"A Comparative Evaluation of Biomimetic Enamel Remineralization Potential on White Spot Lesions: An Ex-Vivo Study"

DISSERTATION

Submitted to the

BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR PRADESH

In the partial fulfillment of the requirement for the degree of

MASTER OF DENTAL SURGERY

In the subject of

CONSERVATIVE DENTISTRY & ENDODONTICS

Submitted by

DR. PRIYANKA MAHAJAN

Under the guidance of

DR. VISHESH GUPTA

Professor

DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

BATCH: 2021-2024 **Enrolment No.: 12103222928**

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LUCKNOW

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"A Comparative Evaluation of Biomimetic Enamel Remineralization Potential On White Spot Lesions: An Ex-Vivo Study "** is a bonafide, & genuine research work carried out by me under the guidance of **Dr.Vishesh Gupta, Professor,** Department of Conservative Dentistry & Endodontics, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

DATE: 19/02/24

PLACE: Lucknow

Priyanka Mahajan

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BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

CERTIFICATE BY THE GUIDE&CO-GUIDE

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DECLARATION BY THE CANDIDATE

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To **Divine Providence**, whose blessings make everything possible.

Thank you Almighty, for the shower of blessings, that gave me strength, patience and zeal to complete my dissertation and thank you for blessing me with such wonderful people in my life.

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At last I would like to apologize to those, whose names I have inadvertently missed but they should find solace that their knowledge has been extended to others by way of this dissertation.

DR. PRIYANKA MAHAJAN

ABBREVIATIONS	FULL FORM
ANOVA	Analysis of Variance Analysis
mm	Millimeter
WSLs	White Spot Lesions
$PO4^{3-}$	Phosphate ion
H^{+}	Hydrogen ion
CPP-ACP	Casein Phosphopeptide Amorphous Calcium Phosphate
nHAP	nano-hydroxyapatite
nm	Nanometer
TH	Thymol
mL	Millilitre
cm	Centimeter
NaF	Sodium Fluoride
%	Percentage
mM	Millimoles
gm	Grams
VHN	Vickers Hardness Number
Ca	Calcium
P	Phosphorus
Zn	Zinc
Mn	Manganese
Se	Selenium
Na	Sodium
PE	Polyethylene
PEG	Polyethylene glycol
SAP	Self-assembling peptide

GIC	Glass ionomer cement
Ca/P	Calcium to Phosphorous ratio
α	Alpha
β	Beta
3-D	Three Dimensional
Si-OH	Silanol
ppm	Parts per million
F^-	Fluoride ion
ACP	Amorphous Calcium Phosphate
Ca^{2+}	Calcium ion
OH^-	Hydroxyl ion
Nano-HA	Nano-hydroxyapatite
BAG	Bioactive glass
SPSS	Statistical Package for Social Sciences
$(HPO_4)^{2-}$	Hydrogen phosphate ions

AIM: The aim of the present ex-vivo study was to evaluate and compare the biomimetic enamel remineralization potential on white spot lesions.

MATERIALS AND METHOD: Forty single rooted human permanent mandibular premolars extracted due to periodontal or orthodontic reasons were chosen for this study. The teeth were decoronated at 1mm apical to Cemento-enamel junction level. The coronal half of the teeth were then split vertically retaining the buccal portion of the coronal part of the teeth. The samples were then mounted in auto-polymerization acrylic resin in standardized circular silicon mould with buccal surface facing the observer. All the samples were subjected to demineralization process to induce white spot lesions. A Pre and Post demineralization Vickers hardness test was performed followed by random allocation of samples in the four groups. The four groups were namely Group A (Curodont Protect Gel), Group B (GC Tooth Mousse Plus), Group C (Dente 91) and Group D (Regenerate Enamel Science). A post remineralization Vickers hardness test was conducted to evaluate the enamel remineralization potential of tested agents.

STATISTICAL ANALYSIS: An analysis of variance (ANOVA) was performed to determine the significant difference of Vickers microhardness.

RESULTS: Group C (Dente 91) showed the highest microhardness value and hence proved excellent biomimetic enamel remineralization potential agent among tested other competitors like Group A (Curodont Protect Gel), Group D (Regenerate Enamel Science) and Group B (GC Tooth Mousse Plus).

CONCLUSION: In the present study Group C (Dente-91) was found more efficient than Group A (Curodont Protect Gel) followed by Group D (Regenerate Enamel Science) and Group B (GC Tooth Mousse Plus) for the biomimetic enamel remineralization potential on white spot lesions.

Keywords: Demineralization, Remineralization, Vicker microhardness, White spot lesions

Dental caries in enamel is unique among diseases, as enamel is both acellular and avascular, hence does not exhibit the potential to repair by a cellular mechanism of its own.[1]

Sub-surface carious lesions or white spot lesions are probably considered as early indication of dental caries disease.[2] White spot lesions (WSLs) is the first clinical sign of enamel demineralization which progress to dental caries or an arrested demineralized area if untreated at an early stage.[3]

In neutral environment, the hydroxyapatite of the enamel is in equilibrium with saliva which is saturated with calcium and phosphate ions.[4] At or below pH 5.5, hydroxyl ions produced by the bacterial metabolites react preferentially with the phosphate group of the enamel crystals, converting phosphate ion (Po_4^{3-}) to $(HPO_4)^{2-}$ ion which once formed can no more form the crystal lattice. At the same time, H^+ ions are buffered. This leads to enamel dissolution or demineralization.[5,6]

However, the demineralization can be reversed if the pH is neutralized and there are sufficient calcium and phosphate ions available in the immediate environment thereby rebuilding the dissolved apatite crystals. This is called remineralization. To maintain the enamel equilibrium, either remineralization must be enhanced or demineralization must be retarded.[7]

Modern dentistry advocates the non-invasive style for the management of non-cavitated, subsurface lesion via remineralization.[8] The most established element for prevention against the caries is fluoride, which aims to harden the mineral surface layer and inhibit its progression.[9]

Fluoride can interact with saliva on the enamel surface and sub-surface and combines with phosphate and calcium ions to form a new and large crystals that contain more fluoride (Fluor-hydroxyapatite) that enhance remineralization.[10,11]

Nevertheless, concerns have been raised recently with the wide array of both prescription and over-the-counter fluoride products that are now being marketed in every country. The total fluoride intake has increased to harmful levels that has resulted in problems in organ system of normal individuals.[12]

Considering the disadvantage of total fluoride intake, there has been a demand for its alternative. A new remineralizing agent CPP-ACP is a bioactive agent with a milk product base that binds to hydroxyapatite and supplies free calcium and phosphate ions, thereby promoting remineralization and reforming into calcium phosphate crystals thereby, preventing demineralization.[13] The casein phosphopeptide (CPP) contain multiphosphoseryl sequences with the ability to stabilize calcium phosphate in nano complexes in solution like amorphous calcium phosphate (ACP). CPP binds to ACP in metastable solution preventing the dissolution of calcium and phosphate ions. They also act as reservoir of bio-available calcium and phosphate ions, and maintains the solution supersaturation state thereby, facilitating remineralization.[14]

Recently, nanotechnology has brought up a great deal of attraction. Nano-hydroxyapatite has been used as bone grafting material, scaffolds for bone tissue engineering, dental implant coatings, soft tissue repair material, desensitizing and remineralizing agents.[15] The basic foundation of enamel unit is found to be composed of hydroxyapatite particle sized 20nm to 40nm. When enamel reaches its maturation, the proteins are almost completely degraded or removed, which leads to the crystallization of apatite, therefore the enamel cannot be biologically remodeled.[16] The Nano-hydroxyapatite has shown to have similar morphology, structure and crystallinity as a biological apatite and hence is placed under biomimetic material.[17] The Nano sized particles directly fill up any small porosity on demineralized surfaces and act as a scaffold for further precipitation that attracts calcium and phosphate from saliva to the enamel surface for the formation of a new apatite layer.[18] They have strong affinity towards enamel and appears be a potent biocompatible and bioactive material that has achieved acceptance in dentistry in the recent years.[19]

The conventional remineralization is the procedure where by calcium and phosphate ions are precipitated from external source to the tooth surface in order to promote ion deposition into the crystal voids of demineralized enamel to facilitate mineral ions gain.

Recently introduced Biomimetic remineralization is an approach that mimics the natural process of mineralization. It is generally accepted that the biomimetic synthesis of enamel like apatite structures under a physiological condition is an attractive bioavailable alternative of replacing defective enamel by stimulating the

biomineralization process.[20] A recent product named Regenerative Enamel Science which is based on NR-5 technology is a mixture of calcium silicate, salts of sodium phosphate and fluoride focused to enhance hydroxyapatite remineralization via minerals nucleation in tooth enamel in vicinity of saliva.[21] Another modern Self-assembling peptides (P11-4), is a biomimetic remineralizing agent that form a three dimensional matrix which helps in the remineralization of subsurface lesions. The Curodont repair technology present in Curodont Protect Gel offers monomeric self-assembling peptides (P11-4) which are able to produce a new hydroxyapatite crystal nucleation and support mineral crystal development in a cycle of biomimetic mineralization.[22,23]

Therefore, the aim of the present ex-vivo study was to evaluate and compare the biomimetic enamel remineralization potential on white spot lesions.

AIM OF THE STUDY:

The aim of this ex-vivo study was to evaluate and compare the biomimetic enamel remineralization potential on white spot lesions.

OBJECTIVES OF THE STUDY:

1. To evaluate and compare biomimetic enamel remineralization potential of Curodont Protect Gel on white spot lesions.
2. To evaluate and compare biomimetic enamel remineralization potential of GC Tooth Mousse Plus on white spot lesions.
3. To evaluate and compare biomimetic enamel remineralization potential of Dente 91 on white spot lesions.
4. To evaluate and compare biomimetic enamel remineralization potential of Regenerate Enamel Science on white spot lesions.

1. Humel MMC, Oliveira MT , Cavalli V , Giannini M (2006) evaluated the effect of storage and disinfection methods (SDM) on bond strength (BS) of enamel among 100% Humidity (HU); Gamma Radiation (GR); Autoclave (AU); 0.1% Thymol (TH); 10% Formalin (FO); Frozen (FR); 0.2% Sodium Azide (SA) and 0.5% Chloramine and concluded that there was no change in the bond strength among the tested agents.[24]

2. Andersson A, Skold-Larsson K, Hallgren A, Petersson LG, Twetman S (2007) compared the effects of a dental cream containing complexes of Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP) and fluoride mouthwashes on the regression of WSLs by remineralization. They concluded that both the treatment could promote the regression of white spot lesions, but CPP ACP provided a more favourable aesthetic outcome.[25]

3. Pai D, Bhat SS, Taranath A, Sargod S, Pai VM (2008) performed an in vitro study to evaluate the remineralization of incipient enamel lesions by the topical application of CPP ACP. They concluded that CPP ACP can prevent demineralization and also bring about remineralization in enamel lesions.[26]

4. Lata S, Varghese NO, Varughese JM (2010) evaluated the remineralization potential of fluoride and ACP-CPP and the combination of ACP-CPP and fluoride on early enamel lesions in vitro. It was concluded that ACP-CPP cream is effective, but to a lesser extent than fluoride in remineralizing early enamel caries at surface level. Combination of fluoride and ACP-CPP does not provide any additive remineralization potential compared to fluoride alone.[27]

5. Jayarajan J, Janardhanam P, Jayakumar P, Deepika (2011) evaluated the remineralization potential of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF) on enamel surface on which artificial caries lesion was created in vitro. It was concluded that CPP-ACPF (Tooth Mousse-Plus) showed more amount of remineralization than CPP-ACP (Tooth Mousse).[28]

6. Najibfard K, Ramalingam K, Chedjieu I, Amaechi BT (2011) tested following remineralizing agents 5% nHAP, 10% nHAP, 1100 ppm fluoride and 10% for remineralization on molars and concluded that nHAP agent caused remineralization and inhibited caries development.[29]

7. Krithikadatta J, Fredrick C, Abarajithan M, Kandaswamy D (2013) evaluated the efficacy of 10% casein phosphopeptide amorphous calcium phosphate complex (CPP-ACP) used alone or with fluoride as compared to fluoride mouthrinse for the remineralization of occlusal white spot lesions in an in vivo pilot study. They concluded that CCP-ACP technique was found highly beneficial for the remineralization of non-cavitated occlusal white spot lesions when compared to 0.5% NaF mouthrinse.[30]

8. Patil N, Choudhari S, Kulkarni S, Joshi SR (2013) assessed remineralization potential of fluoride (NaF 0.2%), CPP-ACPF (Tooth Mousse-Plus) and TCP-F on enamel surface and concluded that CPP-ACP (Tooth Mousse) was effective as compared to other tested agents.[31]

9. Mehta R, Nandlal B, Prashanth S (2013) conducted an in vitro study that evaluated and compared the remineralization potential of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP) on artificial white spot enamel lesions. They inferred that significant amount of remineralization was shown by CPP-ACP and CPP-ACFP only after the 7th day.[32]

10. Brunton PA, Davies RP, Burke JL, Smith A, Aggeli A, Brookes SJ *et.al* (2013) assessed about the safety and potential clinical efficacy of a single application of (P11-4) on early enamel lesions. They concluded that treatment of early caries lesions with (P11-4) is safe, and that a single application is associated with significant enamel regeneration, by promoting mineral deposition within the subsurface tissue.[33]

11. De Carvalho FG, Vieira BR, Santos RL, Carlo HL, Lopes PQ, De Lima BA (2014) analyzed the protective effect of remineralizing agents on enamel caries lesions among fluoride varnish (Duraphat); nHAP paste (DesensibilizeNano P) and CPP ACPF paste (MI Paste Plus). They concluded that nHAP paste has protective effect against in-vitro enamel caries development.[34]

12. Gamal1 M , El-Baily A , Osman M , Marwa S (2017) evaluated the ability of nano-hydroxyapatite (Nano-HAP) to remineralize the demineralized enamel layer around the orthodontic brackets. They concluded that Nano-HAP to be effective in repairing the demineralized enamel around the orthodontic brackets, restoring the enamel surface smoothness and its color.[35]

13. Soares R, De Ataide IN, Fernandes M, Lambor R (2017) evaluated the ability of Casein Phosphopeptide-Amorphous Calcium Phosphate Fluoride (CPP ACPF), Bioactive Glass (BAG), fluoride enhanced Hydroxyapatite (HA) gel and self-assembling peptide (P11-4) to remineralize artificial carious lesions. They concluded that Self assembling peptide (P11-4) was effective in remineralizing the enamel lesions.[36]

14. Kamath P, Nayak R, Kamath SU, Pai D (2017) compared and evaluated the remineralization potential of commercially available agents containing Nano-hydroxyapatite (nano-HA), Casein phosphopeptide amorphous calcium phosphate fluoride (CPP-ACPF), and Tricalcium phosphate (TCP) on artificially induced white spot lesions. All test agents were comparable in their remineralization potential.[37]

15. Eltayeb MK, Ibrahim YE, El Karim IA, Sanhouri NM (2017) evaluated the prevalence, pattern of distribution and contributing factors to WSLs' development. They concluded that the prevalence for each tooth was: 48.1% in the canine, 32.3% in the lateral incisor, 31.6% in both the central incisor and the first premolar, 27.2% in the second premolar and 8.9% in the first molar.[38]

16. Sharma A, Rao A, Shenoy R, Suprabha BS (2017) evaluated and compared the remineralizing efficiency of the paste containing hydroxyapatite and casein phosphopeptide-amorphous calcium phosphate. They concluded that Nano-hydroxyapatite is more effective as compared to Casein phosphopeptide- amorphous calcium phosphate, in increasing the Calcium and Phosphorus content of enamel.[39]

17. Kamh RA, Niazy MA, El-Yasaky MA (2018) evaluated and compared the remineralization potential of different biomimetic materials like Self-assembling peptide (P11-4), highly concentrated sodium fluoride agent and combination of fluoride, hydroxyapatite and xylitol paste on the white spot lesions. Among all the

tested materials had varying remineralization potential though Hydroxyapatite was the most efficient. Self-assembling peptides (P11-4) was beneficial in resisting the acid challenge. All agents were clinically efficient in management of white spot lesions.[40]

18. Indrapriyadharshini K, Madan Kumar PD, Sharma K, Iyer K (2018) assessed the long term remineralizing potential of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) only in paste form compared with fluoride varnish, and or placebo in both naturally occurring and post-orthodontic white spot lesions in vivo. It was concluded that there was high evidence of remineralizing potential of CPP-ACP on naturally occurring white spot lesion and WSL post orthodontic treatment in comparison with placebo/fluoridated toothpaste and fluoride varnish.[41]

19. Madhusudanan SV, Pillai P, Varghese R, George N, Antony S, Abe (2018) evaluated and compared the microhardness of artificially demineralized human enamel treated with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) containing toothpaste, hydroxyapatite containing toothpaste. They concluded that Hydroxyapatite paste showed better remineralization potential when compared to paste which contain calcium and potassium ions.[42]

20. Vijayasankari V, Asokan S, Geetha Priya PR (2019) analysed the remineralization potential among nano-hydroxyapatite (nHAP) paste and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on artificial caries lesions. They concluded that commercially available and experimental nHAP have the potential to remineralizing artificially induced carious lesions.[43]

21. Manchery N, John J, Nagappan N, Subbiah GK, Premnath P (2019) evaluated and compared the remineralization ability of dentifrices containing Nano-hydroxyapatite, NovaMin, and amine fluoride on artificial enamel caries. Nano-hydroxyapatite agent produced significantly better results compared to fluoride and NovaMin containing dentifrices, instigating for its use in the management of early carious lesions.[44]

22. Tahmasbi S, Mousavi S, Behroozibakhsh M, Badiee M (2019) compared the efficacy of NaF, CPP-ACP, MI Paste Plus and Remin-Pro for prevention of enamel demineralization. It was concluded that NaF is more efficient than Remin-Pro and MI Paste for prevention of white spot lesions.[45]

23. Kobeissi R, Badr SB, Osman E (2020) quantitatively and qualitatively compared the effectiveness of the Self-assembling peptide (P11-4) vs tricalcium phosphate fluoride (TCPF) in remineralization of WSLs in young permanent teeth. However, they concluded that WSL recovery was significantly better in the Self-assembling peptide group, reflecting an excellent remineralization potential of the WSLs by the SAP (P11-4) compared to TCPF varnish.[46]

24. Juntavee A, Juntavee N, Hirunmoon P (2021) compared remineralization potential of nano-HA toothpaste (NHT), functionalized tricalcium phosphate toothpaste (TCPT), and fluoride toothpaste (FT) on carious lesions. It was concluded that NHT had the potential to remineralize artificial carious lesion. It was confirmed in potential in the lesion depth reduction and forming a new enamel layer.[47]

25. Tripathi P, Mengi R, Gajare SM (2021) compared the remineralizing efficacy of NovaMin, CPP-ACP, silver diamine fluoride (SDF) and P11-4. They concluded that Self-assembling peptides showed maximum remineralization in tested specimens followed by CPP-ACP, SDF, and NovaMin containing toothpaste.[48]

26. Alaghal E, Samy A (2021) evaluated the impact of two remineralizing agents containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and tricalcium phosphate (TCP) on white spot lesion. It was concluded that CPP-ACP had higher remineralizing effect when compared to TCP.[49]

27. Walaa A , Galal S , Tamer ES (2021) assessed the remineralizing potential of Self-assembling peptide (P11-4), phosphorylated chitosan and nano-hydroxyapatite paste on subsurface carious lesions and they concluded Self-assembling peptide (P11-4) showed the highest biomimetic remineralizing mean value.[50]

28. Sankaranarayanan RR , Navaneethan R (2022) compared the efficacy of remineralization of white spot lesions between two green synthesized nano particle varnishes namely nano silver fluoride and nano-hydroxyapatite varnish. They concluded that green synthesized sodium silver fluoride n-NSF can be considered as an option for arresting white spot lesions in orthodontic patients.[51]

29. Gohar RA, Ibrahim SH, Safwat OM (2023) evaluated the clinical performance of Self-assembling peptides versus fluoride-based delivery systems in post Orthodontic white spot lesions. They concluded that the biomimetic remineralization using Self-assembling peptides and the fluoride-based varnish material showed a similar effect in masking post-orthodontic white spot lesions. Self-assembling peptides reveal higher performance in subsurface remineralization than the fluoride-based varnish material.[52]

30. Amin O, Shaalan O, Riad M (2023) compared the remineralization potential of Curodont Repair Fluoride Plus Versus CPP -ACP in management of white spot lesions. They concluded that Curodont Repair Fluoride Plus is a biomimetic remineralizing agent that provides a therapeutic option for enamel regeneration. Curodont repair fluoride plus provided a better chance for complete healing of incipient lesions.[53]

The present ex-vivo study was conducted in the Department of Conservative Dentistry and Endodontics, Babu Banarasi Das College of Dental Sciences, Lucknow in collaboration with Praj Metallurgical Laboratory, Pune.

Forty human permanent single rooted mandibular premolar teeth were collected as per inclusion and exclusion criteria fulfillment. The collected teeth were cleaned with hand instrument (spoon excavator) and ultrasonic scaler, thereafter polished with wet pumice slurry paste using the low speed contra-angle handpiece. These teeth were stored in 0.1 % thymol solution until further use.

Eligibility Criteria:

Inclusion criteria:

1. Non-carious, sound and intact human single rooted mandibular premolar teeth with normal morphology.

2. Tooth with no enamel surface defect.

Exclusion Criteria:

1. Teeth with any craze line or crack and caries.

2. Teeth with any mineralization defect.

3. Teeth with any non-carious lesions like attrition, abrasion, erosion, abfraction.

4. Teeth with any previous restoration.

Table 1: Materials and Armamentarium

Sr. No	Material & Armamentarium	Manufacturer
1.	Micromotor straight handpiece Micromotor contra-angle handpiece	Marathon, Korea NSK, Japan
2.	Vickers Hardness Machine.	(Reichert Austria hardness tester Sr.No.363798), Austria
3.	Diamond disc & Mandrel (grit No. 180)	Horico, Germany
4.	Cold Cure Acrylic Resin	DPI India
5.	Spoon excavator	GDC, India
6.	Ultrasonic scaler	Biosonic,India
7.	Pumice powder	Ajanta Pvt.Ltd
8.	Silicon circular mould of diameter (2cm)	Epoxy, India

Table 2: Materials and Armamentarium Used for Demineralizing, Rinsing and Storage of the Samples

Sr. No	Material & Armamentarium	Manufacturer
1	2.2 mM Potassium dihydrogen phosphate $KH_2PO_4 \cdot 7H_2O$	M. M. Arochem Pvt Ltd, India
2	2.2mM Calcium chloride, $CaCl_2 \cdot 2H_2O$	J J chemicals, India
3	0.05mM Lactic acid, $C_3H_6O_3$	Novartis Ltd, India
4	Deionized Water	Charco Chemicals, Punjab, India

5	0.1% Thymol solution	Ricca chemicals, India
6.	Artificial saliva	ICPA Health Products Ltd., India
7.	Digital pH meter	Kheraexim, India
8.	Beaker(100mL)	Ajanta Pvt.Ltd, India
9.	Blue check liquid dye	Vista blue, India

Table 3: Materials and Armamentarium Used for Remineralizing the Samples

Sr. No	Material & Armamentarium	Manufacturer
1	Curodont Protect Gel	Credentis, Switzerland
2	GC Tooth Mousse Plus	GC America INC, USA
3	Dente 91	Frimline Pvt.Ltd, India
4	Regenerate Enamel Science	Unilever, UK
5.	Painting brush(Liner 2)	Camel, India

MATERIAL PROFILE

Group A: Curodont Protect Gel (Credentis; Switzerland, Europe)

Composition as per manufacturer- Peptide P11-4 (amino acid sequence: Ace-GIn-GIn-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH_2), Hydrogenated starch hydrolysate, Aqua, Hydrated silica, Cellulose Gum, Sodium Monofluorophosphate, Aroma, Sodium Saccharin, Citric acid, Sodium hydroxide, Dicalcium Phosphate, Oligopeptide-104, Calcium Glycerophosphate, Sodium Chloride, Sodium Sulphate.

Peptide treatment for early caries lesion is the area of current research. Anionic P11-4 is a safe rationally designed self-assembling peptide. Curodont Protect Gel is a novel treatment, developed by Credentis, for the early treatment of tooth decay and other dental lesions (prior to cavitation). The treatment was based on a novel technology

called “Curolox”. When applied to the tooth the peptide diffuses into the subsurface micro-pores and forms a three dimensional scaffold made up of small fibres. The scaffold mimics proteins found in teeth development and supports hydroxyapatite (a calcium phosphate ceramic which make up to 50% of bones) crystallisation around it to regenerate tooth enamel, over a period of three months. The use of biomimetic peptide such as P11-4 has the advantage of effecting natural repair by regenerating the mineral itself.

Group B: **GC Tooth Mousse Plus (GC America INC, USA)**

Composition as per manufacturer -Pure water, Glycerol, RECALDENT (CPP-ACP), D-Sorbitol, CMC-Na, Propylene glycol, Silicon dioxide, Titanium dioxide, Xylitol, Phosphoric acid, Sodium Fluoride, Flavoring, Sodium saccharin, Ethyl p-hydroxybenzoate, Zinc oxide, Propyl p-hydroxybenzoate, Butyl p-hydroxybenzoate. Originated from ‘α’ and ‘β’ casein containing specific sequence acid motif. Consists of three serine phosphate and followed by two glutamic acid residues cluster sequence. At neutral pH these acid motifs are highly charged have ability to bind to minerals Ca, Zn, Mn, Se and Fe. Seryl group which are the main site for calcium. It modulates bio-availability of calcium phosphate levels by maintaining ionic phosphate and calcium super saturation to increase remineralization.

Amorphous Calcium Phosphate (ACP): Control the precipitation of CPP with calcium and phosphate ions. It has the advantage of availability of calcium, phosphate, and fluoride in one product. Each molecule of CPP can bind up to twenty-five Ca^{2+} ions, $PO4^{3-}$ fifteen ions, and five F^{-} ions. Under alkaline conditions the calcium phosphate is present as an alkaline amorphous phase complexed by the CPP referred to as Casein Phosphopeptide- Amorphous Calcium Phosphate (CPP-ACP).

CPP-ACP technology: CPP-ACP binds readily to the surface of the tooth as well as to the bacteria in the plaque surrounding the tooth and deposits a high concentration of ACP near the tooth surface. It diffuses into dental plaque displaying a buffering capacity counteracting pH drop caused by acidogenic bacteria. A high concentration of calcium and phosphate in the dental plaque makes it acid resistant remineralized enamel and reduce the risk of enamel demineralization. CPP keeps calcium and phosphate in an amorphous non-crystalline state which can easily enter enamel. Increase in the

concentration of ions in the lesion results in the formation of Hydroxyapatite or fluorapatite via crystal growth. Direct binding of CPP to plaque bacterial surface results in reduction of calcium and phosphate diffusion into the plaque.

Group C: **Dente 91 (Frimline Pvt. Ltd, India)**

Composition as per manufacturer- Lactoferrin and nano-hydroxyapatite (nHAP)

Nano-hydroxyapatite: A Hydroxyapatite in nano particle crystallite form. Thermodynamically stable form of calcium phosphate nano particles in the 20 nm size ($1/850^{th}$ the width of a human hair) mimic the building blocks of natural enamel. Hydroxyapatite crystals can effectively penetrate the dentin tubules and obturate them and can cause closure of the tubular openings of the dentin with plugs within 10 minutes as well as a regeneration of a surface mineral layer. It is also used as an anticarious agent at pH <7 and to repair enamel.

They produce bioactive calcium and phosphate which penetrates more into porosities beneath the demineralized region as potential remineralizing substances. Nano-hydroxyapatite has the potential to precipitate on the lesion surface because of its strong bioactivity coupled with physical and chemical with natural enamel. A concentration of 10% nano-hydroxyapatite is optimal for remineralization of early enamel caries. Hydroxyapatite has been used in toothpastes (as fillers), Pit-and-fissure sealants, GIC and Composites. Nano-hydroxyapatite containing dentifrices can be recommended in children and those who are concerned with dental fluorosis. It is strongly recommended in to xerostomic patients with diminished amount of saliva. It can be used in the treatment of dentin hypersensitivity.

Group D: **Regenerate Enamel Science (Unilever, UK)**

Composition as per manufacturer - Glycerin, Calcium Silicate, PEG-8, Hydrated Silica, Trisodium Phosphate, Sodium Phosphate, Aqua, PE-60, Sodium Lauryl Sulfate, Sodium Monofluorophospate, AromaFlavour, Synthetic Fluorphlogopite, Sodium Saccharin, Polyacrylic Acid, Tin Oxide, Limonene, C177891

Regenerate Enamel Science is the first oral care system that is able to regenerate tooth enamel mineral, reversing the early enamel erosion process, keeping teeth healthy and strong. 80% of common tooth problems, such as sensitivity, transparency and yellowing, are caused by enamel erosion and acid attacks. Regenerate Toothpaste helps to regenerate enamel minerals and helps to restore natural whiteness while strengthening teeth against cavities.

METHODOLOGY

Sample collection

Forty recently extracted sound permanent single rooted mandibular premolars were collected due to orthodontic and periodontal reasons from private clinics after taking patient consent. The collected teeth were cleaned with hand instrument (Spoon excavator) and ultrasonic scaler, thereafter polished with wet pumice slurry paste using low speed contra-angle handpiece. [PlateI; Figure1] These teeth were stored in 0.1 % thymol solution until further use.

Specimen preparation

The coronal portion of the teeth were separated from the root by sectioning the teeth coronally 1 mm apical to the Cemento-enamel junction using a diamond disc (grit No.180). [PlateI; Figure2, Figure3] Now the retained crown portions were sectioned vertically into buccal and lingual halves using the same diamond disc attached to straight handpiece thereby, retaining the buccal portion of the coronal part of the teeth. [PlateI; Figure4] The sectioned buccal segments were placed in silicon circular moulds of standard dimensions of 2cm and filled with self-cure acrylic resin, until the resin set with buccal surface facing the observer. [Plate I; Figure 5]

Baseline microhardness value using Vickers microhardness testing machine was determined on each sample before demineralizing the samples in a demineralization medium.

Preparation of demineralizing solution [Plate II; Figure 6]

The composition of the demineralizing solution used as follows:

2.2 mM Potassium dihydrogen phosphate, $KH_2PO_4 \cdot 7H_2O$ [Figure 6(a)]

2.2 mM Calcium chloride, $CaCl_2 \cdot 2H_2O$ [Figure 6(b)]

0.05 mM Lactic acid, $C_3H_6O_3$ [Figure 6(c)]

The acetic acid buffer was added to adjust the ultimate pH at 4.5. [Figure 6(d)]

All the samples were subjected to demineralization to induce white spot lesions (WSLs) with the help of demineralization medium. The pH of the demineralizing solution was checked during and after preparation of solution using a digital pH meter. [Plate II; Figure 7]

Preparation of artificial caries-like lesions

All the samples were submerged into a beaker containing 50mL of the prepared demineralizing solution [Plate II; Figure 8] for a period of 96 hours at 37°C to produce an artificial subsurface enamel lesion in all samples. The solution was changed regularly to avoid the build-up of minerals from demineralization and the resulting pH shift.

Blue check liquid was painted onto the sample and blue regions are identified showing the location of demineralization with a high-contrast blue color. [Plate II; Figure 9]

After demineralization, the samples were washed with deionized water [Plate II; Figure 10] for 30 seconds and dried with an air syringe for five seconds and thereafter the samples were subjected to Vickers microhardness test to evaluate the hardness value of all the 40 samples at demineralization level.

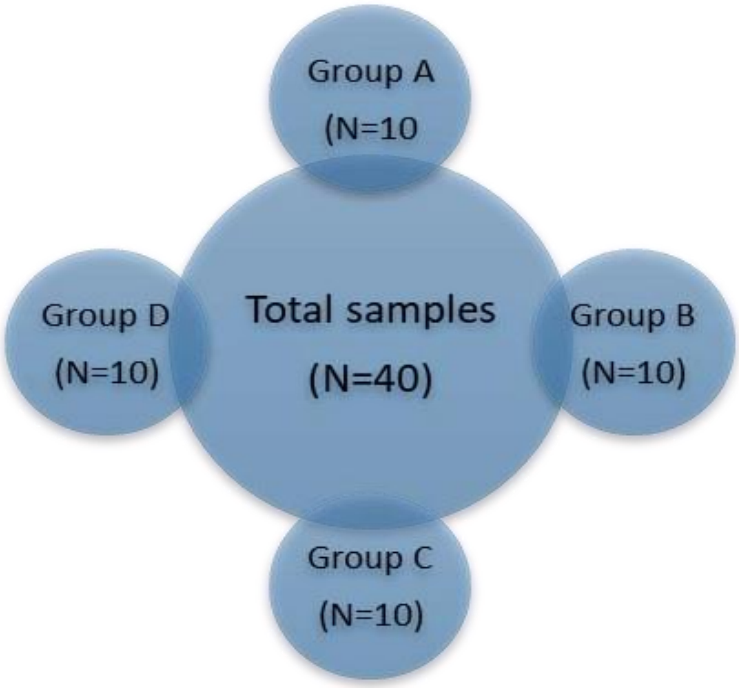
Remineralization

Consequent to this process of demineralization, the forty samples were allocated randomly into one of the following four groups (10 in each group) depending on the remineralizing agents [Plate III; Figure 11]

Table 4: Distribution of Samples

GROUP A (N=10)	**GROUP B (N=10)**	**GROUP C(N=10)**	**GROUP D (N=10)**
Curodont Protect Gel	GC Tooth Mousse Plus	Dente 91	Regenerate Enamel Science

Distribution of samples in each Group



The samples in respective groups were treated with respective remineralizing agent for 3 minutes twice daily using a painting brush (Liner 2) for 14 days. [Plate III; Figure 12] and thereafter were washed with deionized water and stored in artificial saliva in a beaker [Plate III; Figure13, 14]

After 14 days cycles of remineralization, the surface microhardness of the specimens was determined using the Vickers microhardness testing machine. [Plate IV; Figure 15,16]

Vickers Microhardness Testing

The surface microhardness was measured using VHN testing.

The circular mould with embedded sample was kept on the Vickers Microhardness testing machine where the pyramid-shaped indenter (stylus) rested on buccal surface of embedded sample. A load rate of 100 gm (Reference Standardise 6507) was produced for about 20 seconds never close to any edge of the sample. Three indentations were used to assess the specimen's average microhardness.

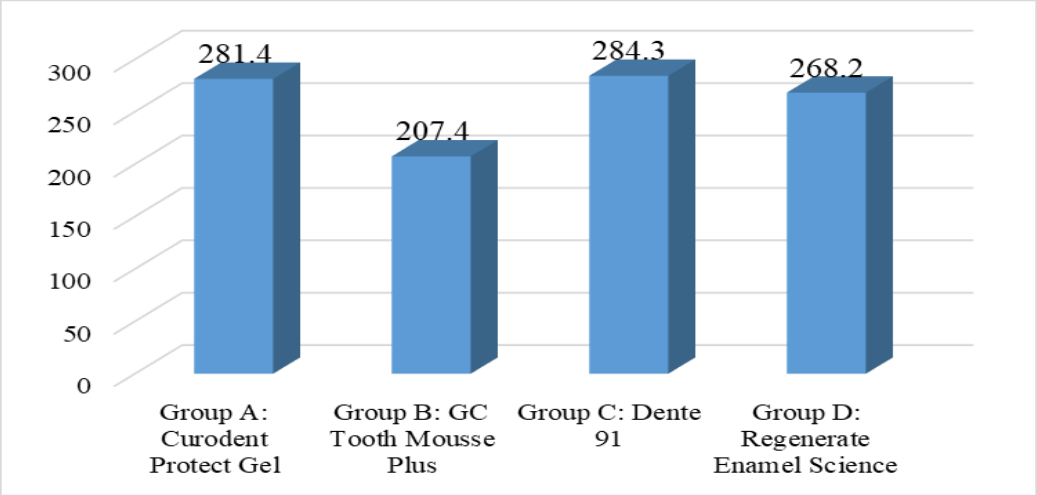
From the objective (sample) a diagonal distance of indentation was measured. The VHN of the specimens was determined by the test, and the difference between the baseline, post-demineralization and remineralization values measured using the identical Vickers indenter settings to calculate the change in the VHN for the surface microhardness investigations.

The observations were laid down and statistically analyzed further.

The one-way analysis of variance (ANOVA) test was used to check mean differences among the groups. **Software:** SPSS (Statistical Package for Social Sciences) Version 24.0 (IBM Corporation, Chicago, USA).

Table 5: Mean and Standard deviation

Groups	Mean	Standard Deviation
Group A: Curodont Protect Gel	281.4	19.979
Group B: GC Tooth Mousse Plus	207.4	32.657
Group C: Dente 91	284.3	49.713
Group D: Regenerate Enamel Science	268.2	30.01



Graph 1: Mean and Standard deviation

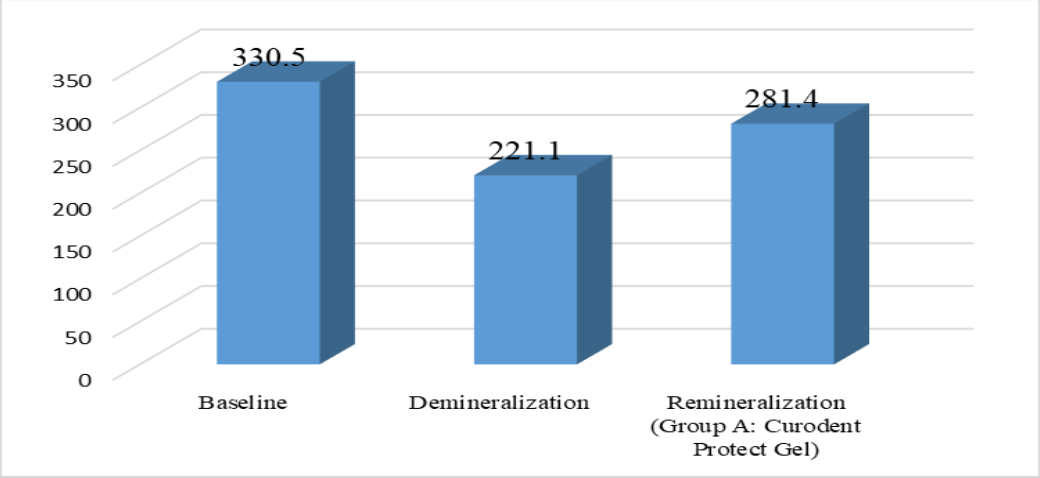
The mean Vickers hardness values provide insights into the demineralizing potential of the tested agents. Among the four groups, DENTE 91 demonstrated the highest mean value at 284.30, indicating a substantial increase in enamel hardness following treatment. This suggests that DENTE 91 has a strong subsequent effect on remineralization, making it a promising entity for increase enamel hardness. On the other hand, GC TOOTH MOUSSE PLUS exhibited the lowest mean Vickers hardness value at 207.40, implying a comparatively lower degree of remineralization.

Thus, GC TOOTH MOUSSE PLUS seems to have a less subsequent impact on enamel remineralization compared to the other tested agents. In summary, based on the mean Vickers hardness values, DENTE 91 appears to be the most effective in promoting remineralization, while GC TOOTH MOUSSE PLUS shows a relatively weaker

subsequent effect in enhancing enamel hardness among the tested demineralizing agents. The following are the mean values for the four categories of dental care products: The means of the following groups are as follows: Group A, which used Curodont Protect Gel, achieved 281.4; Group B, which utilised GC Tooth Mousse Plus, achieved 207.4; Group C, which utilised Dente 91, achieved 284.3; and Group D, which utilised Regenerate Enamel Science, achieved 268.2. The means in question symbolised the average results obtained from each group, thereby offering a graphical depiction of the data's central tendency with respect to each individual dental service product.

Table 6: Mean microhardness of Group A (Curodont Protect Gel) at Baseline, Demineralization and Remineralization stage

	N	Mean	Std Deviation	t Value	p-Value
Baseline	10	330.5	34.433	30.353	0.000
Demineralization	10	221.1	51.47	13.584	0.000
Remineralization (Group A: Curodont Protect Gel)	10	281.4	19.979	44.54	0.000



Graph 2: Mean microhardness of Group A (Curodont Protect Gel) at Baseline, Demineralization and Remineralization stage

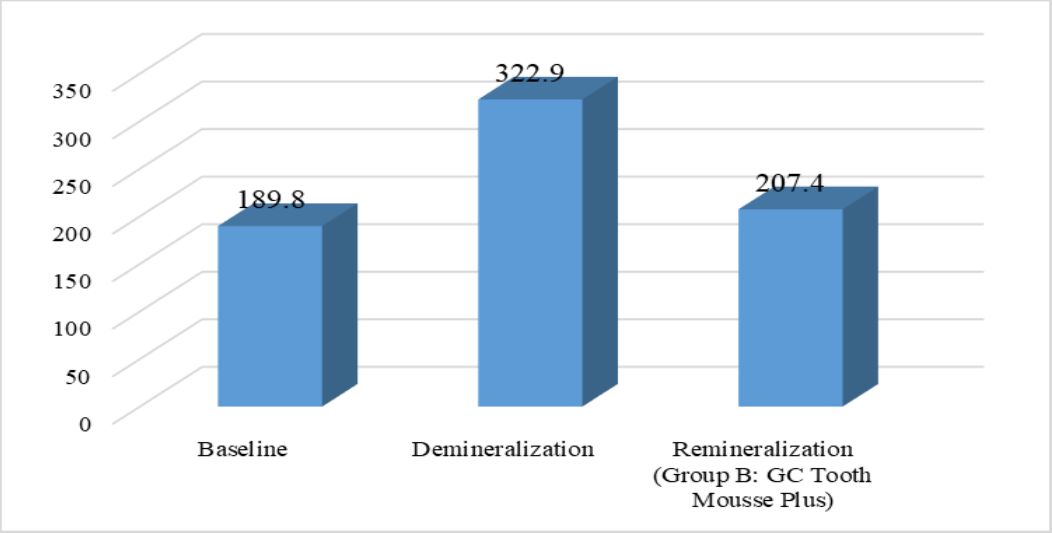
The mean micro hardness of four groups (Group A: Curodont Protect Gel) at the baseline, demineralization, and demineralization stages is compared in the table. The mean basal micro hardness is considerably greater at 330.5 in comparison to the demineralization and demineralization stages (mean = 221.1 and 281.4, respectively).

The standard deviation signifies the degree of variability present within each category, whereby demineralization exhibits the least amount of variability. Comparisons between baseline and demineralization, baseline and remineralization, and demineralization and demineralization have respective t-values of 30.353, 44.54, and 13.584. The analysis of variance (ANOVA) results indicate significant mean differences among groups. Baseline mean was 30.353, Demineralization mean was 13.584, and Demineralization (Group A: Curodont Protect Gel) mean was 44.54. The p-values for all groups were 0, signifying strong evidence of differences in the means.

Formula = $$F = \frac{\text{Between-group variability (MSB)}}{\text{Within-group variability (MSW)}}$$

Table 7: Mean microhardness of Group B (GC Tooth Mousse Plus) at Baseline, Demineralization and Remineralization stage

	N	Mean	Std Deviation	t Value	p-Value
Baseline	10	189.80	33.129	18.117	.000
Demineralization	10	322.90	19.530	52.283	.000
Remineralization (Group B: GC Tooth Mousse Plus)	10	207.40	32.657	20.283	.000



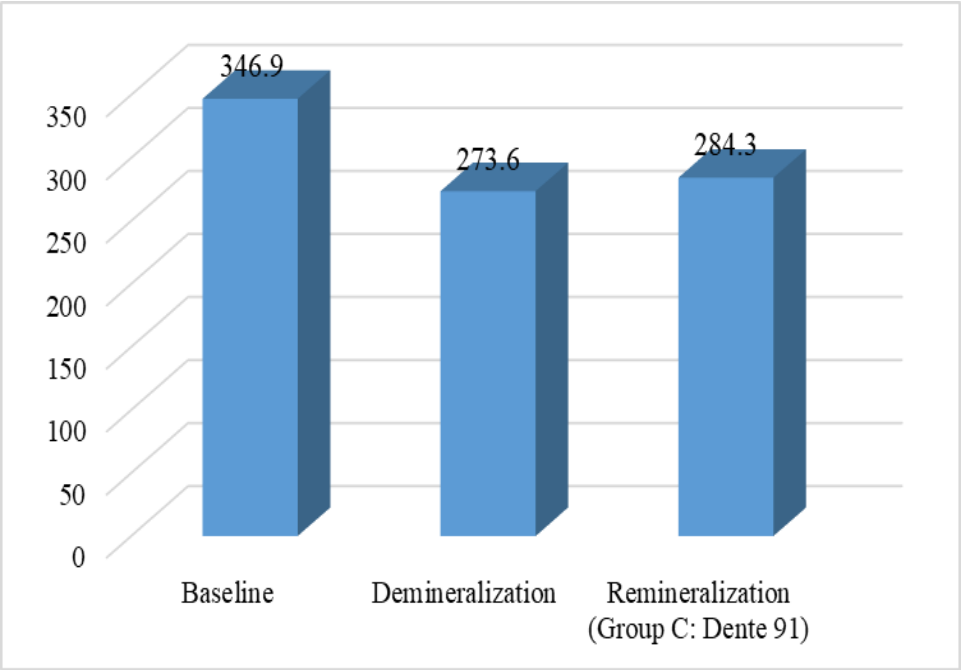
Graph 3: Mean microhardness of Group B (GC Tooth Mousse Plus) at Baseline, Demineralization and Remineralization stage

The mean microhardness of four groups (Group B: GC Tooth Mousse Plus) at the baseline, demineralization, and demineralization stages is compared in the table. The mean of the basal microhardness values is 189.80, which represents the initial level of hardness. As a result of demineralization, the average microhardness of the teeth increased substantially to 322.90, indicating a considerable decrease in their mineral content. The mean microhardness decreased to 207.40 during the remineralization phase, but remained considerably greater than the initial value, indicating a partial restoration of mineralization. The standard deviations of each stage of data represent the dispersion of data points around the mean. The outcomes of the analysis of variance (ANOVA) reveal statistically significant variations in the mean values across the categories. With a p-value of zero, the basal mean (18.117) differs substantially from demineralization (52.283) and demineralization with GC Tooth Mousse Plus (20.283). This indicates that demineralization and demineralization interventions have distinct effects on the parameter under investigation.

Formula- $F = \frac{\text{Between-group various}}{\text{Between-group variance}}$

Table 8: Mean microhardness of Group C (Dente 91) at Baseline, Demineralization and Remineralization stage

	N	Mean	Std Deviation	t Value	p-Value
Baseline	10	346.90	43.144	25.426	.000
Demineralization	10	273.60	50.116	17.264	.000
Remineralization (Group C: Dente 91)	10	284.30	49.713	18.085	.000



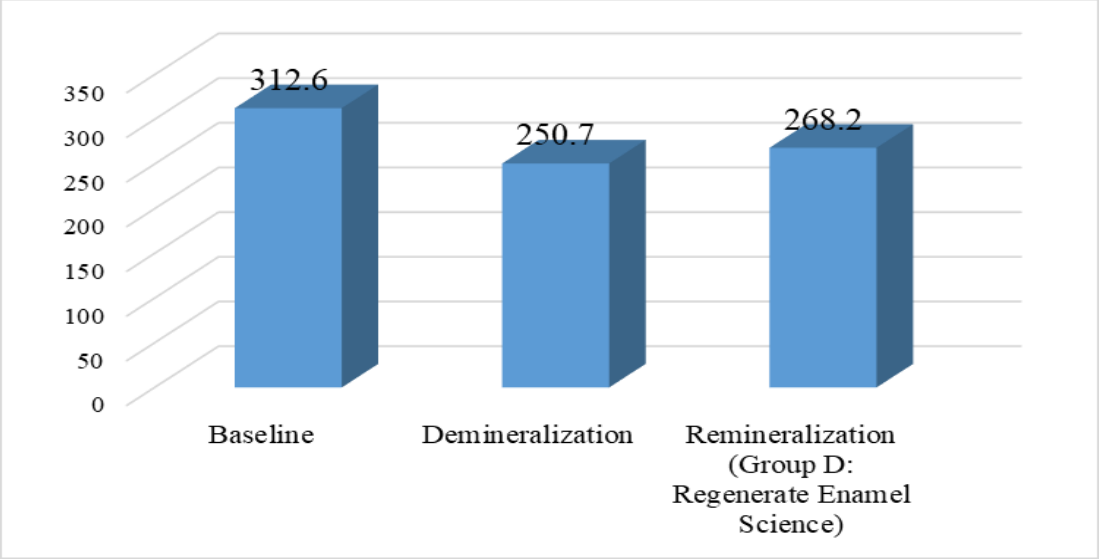
Graph 4: Mean microhardness of Group C (Dente 91) at Baseline, Demineralization and Remineralization stage

The table presents a comparison of mean micro hardness in four groups (Baseline, Demineralization, and Remineralization) at different stages (Group C: Dente 91). Microhardness measurements, expressed as Vickers hardness numbers (VHN), were taken from 10 samples in each group. At baseline, the mean microhardness was significantly higher at 346.90 VHN, with a low standard deviation of 43.144. During the demineralization stage, there was a notable decrease in mean microhardness to 273.60 VHN, accompanied by a slightly higher standard deviation of 50.116. The remineralization stage showed an improvement in microhardness, with a mean value of 284.30 VHN and a standard deviation of 49.713. Statistical analysis using t-tests revealed highly significant differences ($p < 0.001$) between the groups at each stage, indicating that the variations in microhardness were not likely due to random chance. The analysis of variance (ANOVA) results indicate significant differences in mean values among the groups. Baseline group exhibited the highest mean t-value (25.426), followed by Demineralization (17.264) and Demineralization (Group C: Dente 91) (18.085). All p-values are 0, suggesting strong evidence of group differences in the studied parameters.

$$t = \frac{\text{Mean Group 1} - \text{Mean Group 2}}{\sqrt{\frac{S1^2}{N1} - \frac{S2^2}{N2}}}$$

Table 9: Mean microhardness of Group D (Regenerate Enamel Science) at Baseline, Demineralization and Remineralization stage

	N	Mean	Std Deviation	t Value	p-Value
Baseline	10	312.60	36.050	27.421	.000
Demineralization	10	250.70	50.524	15.691	.000
Remineralization (Group D: Regenerate Enamel Science)	10	268.20	30.010	28.261	.000



Graph 5: Mean microhardness of Group D (Regenerate Enamel Science) at Baseline, Demineralization and Remineralization stage

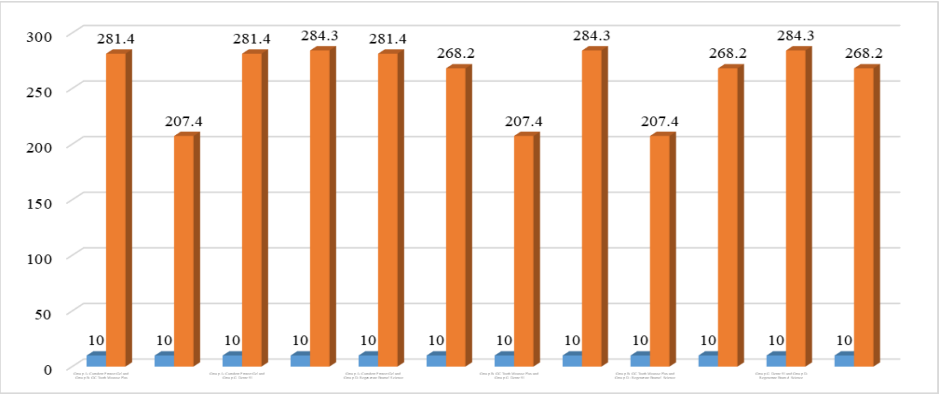
The table illustrates a comparison of the average microhardness of three distinct phases (Baseline, Demineralization, and Remineralization) across four groups, with Group D Undergoing Regenerate Enamel Science receiving particular attention. Distinct patterns of microhardness values, expressed in Vickers hardness units, are observed throughout the phases. The mean microhardness at baseline is 312.60, accompanied by a standard deviation of 36.050. A significant reduction is noted in the mean value during demineralization, which falls to 250.70, accompanied by an increased standard deviation of 50.524. Following this, microhardness recovers substantially during Remineralization, attaining a mean of 268.20 with a standard deviation of 30.010. All

comparisons have substantial t-values, which indicates that the differences are statistically significant. The analysis of variance (ANOVA) results indicate significant differences among the mean values of baseline (27.421), demineralization (15.691), and demineralization (28.261) groups, with all p-values being 0. This suggests that the groups exhibit statistically significant variations in the context of the studied parameters, indicating a meaningful impact of the treatments.

Formula - $$F = \frac{\text{Between-group various}}{\text{Between-group variance}}$$

Table 10: Comparison of mean microhardness among the tested four groups viz Group A: Curodont Protect Gel, Group B: GC Tooth Mousse Plus, Group C: Dente 91 and Group D: Regenerate Enamel Science

Groups	N	Mean	Std Deviation	t value	p value
Group A: Curodont Protect Gel and Group B: GC Tooth Mousse Plus	10	281.4	19.979	6.266	.000
	10	207.4	32.657		
Group A: Curodont Protect Gel and Group C: Dente 91	10	281.4	19.979	-.153	.882
	10	284.30	49.713		
Group A: Curodont Protect Gel and Group D: Regenerate Enamel Science	10	281.4	19.979	1.043	.324
	10	268.20	30.010		
Group B: GC Tooth Mousse Plus and Group C: Dente 91	10	207.4	32.657	-3.648	.005
	10	284.30	49.713		
Group B: GC Tooth Mousse Plus and Group D : Regenerate Enamel Science	10	207.4	32.657	-5.064	.001
	10	268.20	30.010		
Group C: Dente 91 and Group D: Regenerate Enamel Science	10	284.30	49.713	.970	.353
	10	268.20	30.010		



Graph 6: Comparison of mean microhardness among the tested four groups viz Group A: Curodont Protect Gel, Group B: GC Tooth Mousse Plus, Group C: Dente 91 and Group D: Regenerate Enamel Science

The table presents a comparison of mean microhardness among four groups (A, B, C, and D) using different dental products: Curodont Protect Gel (Group A), GC Tooth Mousse Plus (Group B), Dente 91 (Group C), and Regenerate Enamel Science (Group D). The mean microhardness values, standard deviations, t-values, and p-values are provided for pairwise comparisons between the groups. The findings indicate significant differences in microhardness between Group A (Curodont Protect Gel) and Group B (GC Tooth Mousse Plus) ($t = 6.266$, $p < .001$), with Group A exhibiting higher microhardness. Group B also showed lower microhardness compared to Group C (Dente 91) and Group D (Regenerate Enamel Science) ($t = -3.648$, $p = .005$ and $t = -5.064$, $p = .001$, respectively). The significant differences among the groups were identified through analysis of variance (ANOVA) in present study that compared the efficacy of Regenerate Enamel Science, Curodont Protect Gel, GC Tooth Mousse Plus, and Dente 91. Significant differences were observed in pairwise comparisons among Group B (GC Tooth Mousse Plus), Groups C (Dente 91), and Group D (Regenerate Enamel Science), indicating that the dental products exhibited differing degrees of efficacy.

$$F = \frac{\text{Mean Square Between Groups}}{\text{Mean Square Within Groups}}$$

The present ex-vivo study was conducted in the Department of Conservative Dentistry and Endodontics, Babu Banarasi Das College of Dental Sciences, Lucknow in collaboration with Praj Metallurgical Lab, Pune.

The aim of the study was to evaluate and compare the biomimetic enamel remineralization potential of recent remineralizing agents like Curodont Protect Gel, GC Tooth Mousse Plus, Dente 91 and Regenerate Enamel Science on White Spot lesions (WSLs). Single rooted mandibular premolars were chosen considering that these teeth were commonly extracted due to orthodontic or periodontal reasons hence easily available. Moreover, it has been found that the increased prevalence of WSLs was in mandibular first premolars (31.6%) followed by mandibular second premolars (27.2%).[54] In general,white spot lesions(WSLs) ranges from 23.4% to 75.6% and a prevalence ranges from 33.8% to 97%.[55-58]

In the present study, thymol (0.1%) was used to store extracted teeth as it is an antimicrobial solution. It has been shown that the storage of teeth in thymol for a longer period of time (six months) had no significant effect on the enamel permeability.[59,60]

The methodology of the present study has been followed seeking the research done by Madhusudanan P *et.al*.[61]

In the present study forty single rooted human permanent premolars were chosen as per exclusion and inclusion criteria. All the teeth samples were cleaned and then decoronated. The coronal half of teeth were then split vertically retaining the buccal segment. These samples were then mounted in circular silicon moulds of standard dimensions with an auto-polymerization acrylic resin with buccal surface facing the observer.[62]

Baseline microhardness values using Vickers microhardness test were determined for each sample before demineralization. Thereafter, all the groups were subjected to demineralization to induce white spot lesions with the help of demineralization medium.

When considering demineralization, it has been revealed that the caries progression occurs very slowly and intermittently. Also the demineralization is followed by a period

of rest or even remineralization. The progression of incipient caries may take 3 months to 48 months. If we are able to intervene during this time period, remineralization is possible.[63]

A post demineralization Vickers hardness test was performed.[64] Subsequently, all the demineralized samples were randomly allocated into four groups containing 10 samples in each group and further each group was treated by designated remineralizing agent viz Group A:Curodont Protect Gel, Group B:GC Tooth Mousse Plus, Group C:Dente 91, Group D:Regenerate Enamel Science. A post remineralization Vickers hardness test was performed.[65]

Diagnostic tools like Surface hardness, Surface Profilometry can be used to determine the remineralizing potential of the materials.[66] The main disadvantage of Surface Profilometry was that the stylus penetrates the eroded surface, which is either partially demineralized or completely demineralized enamel area. In enamel, Surface profilometry can cause damage to the natural surface area and can lead to an overestimate of early erosion depth.[67] Vickers hardness test was chosen in the present study because it is less sensitive to prevailing surface conditions during the research among the other microhardness measurement methods and is more technique sensitive to measurement errors when equal loads are applied on a sample.[68]

In the present study, Group C (Dente 91) showed the highest remineralization potential amongst the tested Group A (Curodont Protect Gel), Group D (Regenerate Enamel Science) and Group B (GC Tooth Mousse Plus). The major composition of Dente 91 consist of nano-hydroxyapatite. It's mode of action is due to the adsorption of hydroxyapatite particles on the tooth surface due to their large surface areas and weak inter-crystalline bonds. In white spot lesion, there is dissolution of hydroxyapatite crystals from the subsurface, forming a subsurface lesion with a highly mineralized surface layer.[27] The Ca/P nano-crystals are able to penetrate more deeply into the demineralized subsurface thereby, forming a "reservoir-like" deposit of calcium (Ca^{++}) and phosphate ($PO4^{-3}$) ions.[69]

As there chemical composition is analogous to enamel and dentin, this reservoir-like deposit could make these ions available during a subsequent enamel demineralization that help to maintain a state of supersaturation with respect to enamel minerals.[70]

Nano-HA crystals can easily inhibit enamel dissolution acting as a sacrificial layer or buffering agent, being more readily dissolved than the underlying enamel.[71] Accordingly, materials containing nHAP are able to provide calcium (Ca^{++}) and phosphate ($PO4^{-3}$) ions for reducing tooth demineralization and or improving tooth remineralization. Hydroxyapatite nano particles may better penetrate tooth porosities (Mechanical imbrications) and produce a protective layer on the tooth surface. nHAP helps in providing more mineral deposits on the outer layer than the body of the lesion.[72] In support to the results for Group C (Dente 91) obtained in our study, similar result was revealed in the study of Sara M.*et.al*. It was due to nano-hydroxyapatite bioavailability synthesizing bulk of calcium (Ca^{++}) and phosphate ($PO4^{-3}$) ions thereby, promoting remineralization within the enamel structure such as physiological hydroxyapatite.[73]

However, the results obtained in our study are in contrary with studies done by Kirkham J *et.al*, Brunton PA *et.al*, Schmidlin P *et.al.*[74,75,76]

In the present study Group A (Curodont Protect Gel) showed the second highest remineralization potential as it was able to induce better biomimetic remineralization of early caries lesions in comparison to Group B (GC Tooth Mousse Plus) and Group D (Regenerate Enamel Science). Group A majorly consist of Self-assembling peptide (P11-4). P11-4 is an α-peptide that self-assembles into β-sheet amyloids (protein secondary structure).[77] Due to the peptide backbone pattern of hydrogen bonds between amino hydrogens and carboxyl oxygen atoms, β-sheet are formed, which allows for a 3D-matrix. P11-4 has a hydrogel appearance at low pH(pH <7.4) due to β-sheet-forming domains that promote toughness and strength, similar to what is observed in muscle, tissues and amyloid fibrils.[49,77,78] This is a hybrid polymer–peptide conjugate that can self-assemble into a self-supporting soft hydrogel over dry and wet surfaces areas.This allows for diffusion because of its very low viscosity that ensures deep penetration into the subsurface lesion. Thereby, promoting in depth biomimetic remineralization.[79]

The result of the present study was in accordance with the result obtained by study done by Omnia A *et.al* who found that Self-assembling peptide (P11-4) is a biomimetic remineralizing agent that provides a better chance for complete healing of incipient lesions compared to Group B(CPP-ACP).[52,80]

In contrary to the result obtained from the present study, Wahba N *et.al* stated that Group A (self-assembling peptide) and Group B (CPP-ACP) were unable to prevent surface enamel caries.[81]

In the present study the third highest remineralization potential was shown by Group D (Regenerate Enamel Science) followed by Group B (GC Tooth Mousse Plus). It is majorly composed of calcium silicate particles that have a particular affinity for high energy sites, such as scratches and other surface defects.[81] It is thought that the calcium ions in calcium silicate are exchanged with H^+ in the surrounding fluid, which leads to silanol (Si–OH) formation in the surface layer. This causes an increase in pH and eventually the formation of a negatively charged surface with the Si–O functional group.[82] For this reason, the solution in the immediate vicinity of the surface must therefore be depleted of protons and enriched with calcium. When combined with phosphate ions from surrounding saliva, hydroxyapatite precipitation onto the surface of the calcium silicate takes place. Therefore, the protection and remineralization mechanism is due to a combination of calcium release, pH buffering, and hydroxyapatite formation. The nucleated hydroxyapatite acts as a sacrificial layer to the underlying enamel.[83]

The study conducted by Vicente A *et.al* and Wood J *et.al*. were in accordance with the result obtained from the present study. They found greater demineralization prevention with the application of calcium silicate-based paste as found in Group D (Regenerate Enamel Science) compared with a conventional fluoride toothpaste (1450ppm F^-) and with a non-fluoride toothpaste.[84,85]

In the present study the lowest remineralization potential was shown by Group B(GC Tooth Mousse Plus) due to the reason that they act by localizing ACP on the tooth surface, which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel and preventing demineralization and enhancing remineralization. The poor remineralization potential can be due to the limited availability of calcium and phosphate ions.[86] Moreover, degradation of phosphopeptides can lead to dephosphorylation of CPP by phosphatases substantially thus reducing the ability of phosphopeptides to bind calcium ions and phosphate ions, thereby decreasing the remineralization potential.

The result obtained from the present study was in accordance with the results obtained by the study done by Vijayasankari V *et.al.*[42,86]

In contrast to the result obtained in the present study, Pallepati A *et.al* found that CPP-ACP showed the highest microhardness value thus proving potent enamel remineralization agent among the other tested groups.[87]

The present ex-vivo study aimed to evaluate and compare the biomimetic enamel remineralization potential on white spot lesions and concluded that:

1. All the tested remineralizing agents like Group A (Curodont Protect Gel), Group B (GC Tooth Mousse Plus), Group C (Dente 91) and Group D (Regenerate Enamel Science) had the potential to remineralize the white spot lesions.
2. Among the tested groups, nano-hydroxyapatite remineralizing agent (Group C) had highest remineralization potential in white spot lesions.
3. Nano-hydroxyapatite can be recommended as a biomimetic enamel remineralizing agent that could be beneficial for the patients who are at high risk of demineralization (WSLs).

However, seeking the limitations of in-vitro studies, further in-vivo studies are required to infer a confirmative remineralizing agent to be opted in cases of white spot lesions or early demineralized enamel.

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BABU BANARASI DAS UNIVERSITY

BBD COLLEGE OF DENTAL SCIENCES, LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled "**A Comparative Evaluation Of Biomimetic Enamel Remineralization Potential On White Spot Lesions: An Ex-Vivo Study"** submitted by **Dr Priyanka Mahajan** Postgraduate student in the **Department of Conservative Dentistry and Endodontics** for the Thesis Dissertation as part of MDS Curriculum for the academic year 2021-2024 with the accompanying preform was reviewed by the Institutional Research Committee in its meeting held on **14" September, 2022** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Dr. Puneet Ahuja
Chairperson

Dr. Mona Sharma
Co-Chairperson

BABU BANARASI DAS UNIVERSITY
BBD COLLEGE OF DENTAL SCIENCES, LUCKNOW

BBDCODS/IEC/09/2022 **Dated: 16 September, 2022**

Communication of the Decision of the X" Institutional Ethics Sub-Committee Meeting

IEC Code: 06

Title of the Project: A Comparative Evaluation of Biomimetic Enamel Remineralisation Potential On White Spot Lesions: An Er-Vivo Study.

Principal Investigator: Dr Priyanka Mahajan **Department:** Conservative Dentistry and Endodontics

Name and Address of the Institution: BBD College of Dental Sciences Lucknow**.**

Type of Submission: New, MDS Project Protocol

Dear Dr Priyanka Mahajan.

The Institutional Ethics Sub-Committee meeting comprising following members was held on 1s" September, 2022.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry
2.	Dr. Praveen Singh Samant Member	Prof. & Head, Department of Conservative Dentistry & Endodontics
3.	Dr. Jiji George Member	Prof. & Head, Department of Oral Pathology & Microbilogy
4.	Dr. Amrit Tandan Member	Professor, Department of Prosthodontics and Crown & Bridge
5.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics & Dentofacial Orthopaedics

The committee reviewed and discussed your submitted documents of the curent MDS Project Protocol in the meeting.

The comments were communicated to PI, thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Prof. Dr. Puneet Ahuja
Principal 16/9
BBD College of Dental Sciences
BBD University, Lucknow
PRINCIPAL
Babu Banarasi Das College of Dental Sciences
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Lakshmi Bala 16/09/22
Dr. Lakshmi Bala
Member-Secretary
Institutional Ethics Sub-Committee (IEC)
BBD College of Dental Sciences
BBD University, Lucknow
Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

Master Chart

Date: 18/11/2023

Name : **Dr. Priyanka Mahajan**

Name of Institute : Babu Banarasi Das College of Dental Sciences, Lucknow.

Subject of research : A comparative evaluation of biomimetic enamel remineralization potential on white spot lesions : An ex vivo study.

1.0 Micro hardness -Baseline

Machine Specifications: Micro hardness Tester, Reichert Austria Make, Sr.No.363798, Load- 100 g, Reference Standard: ISO 6507

Sr. No.	Sample No.	Microhardness in HV		Sr. No.	Sample No.	Microhardness in HV
1	Sample No.1	356		21	Sample No.21	390
2	Sample No.2	311		22	Sample No.22	327
3	Sample No.3	397		23	Sample No.23	363
4	Sample No.4	327		24	Sample No.24	390
5	Sample No.5	290		25	Sample No.25	344
6	Sample No.6	356		26	Sample No.26	376
7	Sample No.7	353		27	Sample No.27	363
8	Sample No.8	317		28	Sample No.28	275
9	Sample No.9	288		29	Sample No.29	369
10	Sample No.10	310		30	Sample No.30	272
11	Samples No.11	352		31	Sample No.31	398
12	Samples No.12	341		32	Sample No.32	306
13	Sample No.13	344		33	Sample No.33	312
14	Sample No.14	309		34	Sample No.34	280
15	Sample No.15	296		35	Sample No.35	310
16	Sample No.16	310		36	Sample No.36	300
17	Sample No.17	333		37	Sample No.37	340
18	Sample No.18	321		38	Sample No.38	290
19	Sample No.19	325		39	Sample No.39	270
20	Sample No.20	298		40	Sample No.40	320

1.1 Microhardness -Demineralization

Machine Specifications: Microhardness Tester, Reichert Austria Make, Sr.No.363798

Load- 100 g, Reference Standard: ISO 6507

Sr. No.	Sample No.	Microhardness in HV		Sr. No.	Sample No.	Microhardness in HV
1	Sample No.1	266		21	Sample No.21	296
2	Sample No.2	275		22	Sample No.22	320
3	Sample No.3	298		23	Sample No.23	283
4	Sample No.4	233		24	Sample No.24	230
5	Sample No.5	158		25	Sample No.25	300
6	Sample No.6	181		26	Sample No.26	280
7	Sample No.7	160		27	Sample No.27	320
8	Sample No.8	172		28	Sample No.28	187
9	Sample No.9	214		29	Sample No.29	320
10	Sample No.10	254		30	Sample No.30	200
11	Samples No.11	188		31	Sample No.31	321
12	Samples No.12	183		32	Sample No.32	288
13	Sample No.13	156		33	Sample No.33	220
14	Sample No.14	212		34	Sample No.34	186
15	Sample No.15	170		35	Sample No.35	257
16	Sample No.16	165		36	Sample No.36	256
17	Sample No.17	196		37	Sample No.37	318
18	Sample No.18	150		38	Sample No.38	176
19	Sample No.19	220		39	Sample No.39	218
20	Sample No.20	258		40	Sample No.40	267

1.2 Micro hardness -Remineralization

Machine Specifications: Micro hardness Tester, Reichert Austria Make, Sr.No.363798, Load- 100 g, Reference Standard: ISO 6507

Group A : Curodent Protect Gel				**Group B: GC Tooth Mousse Plus**		
Sr. No.	Sample No.	Micro hardness in HV		Sr. No.	Sample No.	Micro hardness in HV
1	Sample No.1	290		1	Sample No.11	200
2	Sample No.2	283		2	Sample No.12	189
3	Sample No.3	317		3	Sample No.13	170
4	Sample No.4	283		4	Sample No.14	250
5	Sample No.5	250		5	Sample No.15	200
6	Sample No.6	270		6	Sample No.16	190
7	Sample No.7	254		7	Sample No.17	210
8	Sample No.8	280		8	Sample No.18	170
9	Sample No.9	287		9	Sample No.19	225
10	Sample No.10	300		10	Sample No.20	270

Group C: Dente 91				**Group D : Regenerate Enamel Science**		
Sr. No.	Sample No.	Microhardness in HV		Sr. No.	Sample No.	Microhardness in HV
1	Sample No.21	309		1	Sample No.31	258
2	Sample No.22	324		2	Sample No.32	278
3	Sample No.23	290		3	Sample No.33	274
4	Sample No.24	240		4	Sample No.34	280
5	Sample No.25	325		5	Sample No.35	275
6	Sample No.26	290		6	Sample No.36	270
7	Sample No.27	330		7	Sample No.37	320
8	Sample No.28	200		8	Sample No.38	220
9	Sample No.29	325		9	Sample No.39	220
10	Sample No.30	210		10	Sample No.40	287

Experiment Certificate

- Testing of Metal, Rubber, Plastic, Gasket, Foam, Dental.
- Calibration of Durometer, Temp. Sensors, Vernier & Load cells.
- Failure analysis.

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Date: 12/12/2023

TO WHOMSOEVER IT MAY CONCERN

This is to certify that Dr. Priyanka Mahajan Final year MDS, Babu Banarasi Das College of Dental Sciences has done her Vickers Micro hardness testing of teeth samples from Praj Metallurgical Laboratory, Kothrud, Pune (Maharashtra).

TEST CONDUCTED BY

SACHIN
TEST ENGINEER

ASHOK BHAGAT
PROPRIETOR

PRAJ METALLURGICAL LABORATORY PUNE

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