polarized microscope. Artificial saliva was used to store all the teeth after 2 months

Ardu et al [21](2000) described microabrasion as minimally invasive technique in which superficial white spot lesion was eliminated by removal of hypomineralized layer on the tooth using abrasive paste containing silica carbide particles and 6.6% Hydrochloric acid. Microabrasion followed by CPP-ACP on

long term daily application on tooth allowed recovery of natural tooth appearance and enamel remineralized.

Hodges S J, Spencer R J, Watkins S J,[40] (2000) reported two cases that developed enamel stains after orthodontic treatment. It was treated by microabrasion using 18% Hydrochloric acid and pumice to restore natural appearance of tooth. He concluded that microabrasion decreased the intensity of stain but did not eradicate it completely.

Gelgor E, Buyukyilmaz T[41] (2003) treated 15 patients with post orthodontic white spot lesion with microabrasion technique. An abrasive gel containing 18 % hydrochloric acid, fine powdered pumice and glycerin was applied using electric brush with small tip for 3-5 minutes. The postorthodontic white spot lesion with mild lesion showed complete regression and moderate lesion and severe lesion showed decrease in size to acceptable level. The brown stains on the lesion was removed completely and also the rough enamel surface became smooth.

Lynch C and Mc Colonnel R J [42](2003) presented a clinical report on a patient with discoloured maxillary anterior teeth. The discoloured teeth was isolated properly and viscous paste containing 18% Hydrochloric acid and pumice powder was applied for ten seconds. It was then removed using water pressure and high-volume aspirator. The technique was repeated 9 more times and bonding agent was applied later it was polished with soft disc. He reported microabrasion was a conservative method which caused improved esthetics.

Bezzera C B et al,[43](2005) quantitatively evaluated two microabrasion technique 1) 18% hydrochloric acid with pumice slurry &2) 37% phosphoric acid with pumice slurry for the treatment of enamel opacities by using an image analysis software for analyzing photo. This photo was taken immediately and 1 month after treatment. they concluded that both can be used and there is colour improvement with time.

Murphy T C, Wilmott D R, Rodd H D[44], (2007) quantitatively assessed the postorthodontic white spot lesion in patients after treating with microabrasion using an image processing software. The post-orthodontic demineralized lesion was treated with abrasive paste made of 18% hydrochloric acid and pumice slurry. This paste was placed on the demineralized lesion for 10 seconds and washed off , the cycle was repeated 10 times. Around 83% of lesion was reduced immediately after treating the lesion with Microabrasion. They concluded that it is an effective technique for long standing WSL.

Sunfield R H et al[45] (2007) studied the current status of microabraded teeth 18 years post treated. They concluded that it is a safe, satisfactory and effective method and it improves esthetic appearance of teeth.

Meirless et al [46](2009) evaluated the surface roughness and enamel loss with two microabrasion techniques using stereomicroscope. The 20 bovine samples were grouped in two groups. The first group was treated with

18%hydrochloric acid and the other was with 37% phosphoric acid. Both the acids were made into paste by adding pumice. The microabrasion procedure using phosphoric acid showed greater roughness but less demineralization than HCl.

Son J H, Aur B, Kim H C and Park J[47] (2011) compared the effectiveness of resin infiltration and microabrasion in treating post orthodontic white spot lesion. The procedure in resin infiltration was by etching with Icon etch containing HCl for 2 min and washed. Then Icon resin infiltrant which contained methylacrylate based resin, was applied on dried enamel and light cured twice. The microabrasion was done using opalustre paste, it contained 6.6% hydrochloric acid, 20-60 µm silicon carbide particle. The opalusture was applied on the tooth and mild pressure was applied for 60-120 sec with opalcups placed on gear reduction handpiece. They concluded that both resin infiltration and microabrasion did not resolve the lesion completely as the selected lesion had deeper subsurface lesion may be more than 80µm and hence careful examination needed before selecting the case

Caglaroglu M, Gelgor I E[48](2011) demonstrated an effective microabrasion technique post debonding. In this technique abrasive paste was made using 18% hydrochloric acid and pumice slurry in the ratio of 2:1 ratio by weight to this glycerin was added until this paste was fluid gel in consistency and stored in air tight container. The powered brush was modified by cutting the outer bristle and inner bristles which produce oscillating movements or rotating movements was taken. The abrasive paste was applied on white spot lesion with

the oscilating tooth brush for three- five minutes. They experienced that the microabrasive technique was effective in removing enamel discolouration, white spot lesion and streaks.

Pliska TB, Warner GA, Tantbirojn D, Larson BE [49] (2012) conducted an in-vitro study to assess the treatment effect of white spot lesion using microabrasion and CPP-ACP paste. 16 artificially induced white spot lesion on bovine teeth were divided into four groups, namely 1) paste group , 2) microabrasion group,3) combination group and 4) control group. The paste only group had 1:1 dilution of MI paste and Recaldent paste and they were placed twice daily for two weeks. The microabrasion group which was treated by placing 35% phosphoric acid for 2 minutes and rinsed. It was then followed by polishing in pumice using rubber cup for 20 seconds after rinsing they were then rubbed with deionized water for 20 sec and this microabrasion procedure was carried for twice daily for two weeks. In the combination group, microabrasion was followed by application of CPP-ACP paste for 2 minutes and rinsed with deionized water and the other group was control which is untreated. After two weeks of treatment the WSL were quantitatively evaluated using fluorescence. The results showed that there was a reduction in white spot lesion when treated by Microabrasion with or without CPP-ACP than lesion treated by CPP-ACP.

Akin M, Dilber E, Bastiftci CI, Ozruk B[50](2013) conducted an invivo study on 20 post debonded patients, to assess the colour improvement of microabrasion treated post orthodontic white spot lesion using spectrophotometer.

They used 18% Hcl and pumice paste and made into slurry. The paste was applied on the teeth and they washed away after 30 sec. The colour change obtain was effective, they reported that microabrasion therapy makes created clinically acceptable colour change.

Yetkiner E et al [51] (2014) evaluated the colour improvement and stability of post orthodontic white spot lesion using fluoride application, microabrasion and resin infiltration. This invitro study was done on 60 bovine samples divided into four groups, each group had 15 samples. 1) The control group was untreated and in 2) the fluoride group the samples were immersed in 2ml of solution for I minute daily for 30 days. 3) The microabrasion group were polished using rubber cup with opalustre paste which contain 6% hydrochloric acid and silicon carbide less than 45% at 300rpm for 1 minute. 4) The resin infiltrant group was treated with 15% hydrochloric acid for 120 seconds and rinsed in water for 30 seconds and air dried. Later resin infiltrant is applied over the teeth using microbrush and let it settle for 180 seconds and then light cured and a second layer of infiltrant is applied. The colour of enamel was assessed using spectrophotometer. The resin infiltrant group showed highest colour improvement followed by microabrasion and fluoride and only resin infiltration showed stability following discolouration.

Jahanbin A, Ameri H, Shahabi M and Ghazi A[52](2015), compared the effectiveness of two microabrasive technique namely hydrochloric acid and phosphoric acid in treating postorthodontic white spot lesion and also evaluated the resistance of treated enamel discolouration. Invitro study was done on 60 extracted

human premolars with were grouped into 3 groups containing 20 samples each. The control group was polished with pumice slurry and the hydrochloric acid group were treated with paste containing 18% hydrochloric acid and pumice for five seconds. The phosphoric acid group was treated with paste containing 37% orthophosphoric acid and pumice for 5 sec. they were assessed for colour change using calorimeter. They was then subjected to staining in tea- coffee solution and colour measurements were taken. They reported that the colour change by hydrocholoric acid was significant but during discolouration they stained more whereas orthophosphoric acid group showed less effect.

4)Laser technique

Myers T and Myer M D[23] (1985) treated the incipient enamel lesion present on pit and fissure with mode locked YAG Neodymium laser and examined macroscopically for surface lesion and also examined using scanning electron microscopy. The invitro study done on 30 extracted human teeth where one half of the teeth were irradiated with mode- locked YAG Neodymium laser of wavelength of 10.6 nm, pulse energy 3.4mJ, pulse duration of 30psec and spot beam size of 50µm, the other half served as control. They reported that laser irradiated enamel significantly removed caries and extrinsic stains and their colour appeared similar to the adjacent caries free enamel surface. They concluded that laser can be used for debridement of incipient caries.

Monseau A et al [53] (2002) carried out an in vivo study to evaluate the effects of argon laser on enamel decalcification during orthodontic treatment,

group 1- Nine patients who had treatment plan of extraction of premolar were included and totally 36 teeth were grouped into 4 groups. They were the group 1(control group) with no treatment. Group 2(pumice-laser group) the teeth were polished with pumice for 3 sec followed by argon laser irradiation with 325 mW, 5mm diameter laser beam for 60 sec, Group3(Pumice etch- laser group), the tooth were acid etched for 30 sec and then lased with argon laser for 60 sec, Group 4(laser only group), the argon laser was irradiated for 60 sec. they were subjected to acid challenge by placing an ill-fitted band for 5 weeks and then they are extracted and analyzed using polarized microscope. The argon laser treated enamel surface showed reduced depth which implies they are effective in reducing enamel decalcification and pumice and acid etching does not reduce the effect of laser on enamel solubility.

Delbem A C B et al [54](2003) evaluated the resistance to enamel demineralization when irradiated with Er:YAG laser and its potential in formation of calcium fluoride after application of APF gel using microhardness method. 120 extracted premolars were divided into 4 groups. The groups were 1)untreated control group,2) Er:YAG laser treated group, 3)APF gel treated group and 4)combination of Er:YAG laser and APF gel group. These groups were subjected to caries challenge for 14 days and microhardness was evaluated in 10 µm, 20 µm & 40 µm depth. The mineral loss in Er:YAG laser treated group showed was less but the microhardness was low. The Er:YAG laser combined with APF gel group presented more microhardness of all groups deposition of calcium fluoride was

influencial in teeth treated with Er: YAG laser and it cases superficial anticariogenic action than in deep caries.

Cecchini R C M et al[55] (2005) studied 70 enamel samples for the acid resistance effect of enamel irradiated with Er:YAG laser with frequency of 2HZ and water spray 5ml/min and different energy density and output power group1) 60 mJ & 3.33J/cm^2 , 2) 80mJ & 44.4J/cm^2 ,3)$1^2$0mJ & 66.6J/cm^2, 4)64mJ &20J/cm^2, 5) 86.4mJ & 29.9J/cm^2,6) 135mJ & 42.2J/cm^2 respectively and group 7) control group. Group 1,2, 4 showed decreased demineralization. They concluded that without severe attrition of the enamel, low energy lasers can decrease enamel solubility.

Kim J, Kwon O, Kim H, Kwon Y H (2006)[25] conducted invitro study and compared the effects of Er:YAG laser ablation and phosphoric acid etching on bovine enamel. The enamel of Er:YAG laser ablated group was irradiated with wavelength 2.94µm, 2 Hz frequency, noncontact mode, energy of 380mJ/cm^2 and acid etching group was treated with 37% phosphoric acid. On evaluating using the x-ray diffraction pattern, the enamel of laser etched group showed least mineral loss and improved crystalline structure and blocking effect of organic matrix.

Ahrari F, Poosti M, Motahari P [56](2012) evaluated the enamel resistance to demineralization following Er:YAG laser etching before bonding orthodontic brackets. The invitro study was done with 50 extracted human premolar, divided into two groups 1) acid etched and 2) laser etched. In acid etched group the teeth

were etched using orthophosphoric acid from 15-30 sec and the laser group were etched using Er:YAG laser with parameters λ= 2.94nm, 300 mJ / pulse, 10 pulse/ sec and irradiated for 10 sec with water spray. The teeth were sectioned and evaluated the surface lesion depth by microphotograph and reported that there was no significance difference between groups. Thus, the Er:YAG laser etching parameters are not effective in reducing the enamel demineralization

Lamser M F, Reher VGS, Lallo R, Reher P[57] (2012) evaluated enamel demineralization and bond strength when etched with Er:YAG laser. 60 extracted premolar teeth were group into 4 groups namely1) control (not treated),2) acid etching group which is etched using 37% phosphoric acid for 30 sec followed by washing for 20 sec,3) the Er:YAG laser group ablated with 80 mJ, 4 Hz, diameter of beam 0.63 pulse duration 250-400μs for 40 sec. 4) the laser + acid group was ablated with laser followed by acid etching. The ceramic and metal brackets were bonded with Transbond XT and Fuji Ortho LC and bond strength were tested using microtensile test and SEM evaluation with EDS was done to evaluate the enamel mineral content. The results showed that Laser irradiated teeth showed less demineralization then other, but bond strength seems to be lower but sufficient to produce clinically efficient retention.

Liu Y, Hsu C Y S, Teo C M J and Teo S H[58] (2012) evaluated the effect of fluoride application followed by Er:YAG laser irradiation on enamel demineralization using micro computed tomography. Ten non-carious extracted human premolars are used and four windows ae created on the teeth. These

windows are assigned as control group, fluoride-laser group, fluoride group and laser group. In the control group it was not treated and in fluoride group topical application of 2.0% NaF applied for four 4 mins. The Er:YAG laser was irradiated on fluoride-laser group after application of fluoride for four min and the parameters were 100mJ, 5 Hz, 5 sec with 5.1 J/cm^2 and the spot size of 0.5mm without water or air spray. the same parameters were used and the laser window was irradiated and all the windows were washed for 10 mins in water. The study revealed that the fluoride – laser group instantaneously formed fluoridated hydroxyapatite crystal and showed the highest resistance to enamel demineralization followed by laser group and fluoride group.

Altan A B , Baysar A , Berkkan A , Goktolga-Akin E [59], (2013) using atomic absorption spectrormetry the effect of Er:YAG laser and acidulated phosphate fluoride on enamel demineralization was studied. Twenty extracted premolars were divided in four groups, they were1) untreated control,2) Application of 1.23% APF gel group,3) Er:YAG laser treated group followed by APF gel and 4) APF gel application followed by laser treatment. The samples were irradiated with Er:YAG laser with wavelength 2.94µm with contact handpiece having frequency of 10HZ, energy output 100mJ/pulse, density of 12.73 J/cm^2 and water spray at rate of 5 mL/min. the groups treated with ablation of Er:YAG laser followed by application of fluoride showed increased resistance to enamel decalcification.

de Souza-e silva C M et al[60] (2013) conducted a study to determine the effects of CO2 laser irradiation combined with fluoride releasing bonding material in reduction of demineralization of enamel. An in vitro study was done using 90 bovine enamel slabs grouped as 5 groups.1) The first group was exposed to noninoculated brain – heart infusion broth, 2) the second group was bonded with non- Fluoride releasing composite resin and3) the third was with resin modified GIC, 4) The fourth group was lased with CO2 laser having wavelength – 10.6µm, pulse duration of 10ms, rest cycle of 10 ms, frequency 5Hz and laser power 0.7 W and

5) the fifth group, initially irradiated with Er:YAG laser & the brackets were bonded using Non- Fluoride releasing composite resin, whereas the other group with resin modified GIC. The results showed that lased enamel surface with CO2 showed increased microhardness of all groups. They presented laser irradiated enamel when bonded with fluoride releasing material do not have any addition effect. This implies laser irradiated enamel with or without fluoride bonding material was effective in reducing enamel demineralization around bracket.

Mathew A et al [61]62(2013) compred the effect of Er:YAG laser and Co2 laser combined with acidulated phosphate fluoride treatment on extracted human premolar using atomic emission spectrometry. The vitro study was done on 30 extracted human premolar. The Er:YAG group, teeth were irradiated with 2.94 µm, pulse energy 200mJ, frquency 7 Hz in noncontact mode and no water flow and Co2 laser group were irradiated using wavelength 10.6 µm, 1 w, 0.75 sec exposure time and 0.3mm beam spot. The other groups were treated with laser followed by

application of APF gel. The results showed that teeth which were exposed to Co2 and Er:YAG laser with APF gel had increased resistance to acid than those with laser irradiation and fluoride therapy alone.

Colucci V et al[62], (2014) determined the enamel mineral loss around the composite restoration when cavity preparation done using Er:YAG laser with the variable frequency of 2, 4 & 6 HZ and water flow rate 2, 5& 8ml/min at 300MJ/min. The mineral loss was evaluated by Knoop microhardness test. They concluded that Er:YAG laser with the parameters of 2Hz, 2ml/min and 300MJ/min shows highest microhardness indicating resistance to demineralization.

Curylofo-zotti FA, Tanta GS, Zugliani, ALCorona SAM et al[63] (2016) evaluated the effect of Er:YAg laser when combined with fluoride in controlling of enamel decalcification. The invitro study was done on freshly extracted bovine enamel and enamel slabs were made, they were divided into 3 groups namely 1) placebo group with no treatment, 2) NaF 0.05% group were slabs are placed in 10 ml of solution for 1 minute, one time a day for 14 consecutive days. 3) Er:YAG laser irradiated with parameter 4) Er:YAG laser followed by fluoride therapy for 1 minute a day and then placed in artificial saliva for rest of hours. The results revealed highest microhardness when Er:YAG laser and 0.05% sodium fluoride was used and no evident difference in between the groups treated with Er: YAG and Er: YAG and 0.05% sodium fluoride. the surface mineral loss was lowered I Er:YAG laser.

Talu S et al[64] (2016) investigated enamel surface nanomorphology using atomic force microscopy after irradiated with Er:YAG laser with densities of 12.7 J/cm2 & 25.5 J/cm2 with and without water irrigation and the other parameters were surface energy of 100-200mJ, frequency 10 Hz, wavelength 2.94µm with beam diameter 1-1.3mm. The obtained results showed that laser irradiated enamel surface with water irrigation showed no cracks with melting area, whereas enamel samples when irradiated without water irrigation showed cracks, microfissure, scratched and melting areas

MATERIALS AND METHODS

MATERIALS AND METHODS

The sample consisted of 90 extracted premolars from the patients who required therapeutic extractions for orthodontic treatment. Informed consent was obtained from the patient for using their teeth for the study. The extracted teeth were washed and stored in saline. Teeth that are intact with no stains were included and teeth which had caries, extrinsic and intrinsic stains, erosion, enamel hypoplasia and fractures were excluded. The ethical clearance was obtained from Institutional Ethical Committee of Vivekanandha Dental College for Women (IEC/VDCW/02/2015)

The teeth were mounted on round acrylic block made with self-cure acrylic resin in vertical position with their roots embedded in acrylic. (Figure 1)

The buccal surfaces of 90 premolar teeth were etched with etchant gel containing 37% orthophosphoric acid (3M ESPE) for 15 seconds. It was then rinsed thoroughly with water until etchant was completely removed. The enamel surfaces were dried with oil and moisture free air and until a whitish frosted appearance was seen on etched enamel surface. Bonding agent (Transbond XT) was applied gently over the etched surface of the enamel using an applicator tip in light strokes without pressure and cured for 5 seconds with the LED light cure unit. Premolar brackets (3M Gemini series) were bonded with nanocomposite (Transbond XT adhesive) and cured for 20 seconds.

The samples were cleaned in deionized water. Baseline (T0) measurements were taken using spectrophotometer (Vita Easyshade Advance 4.0®; Vita Zahnfabrik)) according to manufacturer's instruction[64]. (Figure 2) It uses D-65 illumination for color selection. The spectrophotometer was calibrated before each measurement. The probe

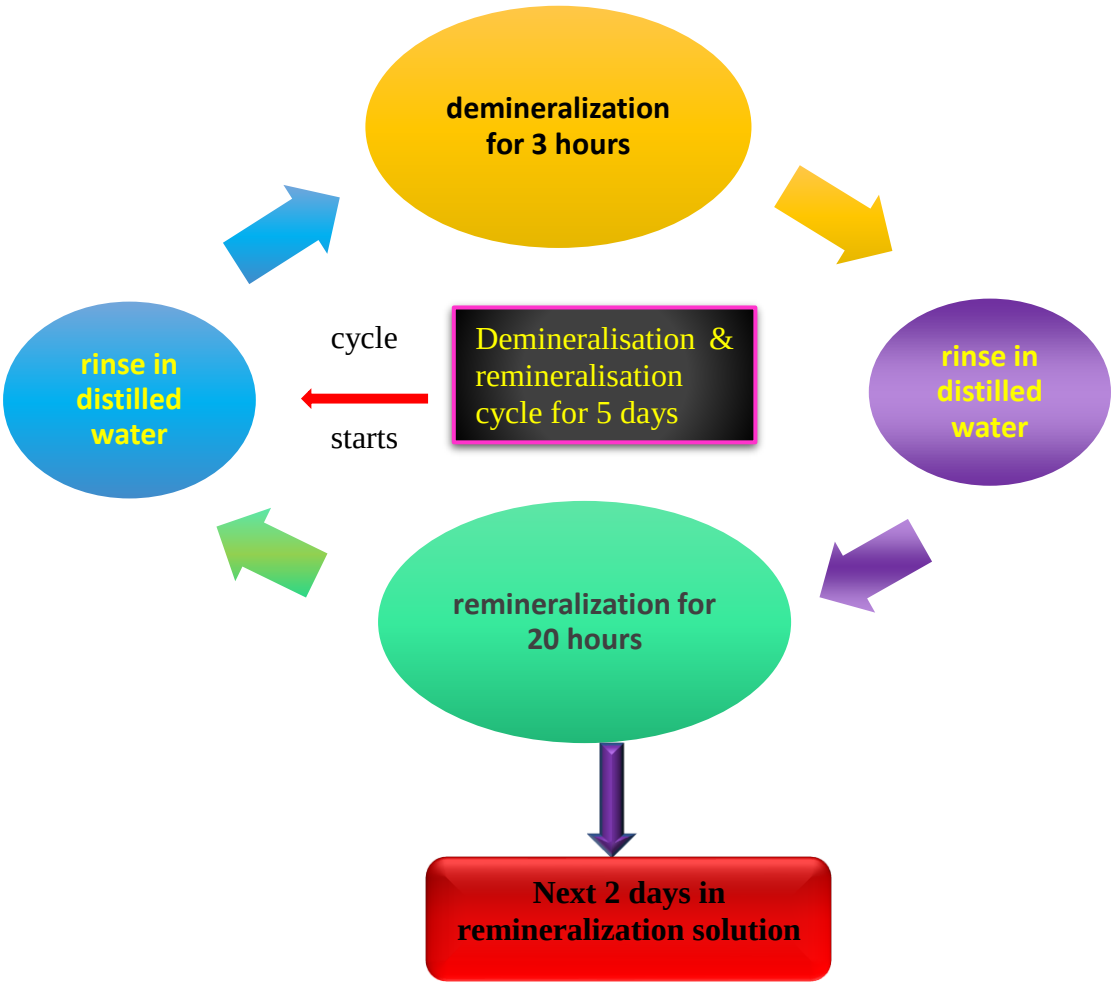
was positioned in contact with the tooth, perpendicular to the cervical and incisal portion of each tooth. To minimize the effect of ambient light, all spectrometer measurements were taken in the same examination room on sunny days between 11 a.m. to 1 p.m. with standardized lighting conditions. This VITA's digital shade determined colour and spectral distributions with precision and measured according to Commission International de l'Eclariage (CIE) L*a*b* system (CIE Colorimetry Publication, 1986)[65]. The L* axis represents the degree of lightness of the teeth. L* measurement ranges from 0(black) to 100 (white) and hence lightness increases as L * increased. The a* denotes is the red/green axis, higher value indicated red colour component and the b* denotes is the yellow/blue axis its higher number indicated yellow colour.

The modified pH cycling model as described by Featherstone et al was used to create the white spot lesion[66] (Table 1, Figure 3). The samples were subjected to pH sampling regimen for 5 days. The samples were kept immersed in demineralized solution for 3 hours at pH of 4.3. (Figure 4) After treating with demineralization solution, the samples were rinsed thoroughly with deionized distilled water for half an hour (i.e approx. 15 sec for each block) and then the samples were immersed in remineralization solution of for 20 hours in pH7.0 at 37ºC. (Figure 5) This cycle was repeated daily for five days. On completion of demineralization – remineralization cycle, teeth samples were placed in remineralization solution for next 2 days [Figure1]. The temperature was maintained at 37ºC throughout the process. The cycle was performed by immersion of the blocks in 2 litre of their respective solution (approx. 20 ml per block). The solution was prepared fresh before subjecting the samples to pH cycle as their shelf life is only 7 days.

Table 1 represent the pH cycling model by Featherstone for formation of incipient lesion-

pH CYCLING MODEL	TIME
For 5 days	
Rinse in deionized distilled water	15 seconds for each sample
Demineralization solution	3 hours
Rinse in deionized distilled water	15 seconds for each sample
Remineralization solution	20 hours
For next 2 days	
Immersed in remineralization solution	

Figure 3:Schematic representation of pH cycling model by Featherstone



On completion of pH cycle, white spot lesion was visible with our naked eye and on drying they exhibited whitish appearance. (Figure 7)

Contents of demineralization solution: (Figure 8)

The demineralization solution contains calcium 2.0 mmol/L [Ca(NO3)2•4H2O mwt = 236.160.4723 g/L], phosphate 2.0 mmol/L[KH2PO4 mwt = 136.09 0.2722 g/L] and acetic acid 75.0 mmol/L [CH3COOH mwt = 60.05 4.5083 g/L]. pH 4.3 was adjusted using sodium hydroxide [NaOH].

Contents of remineralization solution: (Figure 8)

The remineralization solution contains calcium 2.0mmol/L [Ca(NO3)2•4H2O mwt = 236.160.4723 g/L] , Phosphate 2.0 mmol/L[KH2PO4 mwt = 136.09 0.2722 g/L], potassium chloride 130.0 mmol/L [KCl mwt = 74.55 9.6915 g/L], sodium cacodylate 20.0 mmol/L [NaC2H6AsO2•3H2O mwt = 214 4.28 g/L]. pH was adjusted to 7.0 with concentrated hydrochloric acid [HCl].

After the formation of white spot lesion, the brackets were de-bonded using debonding pliers. (GAC International, Inc, Bohemia, NY). The stainless-steel brackets were held mesiodistally by the plier and a shear force was applied. The adhesive resin over the enamel surface was removed by with tungsten carbide bur TCB 012 on a low-speed micromotor with air cooling.

The samples were randomly selected and divided into three groups of 30 each. Group 1 was allocated as control, group 2 as microabrasion treated and group 3 as laser treated.

After the formation of white spot lesion, the brackets were de-bonded using debonding pliers. (GAC International, Inc, Bohemia, NY). The stainless-steel brackets were held mesiodistally by the plier and a shear force was applied. The adhesive resin over the enamel surface was removed by with tungsten carbide bur TCB 012 on a low-speed micromotor with air cooling.

The samples were randomly selected and divided into three groups of 30 each. Group 1 was allocated as control, group 2 as microabrasion treated and group 3 as laser treated.

Table 2 – Grouping of samples

Groups	Sample size	Corresponding treatment
Group 1	30 premolar teeth	No treatment
Group 2	30 premolar teeth	Microabrasion
Group 3	30 premolar teeth	Laser

Randomization was carried out using random number tables to generate sample number for allocating them in three groups. The spectrophotometer measurements of formed white spot lesion (T1) were measured.

In the Group 1 (control group), the samples were not treated.

In Group 2 (Microabrasion treated group), white spot lesion were treated with microabrasion. A paste of 37% orthophosphoric acid with pumice slurry was made. It is then placed using wooden spatula over the white spot lesion for 5 second and followed by polishing the lesion with rubber cup in low speed handpiece for 5 seconds. (Figure 9)

In Group 3 (Laser treated group), the white spot lesion were treated with Er:YAG laser (Doctor Smile PLUSER DENTAL / LA ERT001.1, Italy) in the MSP mode, using a pen (R02), at noncontact mode, pulse energy of 80 mJ and frequency of 2Hz, an output beam diameter of 0.9 mm and under water spray (6 mL/min). During irradiation, the laser beam was held 4mm away and perpendicular to the white spot lesion. (Figure 10,11)

The L, a and b measurements of treated white spot lesion were recorded using spectrophotometer (T2). The colour change between post treatment time was calculated as delta E. The visible colour change (ΔE) was clinically detectable when it exceeded 3.7 and calculated by (CIE Colorimetry Publication,1986)[65]:

$$\Delta E=[(L_1^*-L_2^*)^2 + (a_1^*-a_2^*)^2 + (b_1^*-b_2^*)^2]^{1/2}$$

The colour change delta E1 (ΔE1) represent the colour difference between T1 and T2. It was calculated by

$$\Delta E1=[(T1L^*-T2L^*)^2 + (T1a^*-T2a^*)^2 + (T1b^*-T2b^*)^2]^{1/2}$$

All the samples were placed in artificial saliva for 30 days. The artificial saliva was prepared by Fusayama-Meyer method, the composition was KCI (0.4 g/l), NaCl (0.4 g/l), $CaCl_2$, $2H_2O$ (0.906 g/l), NaH_2PO_4, $2H_2O$ (0.690 g/l), Na_2S, $9H_2O$ (0.005 g/l), Urea (1 g/l), and distilled water at 37° C. (Figure 12,13)

After 30 days in artificial saliva, the samples were rinsed and dried. The spectrophotometer measurement (T3) was taken. The difference of colour change between T2 and T3 was calculated as delta E2 (ΔE2)

$$\Delta E2=[(T2L^*-T3L^*)^2 + (T2a^*-T3a^*)^2 + (T2b^*-T3b^*)^2]^{1/2}$$

To evaluate the colour stability of these treated white spot lesion, the samples were subjected to discolouration by immersing them in tea- coffee solution daily for 5 minutes. For the remaining hours they were immersed in Fusyama-Meyer artificial saliva at pH 7.3. The discolouration process was carried out for 5 days. The discolouration solution was

prepared by mixing 20 g of tea and 20 g of coffee in 2 litre of boiled water and then filtered. (Figure 14)

After five days, the samples were washed in deionized water. The teeth are brushed gently with soft bristles to remove the superficial staining and then rinsed with water thoroughly and dried. The spectrophotometer reading was taken (T4) and delta E3(ΔE3) was calculated to evaluate colour change between T3 andT4.

$$\Delta E3=[(T3L^*-T4L^*)^2 + (T3a^*-T4a^*)^2 + (T3b^*-T4b^*)^2]^{1/2}$$

Figure 1: Mounted samples with acrylic resin

Figure 2: Spectrophotometer equipment – Teethexamined

Figure 4: Immersion of samples in demineralization solution

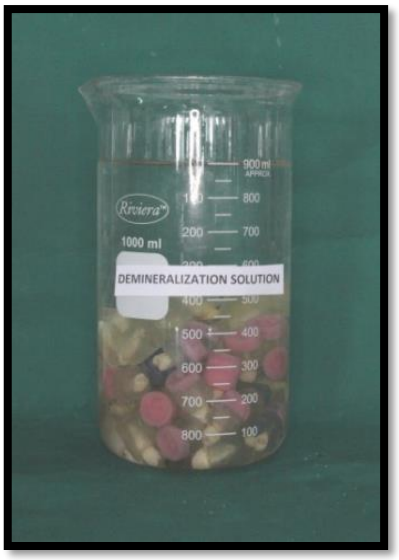


Figure 5: Immersion of samples in remineralization solution

Figure:6 pH meter equipment

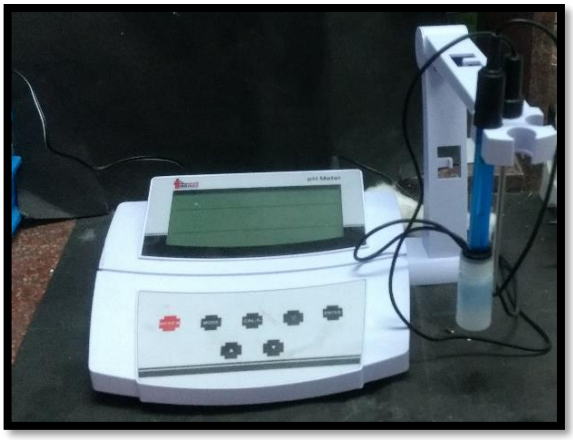


Figure:7 Formation of White Spot Lesion. (WSL)

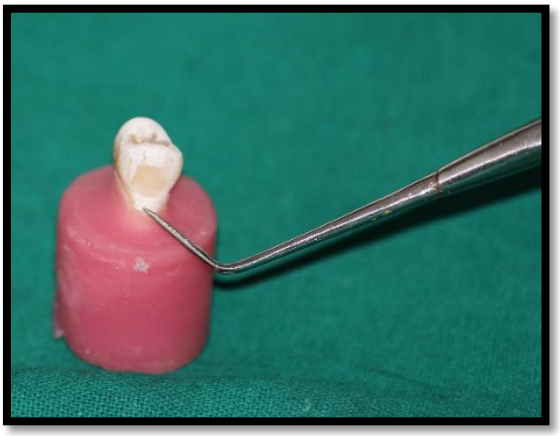


Figure 8: Reagents Of Demineralization And Remineralization Solution

Figure a : Acetic acid

Figure c: Potassium Phosphate

Figure c : Potassium Chloride

Figure d : Sodium Cacodylate

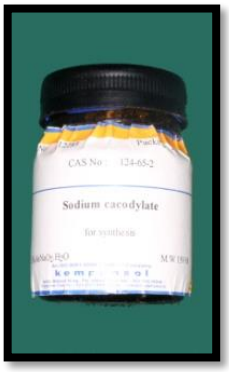


Figure e : Calcium Nitrate

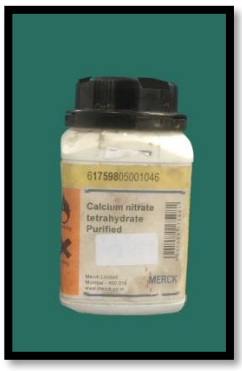


Figure f: Hydrochloric Acid

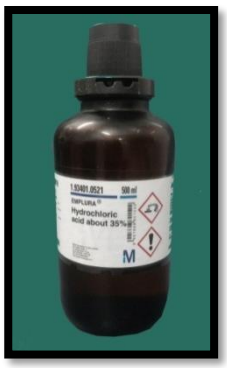


Figure g : Sodium Hydroxide

Figure 9: Microabrasion treatment -Application of 37% Orthophosphoric Acid with Wooden Spatula Rotated with polished rubber cup mounted to contra angle handpiece

Fig 10: Er:YAG laser equipment- Doctor SmillePluser, Italy. (parameter panel)

Fig 11:Laser treatment- treated with Er:YAG laser

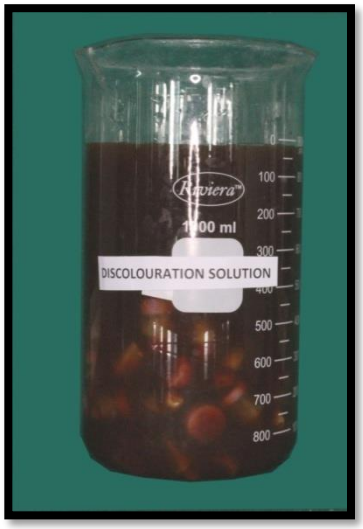
Figure 12: Reagents Of Artificial Saliva

Figure a: Potassium Chloride Figure b : Calcium Chloride Fig c: Sodium chloride

Figure d : Sodium Dihydrogen Monophosphate Figure e : Sodium Sulphide

Figure 13: Immersion in Artificial saliva

Figure 14:Immersion in discolouration solution



STATISTICAL ANALYSIS

STATISTICAL ANALYSIS

Kolmogorov–Smirnov and Shapiro–Wilk tests were used to test normal distribution of the data.

The data were normally distributed. One-way analysis of variance (ANOVA) was used to determine statistically significant differences between the means of three groups 1, 2 & 3.

The formula used for the ANOVA analysis

$$\text{ANOVA} = \frac{\text{BMS- WMS}}{\text{BMS + (n-1) WMS}}$$

Where,

BMS = Between subjects mean sum of squares

WMS = Within subjects mean sum of squares

n = Number of measurements

P value of less than 0.05 was considered to be statistically significant.

Samples were grouped in the following order,

1. Group 1- Control group
2. Group 2 – Microabrasion treated
3. Group 3 – Laser treated.

The Post hoc- Bonferroni correction test was used to determine which specific group showed significance. It is an adjustment made to P values which reduce the chances of obtaining false-positive results as multiple pair wise tests were performed on a single set of data

The colour differences between two treated groups 2 and 3 at various treatments were calculated as ΔE and analyzed by unpaired t test. Unpaired T test used separately for all combinations of two group comparisons as data was not skewed.

RESULTS

RESULTS

The colour change in post- orthodontic white spot lesion was assessed by spectrophotometer. The spectrophotometer measures the L*, a* and b* values

L*- denotes degree of lightness of sample,

a*-denotes red/green chromaticity

b*-denotes blue/yellow chromaticity

Five readings were taken from teeth prior to demineralization to post-treatment white spot lesion discolouration at various intervals. The five readings are as follows

T0- baseline- prior to bonding

T1- white spot lesion

T2- post-microabrasion / laser treatment

T3- after 30 days storage in artificial saliva

T4- after discolouration cycle.

The mean values of three groups (Control, Microabrasion, Laser) were compared at different time intervals from T0 – T4.

At all readings, the “L”, “a” & “b” values were noted.

TABLE- 3- Comparison of "L*" (lightness parameter) at different time intervals between the groups 1,2 & 3– (Control, Microabrasion and Laser).

Groups		***Baseline T0***	***WSL T1***	***Corresponding treatment T2***	***Art. Saliva T3***	***Discolouration T4***
*L**	1-Control	53.46	77.37	77.37	69.06	46.93
	2- Microabrasion	55.98	77.44	59.32	58.61	45.97
	3- Laser	56.61	77.20	61.07	58.30	61.93

With the formation of WSL(T1) there was an increase in L * from T0 to T1, indicating an increase lightness of sample. On treatment with microabrasion and laser group 2 and 3 showed a significant decrease in L *whereas control group showed no change. This showed a significant colour improvement in the laser and microabrasion treated groups. At T3, there was a mild decrease in L* in group 2 and 3. The whitish appearance of the lesion decreased further after immersing in artificial saliva and the lightness was almost back to baseline (T0) level in both the treated groups. The group 1 showed a decrease at T3, which indicated that after 30 days in artificial saliva there is a regression of whitish appearance of lesion but it was higher than the baseline(T0). After discolouration (T4), L*of group 1 and 2 decreased lower than the T0 value, this reduction in L* denotes that the darkness of the teeth increased for both control and microabrasion treated group. The group 3 showed slight increase indicating significant colour improvement.

TABLE-4- Comparison of "a*" (red/green axis parameter) at different time intervals between the groups 1, 2 & 3– (Control, Microabrasion and Laser).

Groups		***Baseline T0***	***WSL T1***	***Corresponding treatment T2***	***Art.Saliva T3***	***Discolouration T4***
a *	1 -Control	-0.80	-1.30	-1.30	-0.96	3.93
	2 -Microabrasion	-0.51	-1.52	-0.79	-0.67	2.73
	3- Laser	-1.02	-3.80	-2.43	-1.15	-1.50

On formation of white spot lesion (T1) a* showed a decrease in all three groups which denotes there was a shift to green component of the spectra. With corresponding treatment microabrasion and laser, group 2 and 3 (T2) significantly increased this indicates that the chromaticity change occurred towards red but not similar to baseline (T0). After 30 days of immersion in artificial saliva (T3), all the three groups showed an increase in a*and in group 2 and 3, which was a significant shift to red which was near the spectra of baseline (T0) indicating colour improvement. At (T4) significant increase in a* seen in group 1 and 2. Thus the a* of control group1 and microabrasion group2 represent that there was an increase chroma towards red spectra of colour. The laser treated group 3 showed mild increase which was insignificant it denotes that there no visible change after T4

TABLE- 5- Comparison of ‘’b*” (blue/yellow axis parameter) at different time intervals between the groups 1,2 & 3– (Control, Microabrasion, and Laser).

Groups		***Baseline T0***	***WSL T1***	***Corresponding treatment T2***	***Art.Saliva T3***	***Discolouration T4***
b *	1 -Control	-03.15	-4.68	-4.68	-2.92	9.03
	2 -Microabrasion	-3.60	-4.50	-2.27	-2.81	6.57
	3- Laser	-3.81	-5.80	-3.12	-2.64	0.18

The b* showed a decrease which denoted a shift towards the blue spectra on formation of white spot lesion(T1). Treatment groups 2 and 3 showed an increase in b* at (T2) denoting these treated group exhibit shift towards yellow spectra. The groups 1, 2 and 3 showed increase in b* at T3. After immersing in tea coffee solution (T4), the microabrasion group 2 and control group 1 showed a significant shift to the yellow component due to discolouration and where as it was mild in laser treated group3.

TABLE 6- Comparison of post treatment measurements (T2L*) in group 1, 2 and 3

Groups	N	L * Mean (S.D)	95% confidence interval		ANOVA	P
			Min	Max		
1.Control	30	77.37 (6.44)	77.97	79.77	89.464	0.000**
2. Microabrasion	30	59.62 (6.28)	59.86	61.56		
3. Laser	30	61.07 (4.42)	59.42	62.77		

** Highly Significant ,P <0.005- significant

The mean, S.D and confidence interval of T2L are shown in Table 6. According to ANOVA, there was a statistically significant difference among the groups. The mean of microabrasion group 2 is least indicating better colour improvement.

TABLE 6 .1 – Post- Hoc test-Bonferroni correction between the groups in T2L*

Comparison of groups	Mean difference	Std error	Sig
1.Control 2.Microabrasion	18.1500(*)	1.49334	.000
2.Microabrasion 3.Laser	-1.8500	1.49334	.656
1.Control 3. Laser	16.3000(*)	1.49334	.000

** Highly Significant ,P <0.005- significant

The Post Hoc – Bonferroni test revealed that there was a statistically significant difference between group1&2 and also between group1 & 3 showing P (0.000) and it signifies more colour improvement in treated groups 2 and 3. There is no statistically significant difference between laser treated group 2 and microabrasion treated group 3. The microabrasion group showed the better colour improvement than laser. (Table 6.1)

TABLE 7 – Comparison of post treatment measurements (T2a*) in group 1, 2 and 3.

Groups	N	a * Mean (S.D)	95% C.I		ANOVA	P
			Min	Max		
1.Control	30	-1.30 (0.49)	-1.48	-1.12	61.719	0.000**
2. Microabrasion	30	-0.79 (0.30)	-0.90	-0.68		
3. Laser	30	-2.43 (0.83)	-2.73	-2.11		

** Highly Significant ,P <0.005- significant

The means and standard deviation of T2a* showed in table 7. The mean of microabrasion group 2 is highest than the other groups. According to ANOVA, there was a statistically significant difference between the three groups.

TABLE 7 .1 – Post Hoc -Bonferroni correction between the groups in T2a*

Comparison of groups	Mean difference	Std error	Sig
1.Control 2.Microabrasion	-.5110(*)	.15034	.003
1.Control 3.Laser	1.1217(*)	.15034	.000
2. Microabrasion 3.Laser	1.6327(*)	.15034	.000

** Highly Significant ,P <0.005- significant

Post Hoc-bonferroni correction showed a statistical significant difference between group1& 2 and also group 1 &3. This signifies that microabrasion treated group2 and laser treated group 3 has better improvement in their chromaticity when compared with control. There is statistically significant difference between group 2 and 3 and the difference of mean for microabrasion is least signify that there was a better improvement in chromaticity in microabrasion group and their spectral shift was towards red. (shown in table 7)

TABLE 8 – Comparison of post treatment measurements (T2b*) in group1,2 and 3

Groups	N	b * Mean (S.D)	95% C.I		ANOVA	P
			Min	Max		
1.Control	30	-4.68 (0.51)	-4.86	-4.49	113.797	0.000**
2. Microabrasion	30	-2.27(0.76)	-2.55	-1.98		
3. Laser	30	-3.12 (0.60)	-3.34	-2.90		

** Highly Significant ,P <0.005- significant

Table 8 shows the mean, S.D, and Confidence interval of T3b. the microabrasion group 2 showed the highest mean. Three groups statistically compared using ANOVA showed significance.

TABLE 8.1 – Post Hoc- Bonferroni correction between the groups in T2b*

Comparison of groups	Mean difference	Std. Error	Sig
1.Control 2.Microabrasion	-2.4110(*)	.16207	.000
1.Control 3.Laser	-1.5573(*)	.16207	.000
2.Microabrasion 3.Laser	.8537(*)	.16207	.000

**Highly Significant ,P <0.005- significant

Post hoc test – Bonferroni correction applied, the results showed there was a significant difference among groups. The mean difference was least in the microabrasion group2 followed by laser group3. The microabrasion group2 indicated shift from blue towards yellow spectra which signifies improvement chromaticity. (shown in table 8.1)

TABLE 9– Comparison of T3L* in all the 3 groups(control, microabrasion and laser)

Groups	N	L* Mean (SD)	95% C.I		ANOVA	P
			Min	Max		
Control	30	69.96 (7.74)	67.07	72.85	29.887	0.000**
Microabrasion	30	58.61 (6.32)	56.24	60.96		
Laser	30	58.30 (5.76)	56.15	60.45		

** Highly Significant ,P <0.005- significant

Table 9 shows the distribution of mean, S.D and confidence interval of T3L among three groups. The laser group3 showed the least mean and there is a statistically significant difference between three groups with p<0.005.

TABLE 9..1 –Post Hoc- Bonferroni correction between the groups in T3L*

Comparison between groups	Mean differerence	Std error	Sig
1.Control 2.Microabrasion	11.3567(*)	1.71924	.000
1.Control 3.Laser	11.6600(*)	1.71924	.000
2.Microabrasion 3.Laser	.3033	1.71924	1.000

**Highly Significant ,P <0.005- significant

According to Post Hoc- Bonferroni correction there is a significant difference between group1 &2 and group1&3. Both the treated group2 and 3 showed a statistically significant difference when compared with control group1. The mean difference of laser showed significance denoting the colour improvement. The enamel colour of teeth becomes translucent as the lightness decreased. There was no statistically significant difference between group 2 and 3.(shown in table 9.1)

TABLE 10– Comparison of T3a* in all the 3 groups(control, microabrasion and laser)

Groups	N	a * Mean (S.D)	95% C.I		ANOVA	P
			Min	Max		
1.**Control**	30	-0.96(0.37)	-1.07	-0.84	7.024	0.001**
2. Microabrasion	30	-0.67(0.14)	-0.71	-0.661		
3. Laser	30	-1.15(0.65)	-1.39	-0.91		

****** Highly Significant ,P <0.005- significant

In Table 10ANOVA showed a statistically significant difference between all three group with P 0.001 and the mean of microabrasion was least among the group.

TABLE 10 .1 – Post Hoc- Bonferroni correction between the groups in T3a*

Comparison of groups	Mean difference	Std error	Sig
1.Control 2.Microabrasion	-.1923	.13088	.436
1.Control 3.Laser	.2947	.13088	.081
2.Microabrasion 3.Laser	.4870(*)	.13088	.001

** Highly Significant, P <0.005- significant

In table 10.1 Post Hoc test-Bonferroni correction showed a significant difference between microabrasion treated group2 and laser treated group 3 and it signifies the microabrasion group showed better improvement in chromaticity in red- green axis. (shown in table 10.1)

TABLE 11– Comparison of T3b* in all the 3 groups(control, microabrasion and laser)

Groups	N	b * Mean (S.D)	95% C.I		ANOVA	P
			Min	Max		
1.Control	30	-2.92 (1.26)	-3.08	-2.14	0.384	0.682
2. Microabrasion	30	-2.81(0.44)	-2.95	-2.64		
3. Laser	30	-2.64(0.85)	-2.95	-2.32		

** Highly Significant ,P <0.005- significant

On comparison between three groups at T3 they showed no statistical significance difference (P =0.682).the laser treated group showed the least. (Shown in table 11)

TABLE 11.1 –Post Hoc- Bonferroni correction between the groups in T3b*

Comparison of groups	Mean difference	Std error	Sig
1.Control 2.Microabrasion	.1883	.23557	1.000
1.Control 3.Laser	.0210	.23557	1.000
2. Microabrasion 3.Laser	-.1673	.23557	1.000

** Highly Significant ,P <0.005- significant

Post Hoc test-Bonferroni correction showed no statistical significant difference on comparison between the groups.(shown in table 11.1)

TABLE 12– Comparison of T4 L* in all the 3 groups(control, microabrasion and laser)

Groups	N	L* Mean (SD)	95% C.I		ANOVA	P
			Min	Max		
1. Control	30	46.93(5.16)	44.99	48.85	133.559	0.000**
2. Microabrasion	30	45.97(1.98)	45.22	46.70		
3. Laser	30	61.93(4.84)	60.11	63.73		

** Highly Significant ,P <0.005- significant

Five days after discolouration T4,

On analysis, the data using ANOVA, there is a statistical significant difference. The mean of laser was highest which signifies least discolouration (shown in table 12)

TABLE 12.1–Post Hoc- Bonferroni correction between the groups in T4L*

Comparison of groups	Mean difference	Std error	Sig
1.Control 2.Microabrasion	.9600	1.09525	1.000
1.Control 3.Laser	-15.0000(*)	1.09525	.000
2.Microabrasion 3.Laser	-15.9600(*)	1.09525	.000

** Highly Significant, P <0.005- significant

In table 12.1-On Bonferroni correction there is a significant difference between the groups 1 & 3 and group 2 & 3. This signifies the laser treated group 3 was showing resistance to discolouration.

TABLE 13– Comparison of T4 a* in all the 3 groups (control, microabrasion and laser)

Groups	N	a* Mean(SD)	95% C.I		ANOVA	P
			Min	Max		
1.Control	30	3.93(0.92)	3.59	4.27	275.182	0.000**
2. Microabrasion	30	2.73(1.13)	2.30	3.15		
3. Laser	30	-1.50(0.74)	-1.77	-1.22		

** Highly Significant ,P <0.005- significant

On comparing the three groups at T4 a* there is a statistically significant association between them. The laser treated group3 least mean indicating minimal colour change with shift towards red spectra.

TABLE 13 .1 –Post Hoc- Bonferroni correction between the groups in T4a*

Comparison of groups	Mean difference	Std error	Sig
1.Control 2.Microabrasion	1.2037(*)	.24323	.000
1.Control 3.Laser	5.4323(*)	.24323	.000
2.Microabrasion 3.Laser	4.2287(*)	.24323	.000

** Highly Significant, P <0.005- significant

In table 13.1, the Post Hoc test-Bonferroni correction in between all three groups showed a statistical difference. The mean difference laser is least which signify the chromaticity in red / green axis shift was minimal.

TABLE 14– Comparison of T4 b* in all the 3 groups(control, microabrasion and laser)

Groups	N	b* Mean (SD)	95%C.I		ANOVA	P
			Min	Max		
1.Control	30	9.03(2.66)	8.03	10.02	190.215	0.000**
2. Microabrasion	30	6.57(1.27)	6.09	7.04		
3. Laser	30	0.18(1.09)	-0.2	0.58		

** Highly Significant ,P <0.005- significant

At T4, 5 days after discolouration,

In the table 14, there is a statistical significant difference among three groups in b*. laser treated group 3 showed the least indicating mild shift to yellow spectra

TABLE 14 .1 – Post Hoc-Bonferroni correction between the groups in T4b*

Comparison of groups	Mean difference	Std error	Sig
1.Control 2.Microabrasion	2.4560(*)	.46842	.000
1.Control 3.Laser	8.8490(*)	.46842	.000
2.Microabrasion 3.Laser	6.3930(*)	.46842	.000

** Highly Significant ,P <0.005- significant

The three groups showed statistical significant difference between them. The mean difference of control group 1 was less followed by microabrasion treated group2 and laser group 3. This shows laser treated group 3 has minimal shift towards yellow whereas the control and microabrasion group showed significant shift towards yellow chromaticity of spectra.

The colour change between post treatment time was calculated as delta E. The visible colour change (ΔE) was clinically detectable when it exceeded 3.7and calculated by (CIE Colorimetry Publication,1986):

$$\Delta E=[(L_1^*-L_2^*)^2 + (a_1^*-a_2^*)^2 + (b_1^*-b_2^*)^2]^{1/2}$$

Table 15 – Comparison of colour change ΔE1(T1-T2) groups 2 and 3. ΔE1 shows the colour change between white spot lesion (T1) and post microabrasion / laser treatment (T2).

$$\Delta E1=[(T1L^*-T2L^*)^2 + (T1a^*-T2a^*)^2 + (T1b^*-T2b^*)^2]^{1/2}$$

Groups	***N***	***ΔE1 Mean***	***SD***	***Std error***	***T***	***P***
2- Microabrasion	30	18.43	8.84	1.61	0.963	0.339
3 -Laser	30	16.51	6.40	1.17		

** Highly Significant, P<0.005- Significant

The colour change (ΔE1) among group 2 and 3 was statistically analyzed using unpaired t test and Post hoc- Bonferroni test. ΔE1 shows no significant difference between the groups. The microabrasion treated group shows more colour change than the laser group but it is not statistically significant (table 15)

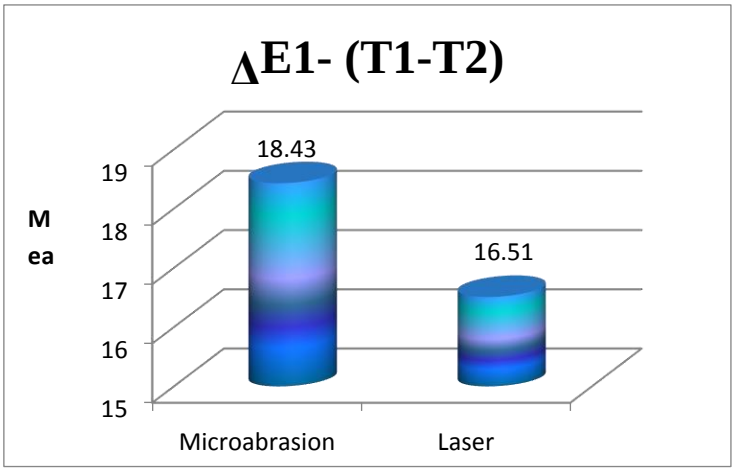


Table 16 – Comparison of colour change ΔE2(T2-T3) groups 1, 2 and 3. ΔE2 - shows the colour change between posttreatment T2 and after 30 days storage in artificial saliva (T3)

ΔE2=[(T2L*-T3L*)2 + (T2a*-T3a*)2 + (T2b*-T3b*)2]1/2

Groups	***N***	***ΔE 2 Mean***	***SD***	***Std. Error***	***T***	***P***
2.Microabrasion	30	5.24	3.69	0.67	0.366	0.716
3.Laser	30	5.59	3.77	0.69		

** Highly Significant, P<0.005- Significant

The colour change (ΔE2) was analyzed between treated groups. Unpaired T test with Bonferroni correction was used for comparing the groups and there is no statistically significant difference between treated groups 2 and 3. The laser treated group showed more colour change than the microabrasion group but it is not statistically significant.

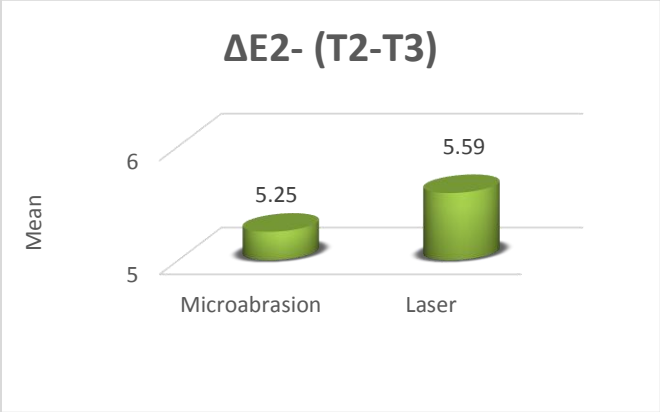


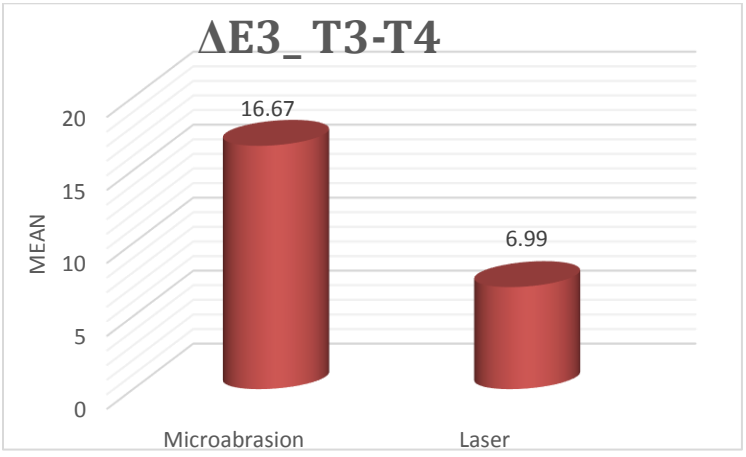
TABLE 17– Comparison of colour change ΔE3(T3-T4) groups 1, 2 and 3 and ΔE3 - shows the colour change after 30 days storage in artificial saliva T3 and after discolouration cycle (T4)

$$\Delta E3=[(T3L^*-T4L^*)^2 + (T3a^*-T4a^*)^2 + (T3b^*-T4b^*)^2]^{1/2}$$

Groups	***N***	***ΔE3 Mean***	***SD***	***Std error***	***T***	***P***
2.Microabrasion	30	16.67	4.31	0.79	8.39	0.000**
3.Laser	30	6.99	4.61	0.84		

** Highly Significant, P<0.005- Significant

The ΔE3 data are seen in table 17. The t test with post hoc Bonferroni correction was done. The comparison of delta E3 between microabrasion treated group2 and laser group3 there is a statistically significant difference. The mean the microabrasion group is highest and the laser treated group 3 was less, signifying that the laser group 3 shows the least colour change after discolouration.



DISCUSSION

DISCUSSION

The post orthodontic white spot lesion which occurs due to poor oral hygiene maintenance during orthodontic treatment detracts the esthetics of a patient's smile. Prevention of these white, opaque areas during and after orthodontic treatment is the first line of defense in providing patients with the most esthetic outcome. Occurrence of white spot lesion even after the effective preventive measures, has become a major concern for the orthodontist as these white spot lesions cause unesthetic appearance on the surface of newly debonded teeth.

WSL are surface lesion that are reversible, can be treated to revert back to their original enamel colour. Some of the treatment modalities are fluoride/CPPACP therapy (varnish, tooth paste, mouthwashes etc.,) microabrasion, resin infiltration.[19] In recent studies laser treated teeth has shown a significant resistance to enamel demineralization and also aids in uptake of more fluoride and calcium/phosphate ions[60,62].

The present invitro study was done on 90 extracted human premolar stored in saline until the commencement of study[51]. In the present study, the white spot lesion was created artificially by exposing teeth to Modified pH cycle model by Featherstone 1998 which showed a clinical correlation with the caries development. Many investigators have followed this technique to create enamel demineralization for studying the effects of various remineralizing agents.[45,62.]

In the present study, the colour improvement and its resistance to discolouration of the WSL was evaluated using spectrophotometer. The spectrophotometer are accurate and reliable method, it provides 33% increase in accuracy and also on objective colour

match they were showed 93.3% reliability when compared with visible eye detection and conventional method. Vita Easyshade advanced 4.0 (Vita Zahnfabrik), which is cordless, small, portable, cost efficient, battery operated, contact-type was used in the present study.[49]

CIE Lab color system developed the measurement and evaluation of colour differences of materials. This colour system developed by CIE lab is most acceptable method as each color occupies a unique location in the 3D CIE Lab color space. The colour difference ΔE is used to evaluate perceptibility of colour differences.[65,66]

Compared to the natural unaffected teeth, the teeth with white spot lesion are opaque and have their chromaticity shifted more towards green and blue.

Microabrasion is a conservative and effective method. The surface of the enamel is polished with acid-abrasive paste which removes the discoloured enamel lesion and leaves a glossy enamel surface.[44,46] Murphy et al[22] reported that there is 83% reduction in the size of lesion and the treatment is long standing. Microabrasion with 37% phosphoric acid was used in the current study. [43,45]

Laser treatment of incipient carious lesion is a conservative approach and is used widely. The laser treated teeth were resistance to acid.**[54,60]** For the past 30 years, with the intention of choosing the best laser wavelength and parameters to effectively promote selective caries removal with minimal thermal consequences, various investigation were carried out and laser treated with different laser like Er:YSGG, Er:YAG, CO2 laser showed significant resistance to demineralization in subablative level with minimal

thermal effect. In the present study, the parameters used for irradiation of white spot lesion is by Curylofo-Zotti F A, et al[62]

At post microabrasion and laser treatment T2, The L* of microabrasion group 2 showed more changes than group1 and 3, which implies that the whitish enamel lesions became more translucent. The a* of microabrasion group 2 showed a significant shift from green of spectra towards the red. This signifies the chromaticity of enamel from green has reduced showing improvement in colour. The b* of microabrasion group 2 showed a significant shift from blue axis towards yellow, denoting that the chromaticity improvement.

Thus, the microabrasion treated enamel becomes translucent, the chromaticity of the spectra also increased denoting diminishing post orthodontic white spot lesion. This result was in accordance with Akin et al[49]and Yetkiner et al[50]

Microabrasion technique improves esthetic results by removing a thin physical discolored enamel layer and significant colour improvement immediately after the treatment.[38]Smooth and lustrous surface is obtained shows different optical properties can enhance the esthetic appearance.[22]

This is attributed to the fact that the colour of teeth we visualize is due to refraction of light, that occurs in the dentin through interprismatic spaces of enamel and they are transmitted back. On microabrasion the minerals of enamel are compacted into interprismatic spaces, resulting in polished surface causing improvement in colour of teeth.[49] The study by Waggoner[69] stated that on initial application of the microabrasion

procedure 12µm of enamel thickness is removed and on subsequent application 26 µm is removed. 5-10 applications are done to successfully eliminate the lesion.[38]

The laser group 3 showed a decrease in L* but it was less than microabrasion group 2. Laser group 3 shows a shift towards red in a* and towards yellow in b.*The parameter used for irradiating laser was subablative and they showed minimal temperature change. The colour improvement signifies the diminution of whitish appearance of WSL. This was similar to the study by Myer[23] et al who irradiated mode locked YAG- neodymium laser on pit and fissure and the discolouration in the incipient lesion was removed on irradiation and the colour of teeth was similar to adjacent enamel. The control group 1 showed no change as it was untreated.

The colour change delta E1 between WSL (T1) and post microabrasion and laser treatment (T2) does not have significant difference but colour improvement in microabrasion group 2 was better which was similar to the study by Akin et al,[49] Yetkinger et al [50]and Jahanbin et al.[51]

After 30 days storage in artificial saliva, (T3) the L* of microabrasion slightly increased but was less when compared to laser, the chromaticity a* and b*showed mild shift to yellow and blue respectively.

The L* of laser showed significant difference with showing increase in lightness which means the teeth become more translucent. The chromaticity of b* improved in laser showing mild shift towards blue. The tooth colour improvement after storage in artificial saliva signify that remineralization has occurred. The control group showed a significant colour improvement but not close to baseline value.

The colour change delta E2 between post treatment (T1) and post treated teeth placed in artificial saliva(T3) did not show any statistically significance change but the laser treated group 3 showed more colour improvement than the microabrasion group 2. This may attribute to remineralization as stated by Duncan[70]who stated that lased enamel has increased affinity towards free ions calcium, fluoride & phosphate and they lead to remineralization.

After discolouration T4, The L* of T4 showed significant difference with laser group 3 showing increase in lightness which means the teeth become more translucent but microabrasion group 2 and control group 1 showed a significant decrease leading to decrease in lightness. The chromaticity of a* improved in laser group 3 but increased in control group 1 and group 2 with shift towards red spectra. The b* showed increase in indicating mild shift towards yellow and in group1 and 2, b* readings are high indicating more discolouration.

The colour change delta E3 between post treated teeth stored in artificial saliva (T3) and after discolouration (T4) showed significant colour change. The laser treated group showed more colour improvement than the microabrasion group2. The microabraded group 2 picked up more discolouration and it is in accordance with Jahanbin et al[52]and Yetkiner et al[51.]

Immediately after treatment with microabrasion group 2 and laser group 3 shows improvement in colour. Post discolouration laser group3 showed less discolouration than microabrasion group2 as laser treated teeth were resistant to pick up stains.

SUMMARY AND CONCLUSION

SUMMARY & CONCLUSION

This study has been done

1) To compare the colour masking effect of microabrasion (orthophosphoric acid with pumice) and laser (Er: YAG) on post orthodontic white spot lesion using spectrophotometer and

2) To compare resistance of microabrasion and laser treated enamel surface of white spot lesion to discolouration (tea and coffee solution) using spectrophotometer.

90 human extracted premolar teeth were divided into three groups of 30 each, they were group1 – control group, group 2 – microabrasion treated group and group 3- laser treated group. The samples were bonded with premolar brackets and they were subjected to modified pH cycle model to form white spot lesion.

The white spot lesions in group 2 were treated with microabrasion and group3 were treated with laser and their colour improvement was measured using spectrophotometer(T2). The colour difference ΔE1 between WSL(T1) and post microabrasion/laser treatment (T2) was assessed and the colour improvement of the treated groups2 and 3 were significant and on comparison between the treated groups 2 and 3 they show no statistical significant difference

The samples were then stored in artificial saliva for 30 days and colour was assessed (T3). The colour difference ΔE2 between post microabrasion/laser treatment (T2) and after storage in artificial saliva (T3) was assessed and the colour improvement of the treated groups2 and 3 were seen but not statistically significant.

After samples were subjected to discolouration, the readings were taken as(T4). The colour difference ΔE3 between the two measurements, after storage in artificial saliva (T3) and discolouration(T4) were assessed. The microabrasion group2 picked up stains and laser group3 showed less colour change.

In conclusion,

1. Microabrasion and laser treatment was effective in treating post-orthodontic white spot lesion.
2. Both laser and microabrasion treated WSL, stored in artificial saliva shows colour improvement indicative of remineralization.
3. Microabrasion treated WSL, when subjected to discolouration stained more than the laser treated WSL which may necessitate repeated microabrasion on long term follow up.
4. Laser treated WSL shows resistance to staining when subjected to discolouration, this is indicative that lasers can be used for treating WSL.

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ANNEXURE

INSTITUTIONAL ETHICS COMMITTEE
VIVEKANANDHA DENTAL COLLEGE FOR WOMEN

SPONSORED BY : ANGAMMAL EDUCATIONAL TRUST

Ethics Committee Registration No. ECR/784/Inv/TN/2015 issued under Rule 122 DD of the Drugs & Cometics Rule 1945.

Dr. J. Baby John	**Chair Person**	**Dr. (Capt.) S. Gokulanathan**	**Member Secretary**
Mr. K. Jayaraman	Social Scientist	Mr. A. Thirumoorthy	Legal Consultant
Dr. R. Jagan Mohan	Clinician	Dr. N. Meenakshiammal	Medical Scientist
Dr. B.T. Suresh	Scientific Member	Dr. R. Natarajan	Scientific Member
Dr. Sachu Philip	Scientific Member	Mr. Kamaraj	Lay Person

No: VDCW/IEC/02/2015 Date: 14.12.2015

TO WHOMSOEVER IT MAY CONCERN

Principal Investigator: Dr. K.Preethi

Title: Assessment of treatment of Post-orthodontic White spot lesion with microabrasion and laser using spectrophotometer: An invitro study.

Institutional ethics committee thank you for your submission for approval of above proposal .I t has been taken for discussion in the meeting held on 04 .12.15.The committee approves the project and it has no objection on the study being carried out in Vivekanandha Dental College For Women.

You are requested to submit the final report on completion of project. Any case of adverse reaction should be informed to the institutional ethics committee and action will be taken thereafter.

CHAIRMAN
INSTITUTIONAL ETHICS COMMITTEE
VIVEKANANDHA
DENTAL COLLEGE FOR WOMEN
Elayampalayam-637 205
Tiruchengode (Tk) Namakk
Tamilnadu.

SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
VIVEKANANDHA
DENTAL COLLEGE FOR WOMEN
Elayampalayam-637 205.
Tiruchengode (Tk) Namakkal (Dt),
Tamilnadu.