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A comparative evaluation of biomimetic remineralization potential of enamel white spot lesions: A Pilot in-vitro study

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Abstract

Introduction: The remineralization potential of three recent remineralizing agents on white spot lesions were compared and evaluated using the Vicker's microhardness testing machine. This short study aims to evaluate and compare the remineralization potential of recently introduced remineralizing agents like Curodont Protect Gel, GC Tooth Mousse Plus, Dente 91 on surface hardness of white spot lesions in human teeth[1].

Method: Thirty single rooted human premolars extracted due to periodontal reasons or orthodontic reasons were chosen for this study. The teeth were decoronated at Cemento-enamel junction level. Samples were then mounted in auto-polymerization acrylic resin. All the groups were subjected to demineralization process to induce white spot lesions. A post demineralization vicker hardness test was performed followed by random allocation to the three groups. The three groups were namely Self-assembling peptide (P11-4), Casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF) and Nano-hydroxyapatite. A post remineralization Vickers hardness test was conducted to evaluate the remineralization potential of tested agents.

Result: An analysis of variance (ANOVA) was performed to determine the significant difference of Vickers microhardness. Self-assembling peptide showed the highest microhardness value.

Conclusion: Self-assembling peptide (Curodont Protect Gel) was more efficient than nanohydroxyapatite(Dente-91) and CPP-ACP (GC Tooth Mousse Plus) for the prevention of white spot lesions(WSLs).

Keywords: Tooth, Enamel, Health, Dental.

Introduction

Despite of implementing various oral and dental health promotion programs, dental caries still remains the most common and a unique infectious disease of dental hard tissues[1].The most common initial clinical sign of the

dental caries is the appearance of 'white spot lesions' or areas of demineralization, which are reversible in nature. The bidirectional dynamic process of demineralization followed by remineralization affects the enamel surface during its life span[2].

At the stage of demineralization, dental caries progress can be prevented by shifting the reaction towards remineralization[1,2].

Today, in dentistry more emphasis is given on caries interception at an earliest by non-invasive remineralization of the lesion rather than opting for invasive preventive techniques[1]. Till date, many commercial remineralizing agents in market are being used for remineralization of white spot lesions such as fluoride compounds, tricalcium phosphate, casein phosphopeptide-amorphous calcium phosphate and a lot more. Recently, the use of biomimetic approach has been introduced as an innovative strategy for enamel remineralization[4].

This short study aims to evaluate and compare the remineralization potential of recently introduced remineralizing agents like Curodont Protect Gel, GC Tooth Mousse Plus, Dente 91 on surface hardness of white spot lesions in human teeth[1].

Materials & Methods

Thirty sound single rooted premolars extracted were collected seeking exclusion and inclusion criteria. Non carious, sound and intact human single rooted premolar teeth with normal morphology were included. Teeth with any craze line or crack, caries as well as any mineralization defect were excluded[5].

The collected teeth were cleaned and polished with wet pumice slurry paste and were stored in 0.1 % thymol solution until further use[6,7]. The teeth were decoronated at Cemento-enamel junction level. The coronal half of teeth were then split vertically using a Isomet diamond saw(1-0.25micrometre), mounted on a contra-angle handpiece thereby, retaining the buccal portion of the coronal part of the teeth. The samples were then mounted in auto-polymerization acrylic resin with buccal surface facing the observer. These samples were stored in deionized water. All the samples were subjected to demineralization to induce white spot lesions with the help of demineralization medium. The demineralizing solution was prepared using composition as follows: 2.2 mM potassium dihydrogen phosphate, $KH_2PO_4{\cdot}7H_2O$; 2.2 mM calcium chloride, $CaCl_2{\cdot}2H_2O$; 0.05 mM lactic acid, $C_3H_6O_3$. The pH of the demineralizing solution was checked during and after preparation of solution using a digital pH meter.

The acetic acid buffer was added to adjust the ultimate pH at 4.5. All the samples were immersed into a glass container containing 50mL of the prepared demineralizing solution for a period of 96 hours at 37°C to produce an artificial subsurface enamel lesion in all samples. After demineralization, the teeth were washed with deionized water and dried[5,6].

Consequent to this process of demineralization, the 30 premolar tooth were allocated at random into one of the following three groups (10 in each group) depending on the remineralizing paste used for application:

Group A: CURODONT PROTECT GEL (N=10)

Group B: GC TOOTH MOUSSE PLUS (N=10)

Group C: DENTE 91 (N=10)

The samples from group A to group C were treated with respective remineralizing agent for 3minutes twice daily for 14 days. The samples were applied with respective remineralizing agent using a cotton applicator tip for 3 minutes twice daily, washed with deionized water and stored in artificial saliva. The artificial saliva was formulated with the subsequent substances in specific proportions—potassium dihydrogen phosphate

(KH2PO4) 0.9 mmol/L, potassium chloride (KCl) 50 mmol/L, calcium chloride (CaCl2) 1.5 mmol/L, and tris buffer 20 mmol/L[6,9]. After 14 days cycles of remineralization, the surface microhardness of the specimens was determined using the Vickers microhardness testing machine. A load of 100 g was applied to the surface of specimen for a period of 10seconds using Vickers elongated diamond pyramid indenter under a x40 objective lens. The accuracy of values of diagonal length of indentations was determined under high power magnification of x400. The depth of the indentation was measured, and the values were converted to Vickers microhardness values.Five indentations were placed on the surface and the mean value was considered for each specimen[6-8].

Statistical Analysis

Descriptive and analytical statistics were done. The data were presented in mean and standard deviation. The paired sample t-test and one-way analysis of variance (ANOVA) test were analyzed with the help of SPSS (Statistical Package for Social Sciences) Version 24.0 (IBM Corporation, Chicago, USA).

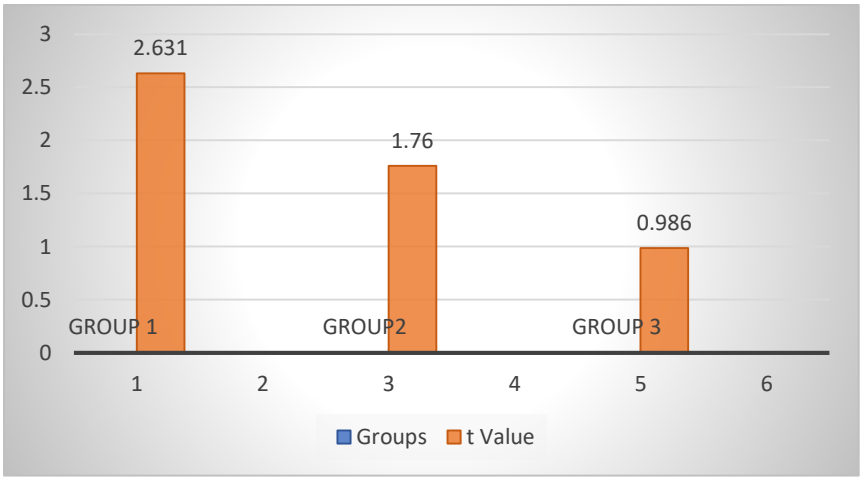
The level of significance was kept at $p<0.05$.

Results

Table 1 Comparison of mean Microhardness in HV at Demineralization and Remineralization stage for each of the three groups

Groups	Stages	N	Mean	Std Deviation	t Value	p- Value
Group A self-assembling peptide-Curodont repair gel	Demineralization	10	389.2	5.534	2.631	0.02
	Remineralization	10	319.4	30.544		
Group B CPP-ACP GC Tooth Mousse Plus	Demineralization	10	368.8	8.039	1.76	0.01
	Remineralization	10	271	26.34		
Group C Nano-hydroxyapatite Dente-91	Demineralization	10	315.3	25.721	0.986	0.334
	Remineralization	10	277.2	28.635		

Significant at $p< 0.05$



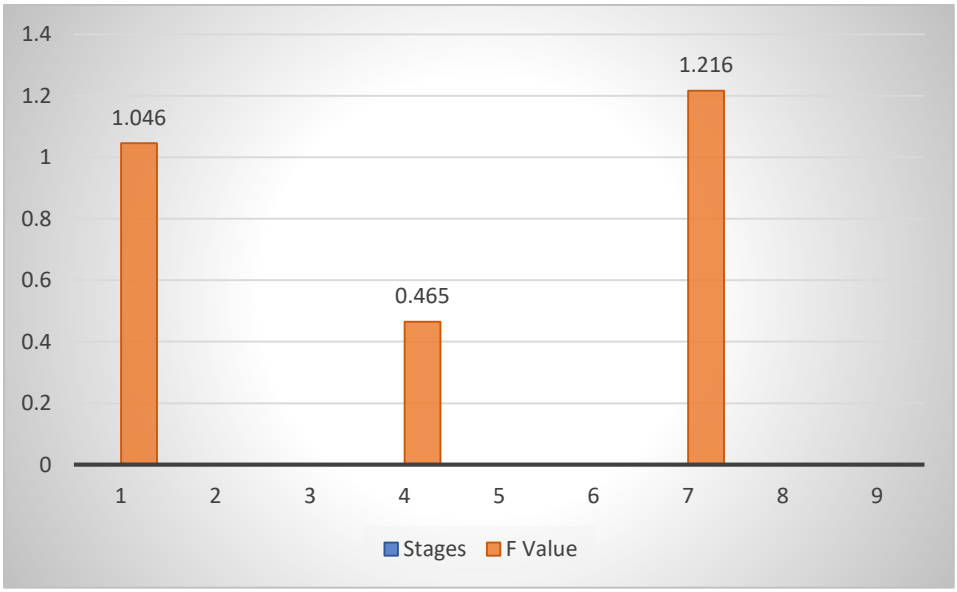
Graph 1: Comparison of mean Microhardness in HV at Demineralization and Remineralization stages

The table 1 compares the mean microharness of each group at demineralization and remineralization stage (X-axis Group A: self-assembling peptide-Curodont repair gel, Group B: CPP-ACP GC Tooth Mousse Plus, Group C: Nano-hydroxyapatite Dente-91 and on Y- axis t values) on demineralization and Remineralization.

Group A shows the highest t-value (2.631) and a significant p-value (0.02) in demineralization, indicating its effectiveness compared to other groups. Notably, Group A also displays a higher mean in Remineralization (319.4) suggesting its potential in promoting tooth Remineralization. Conversely, Group B and Group C exhibit lower t-values and p-values. Overall, the self-assembling peptide-Curodont repair gel (Group A) stands out with promising Remineralization capabilities, emphasizing its potential dental health benefits.

Table 2 Comparison of mean Microhardness in HV among the three groups at baselines, Demineralization and Remineralization stages

	Group	N	Mean	Std Deviation	F Value	p- Value
Baseline	self-assembling peptide-Curodont repair gel	10	350.7	23.348	1.046	0.342
	CPP-ACP GC Tooth Mousse Plus	10	366	4.086		
	Nano-hydroxyapatite Dente-91	10	312	19.711		
Demineralization	self-assembling peptide-Curodont repair gel	10	389.2	5.534	0.465	0.928
	CPP-ACP GC Tooth Mousse Plus	10	368.8	8.039		
	Nano-hydroxyapatite Dente-91	10	315.3	25.721		
Remineralization	self-assembling peptide-Curodont repair gel	10	319.4	30.544	1.216	0.507
	CPP-ACP GC Tooth Mousse Plus	10	271	26.34		
	Nano-hydroxyapatite Dente-91	10	277.2	28.635		



Graph 2: Comparison of mean Microhardness in HV among the three groups at baselines, Demineralization and Remineralization stages

The Table 2 presents a comparison of stages of mean micro hardness (HV) among three dental treatment groups – self-assembling peptide-Curodont repair gel, CPP-ACP GC Tooth Mousse Plus, and Nano-hydroxyapatite Dente-91 – on X- axis Baseline, Demineralization, and Remineralization stages vs on Y-axis F value. Micro hardness reflects material strength. At Baseline, mean HV values were 350.7, 366, and 312 for the three groups, respectively. No significant differences emerged, as indicated by p-values above 0.05. Similarly, during Demineralization (mean HV: 389.2, 368.8, 315.3) and Remineralization (mean HV: 319.4, 271, 277.2) stages, p-values remained above 0.05, suggesting no substantial microhardness variation. The study implies that the examined materials exhibited consistent microhardness levels across these stages and did not exhibit statistically significant distinctions in their hardness properties.

Discussion

This study highlights the remineralization effects of different remineralizing agent on demineralized enamel surface of the extracted teeth. In this research, greatest mean remineralizing potential was noted in Curodont protect gel (GROUP A) for the lost enamel structure in induced white spot lesion. This result can be due to its hydrogel appearence formed by beta-sheet amyloids that creates a 3D- matrix and promotes strength, toughness similar to muscle tissues. This biomimetic remineralization approach that spyron the biomineralization process is in gleam to replicate the natural mineralization process. The monomeric self-assembling peptide(P11-4) offered by curodont repair technology can generate new hydroxyapatite crystals as a foci and speed up the mineral crystal development in a cycle of biomimetic mineralization [10]. Dente-91 (GROUP C) has shown the second highest remineralization potential. The reason behind this can be attributed to the similar morphology, structure and crystallinity of this synthetic nano hydroxyapatite as that of a biological apatite. The nano-sized particles diffuse into the demineralized areas of enamel and form a new apatite layer. Being a potent biocompatible, bioactive substance with greater affinity for enamel it has its own bench mark in dentistry now days[11].

GC TOOTH MOUSSE PLUS (GROUP B) showed the third highest remineralization potential for white spot lesions in the present study. This result can be postulated due to its degradation of the phosphopeptides which in turn leads to dephosphorylation of Casein phosphopeptide-amorphous calcium phosphate by phosphatases. This results in substantially reduction of the peptide ability to bind calcium and phosphate ions thereby decreasing the remineralizing potential[12].

Conclusions

The results of this in-vitro study suggest that among the three agents tested, self-assembling peptide (Curodont Protect Gel) is the most effective remineralizing agent followed by nanohydroxyapatite (Dente-91) and Casein phosphopeptide-amorphous calcium phosphate (GC Tooth Mousse) when tested under the conditions mentioned in this study.

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