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Evaluation of the Effect of Self Assembling Peptide - Curodont on Microhardness of Bleached Enamel Surface: An In Vitro Study

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Abstract
Introduction: *Hydroxyapatite is the mineral crystalline form of calcium and phosphorus found in the enamel, dentin, cementum, and bone. Bleaching has been known to alter the enamel matrix because of the strong oxidizing effect of hydrogen peroxide and can cause significant mineral loss and decrease in surface hardness. The microscopic loss of tooth structure resulting from this procedure is reversible, and remineralization may be attempted.The aim of this study was to evaluate the remineralization efficacy of self-assembling peptide curodont on bleached enamel.*
Materials and method:
Sixty six freshly extracted premolars were cut sagittally and reduced into fragments measuring 4mm X 4mm. These were impregnated in cold cure acrylic resin with the buccal surface of the crown facing upwards. Specimens were kept in artificial saliva to prevent dehydration. Teeth were randomly divided into three groups. Teeth in group A were not treated with any agent (positive control). 30-40% Hydrogen peroxide was used to bleach the teeth in group B and C. Teeth in group C were treated with curodont daily for seven days . All 3 groups were checked for microhardness of enamel surface.
Result: *The mean microhardness values in descending order: Positive control > Curodont repair > Negative control.*
Conclusion: *Bleaching significantly reduced enamel microhardness. Self assembling peptide P11-4 (CURODONT REPAIR) exhibited a significant difference in remineralizing bleached enamel surface.*
Keywords: *Self assembling peptide P11-4, Vickers microhardness testing machine, curodont, bleaching .*

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I. Introduction

Bleaching is a simple method to treat discolouration of teeth. However it has been known to alter the enamel matrix because of the strong oxidizing effect of hydrogen peroxide and can cause significant mineral loss and decrease in surface hardness.[1,2] Remineralising treatment is well recognized and recieved lot of attention by both clinician and researchers.[3] Present research focuses on peptide treatment by self assembling peptide P11-4, as it can be helpful in the treatment of early carious lesions. [4] Peptide treatment is safe and non invasive and can be a potential treatment for early caries lesions and acceptable to patients.[5] These agents promote restoration of altered enamel structure thus increasing its hardness.

Curodont made up of monomeric self assembling peptide P11-4, which diffuses into subsurface of white spot lesion. The assembled P11-4 scaffold is capable of promoting hydroxyapatite crystals and sustains mineral crystal growth in a process of biomimetic mineralization.[6] Curodont proved to be efficacious for regenerating demineralized tooth tissues on smooth surfaces.

The study aims to evaluate the effectiveness of a remineralising agent CURODONT on bleached enamel surface.

II. Materials And Methods

The present *in vitro* study was conducted in the Department of Conservative Dentistry and Endodontics. The sample collection and study methodology were approved by the Institutional Review Board and Ethics Committee of the Yenepoya Deemed to be University.

Method of Collection of Data

Extracted permanent premolar teeth were collected from the Department of Oral And Maxillofacial Surgery,

Yenepoya Dental College.

Sample Size: 66 extracted teeth
Sample size calculation and selection: Sample size is determined using G* Power software.Effect Size = 0.4 .Minimum sample size required is 66 ie 22 per group with Level of significance = 5% and Power = 80%
Inclusion Criteria

- Human premolars

Exclusion Criteria

- Carious teeth.
- Teeth with anomalies.

Statistical Analysis
Data was expressed in mean and standard deviation. 3 group ANOVA was used to ascertain significant difference between each group. Data was analysed using SPSS ver.22.software ,$p<0.05$ were considered significant.
Division of Groups:
Group A:Unbleached teeth
Group B:Bleached teeth ,not treated with remineralising agent.
Group C:Bleached teeth , treated with curodont
A total of 66 human premolars which were extracted for orthodontic purposes were selected for the study. Teeth with any visible or detectable caries, restorations, hypoplastic lesions, stains, cracks were excluded from the study.After removal of debris, calculus, from the tooth surface, the teeth were stored in artificial saliva until further use.The teeth were decoronated using a carborundum disk attached to a straight hand piece, and the crowns were used for the study. L shaped moulds were used to prepare the self cure acrylic resin block.The crowns were cut sagittally and reduced into fragments measuring 4mm X 4mm. It was impregnated in cold cure acrylic resin with the buccal surface of the crown facing upwards. Specimens were kept in artificial saliva to prevent dehydration. Teeth were randomly divided into three groups. 30-40% Hydrogen peroxide was used to bleach the teeth in group B and C. Teeth in group C(n=22) was treated with curodont daily for seven days . All 3 groups were checked for microhardness of enamel surface.

Evaluation of Surface Micro Hardness after 3 weeks

After cycle of remineralization, the microhardness of the specimens was determined using Vickers hardness testing machine. A load of 100g was exercised gradually to the surface of specimens for 15s using Vickers elongated diamond pyramid indenter under a 40x objective lens. Three indentations were placed on the surface and the average value was considered for each specimen.

III. Results

The mean microhardness values in descending order: Positive control > Curodont repair > Negative control. Bleaching significantly reduced the microhardness of enamel and curodont repair helped in remineralisation. However the hardness value was less than that of positive control in both negative control and curodont group.

The statistical parameters such as mean and SD of microhardness of samples were obtained for each group as shown in Table 1.The mean for positive control was the highest 332.78 ± 3.05 MPa, while that of negative control was the lowest 239.94 ± 4.94 MPa

In case of remineralising material used (CURODONT REPAIR) indicated higher mean 297.07 ± 5.3 MPa, which is higher than that of the negative control (bleached group) Analysis of variants was highly significant with the $P=0.000 \leq 0.001$.

Pairwise analysis result showed that hardness value of Group C is significantly higher compared to Group B. However the hardness value of Group A is significantly higher than that of Group B and C.

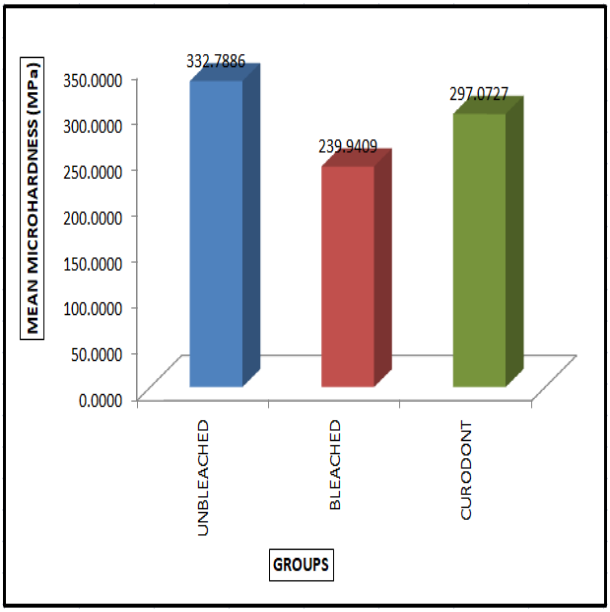
Table 1: Comparison of level of hardness (MPa) in different group

Groups	N[a]	Minimum	Maximum	Mean	Std. Deviation
Unbleached	22	328.50	339.40	332.7886	3.05329
Bleached	22	231.40	246.85	239.9409	4.94353
Curodont	22	287.60	307.05	297.0727	5.30318

Table 2: Pairwise analysis of different groups (microhardness)

Multiple Comparisons
Tukey HSD

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	p
microhardness	Unbleached	Bleached	92.84773	.000
		Curodont	35.71591	.000
	Bleached	Curodont	-57.13182	.000



IV. Discussion

Bleaching is a most common method to treat discolouration of teeth. However the use of highly oxidizing agents causes surface loss of enamel.[1] It also causes porosity of enamel surface and thereby increasing its stain susceptibility. [2] During the process of demineralization calcium, phosphate, sodium, magnesium gets displaced from the enamel surface to the exterior result the formation of crystal voids. One of the common method which can be adapted is theapplication of remineralising agent after bleaching.. [3] There are numerous remineralising agents available in the market. Post bleaching such agents should be applied to the bleached teeth by the dentist and at home patients can incorporate remineralising tooth pastes to their oral hygiene practice.

Self assembling peptide P11-4(CURODONT) developed by Credentis Switzerland, mainly focuses on biomimetic remineralization of initial carious lesion as a minimal invasive therapy. [7]

In this study bleaching significantly reduced enamel microhardness. Self assembling peptide P11-4 (CURODONT REPAIR) exhibited a significant difference in remineralizing bleached enamel surface. The self assembling peptide in Curodont repair was rationally designed with the aim, to present surface changes in such an arrangement, that de-novo nucleation and crystal growth of hydroxyapatite is triggered and favoured over other forms of calcium phosphate. [8]

In order to analyze the surface intactness, SMH measurement was a suitable and rapid method for this study. Therefore, the microhardness values for each specimen were measured using Vickers microhardness testing machine. [9]

The main advantage of Vickers test is repeated measurements of the same specimen can be taken over a given period of time so there won't be any experimental variation. In accordance with the studies conducted by Mehta AB et al, the SMH measurement is a suitable technique for studying demineralization or remineralization process and was therefore employed in this study.[10]

V. Conclusion

The detrimental effects of bleaching on enamel surface could be easily reversed by the application of the remineralising agents. Though bleaching has negative effect on microhardness of enamel , its benefits outweigh the adverse effects. However the hardness of enamel will not match up to the unbleached enamel by the application of remineralising agent after bleaching the adverse effects on enamel can be made negligible. The stain susceptibility of bleached enamel can be significantly reduced by this method. So the dental

practitioners must spend some time and effort to restore the enamel surface mineral loss.

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