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Original Research

Comparative assessment of different remineralizing agents on artificial caries like lesion using surface microhardness- An invitro study

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ABSTRACT:

Aim: The aim of the study was to evaluate and compare the microhardness of demineralized enamel after topical application of different remineralizing agents. **Methodology:** This in vitro study involves 80 enamel samples divided into 4 groups of 20 samples each. The group were subjected to demineralization following which of these groups were remineralized using remineralizing agents' casein phosphopeptide amorphous calcium phosphate with fluoride CPP-ACPF [GC tooth mousse plus], Enafix, silver diamine fluoride, Conybio.The groups treated with remineralizing agents were subjected to pH cycling over a period of 28 days. This was followed with assessment of surface microhardness. **Results:** One-way analysis of variance test and post hoc Tukey test were conducted for multiple group comparison. **Conclusion:** All the four remineralizing agents showed improved surface remineralization. GC Tooth Mousse Plus (CCP-ACPF) showed highest remineralization potential amongst all agents.

Keywords: Casein Phosphopeptide Amorphous Calcium Phosphate Fluoride, Silver Diamine Fluoride, Surface Microhardness, Vickers Hardness Number.

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INTRODUCTION

Dental caries is the most common disease affecting the oral cavity[1]. It is a microbial disease of the calcified tissue, characterized by demineralization of inorganic portion and destruction of organic portion of the tooth. Enamel is a highly mineralized tissue of the body which is composed of 96% of inorganic salts and 4% organic salts[2]. Various causes of demineralization of tooth are acid dissolution by acidic sources like carbonated drinks, citric fruits juices etc. The most effective way to prevent tooth decay is to remineralize it in its most incipient early non cavitated stage[3]. These remineralizing agents are part of a new era of dentistry aimed at controlling the demineralization/ remineralization cycle, depending upon the micro environment around the tooth. Various agents that enhance and/or promotes remineralization are fluorides, casein phosphopeptide–amorphous calcium phosphate (CPP ACP), calcium fluoride nanoparticles, xylitol, alpha tricalcium phosphate etc. Some of the newer commercially available agents are chitosan, silver diamine fluoride, bioactive glass, nano hydroxyapatite particles, theobromine, ozone etc. that aid in remineralization of the tooth structure.

One of the most effective agents in remineralization is casein phosphopeptide amorphous calcium phosphate (CCP-ACP) technology that is based on the stabilizing properties of milk and salivary proteins. The probable anticariogenic mechanism of CPP-ACP is due to its ability to localize ACP at the tooth surface, which brings about buffering of calcium and phosphate free ion activities, thereby helping to maintain a state of super saturation with respect to tooth enamel negating demineralization and enhancing remineralization[4]. CPP-ACPF due to its

added fluoride content has shown improved ability to remineralize initial caries[4].

Silver diamine fluoride (SDF) is one unique fluoride with the added benefit of silver metal that promotes remineralization[5]. Upon application of silver diamine fluoride to a decayed surface, a squamous layer of silver-protein conjugates forms, increasing resistance to acid dissolution and enzymatic digestion[6]. Another agent Enafix toothpaste is a mixture of calcium salts of sucrose phosphate esters, complexed with inorganic calcium orthophosphate that quickly break down and releases calcium, phosphate, and sucrose phosphate ions into the saliva. It is composed of 10-12% calcium and 8-10% phosphorous by weight[7]. It acts by adsorption of sucrose phosphate ions rapidly onto the enamel surface, thereby reducing the rate of acid dissolution of hydroxyapatite and quick remineralization by calcium and phosphate[8].

Chitosan is a copolymer of N-Acetyl-D-Glucose and D-glucosamine which is found in insect's skeleton and cellular wall of the fungus[9]. According to research chitosan is an antibacterial agent that degrades bacterial cell walls and is nontoxic and biocompatible in nature[9,10].Thus, this study was conducted to compare the remineralization potential of GC tooth mousse plus (CCP-ACPF), FAgamin (Silver Diamine Fluoride), Enafix (Calcium sucrose phosphate), Conybio Plus(chitosan) in early caries lesions prepared using pH cycling model on demineralized tooth enamel.

METHODOLOGY

Eighty freshly orthodontically extracted human premolars were included in the study. Teeth with enamel and dentinal defects like erosions, abrasions, microcracks, developmental anomalies, carious lesions, restorations were excluded. The samples were decoronated at cemento-enamel junction with a high-speed diamond disc and were randomly divided into four groups 20 samples each group (n=20)

- Group A- CCP-ACPF (n1=20)
- Group B-Silver Diamine Fluoride (n2=20)
- Group C-Calcium Sucrose Phosphate (n3=20)
- Group D-Chitosan (n4=20)

A Vickers micro hardness indenter was used to evaluate the baseline micro hardness under 100 grams loads applied for 10 seconds at 3 different points each 1 mm apart and the mean was measured.

LESION PREPARATION

After recording baseline microhardness each of the enamel samples was then immersed in 20 ml of demineralizing solution (pH 4.5) for a period of 4 days (96hours) at a constant temperature of 37°C, in an incubator to induce artificial caries formation, Surface micro hardness measurement were made using Vickers microhardness indenter under similar conditions.

REMINERALIZATION PROCEDURE

Slurry of agents was prepared by agitating the GC Tooth Mousse Plus (CCP-ACPF), FAgamin (Silver Diamine Fluoride), Enafix (Calcium Sucrose Phosphate), Conybio Plus (Chitosan toothpaste) in deionized water in the ratio of 1:3.

pH CYCLING MODEL

Each of the enamel sample were treated with the respective remineralizing agents according to the groups divided (20 samples each) for a period of 3 min, following which the samples were individually immersed in 20 ml of demineralizing solution for a period of 3 hours. This procedure was followed-up with treatment of the samples again with the respective remineralizing agents for 3 min. All the enamel samples were individually immersed in 30 ml of remineralizing solution for a period of 17 hours. The pH cycling regime was carried out for a period of 28 days.

After the completion of the process of pH cycling, all the groups of enamel samples were assessed for surface microhardness using Vickers elongated diamond pyramid indenter. A load of 100 grams was applied to the surface for 10 seconds. Three indentations were placed on the surface and the average value were recorded.

STATISTICAL ANALYSIS

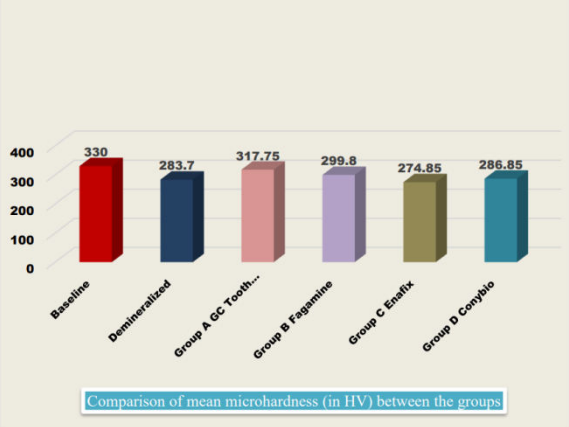
The groups were subjected to Vickers microhardness testing and the data was recorded as baseline, demineralized and remineralized. The data obtained was entered into Microsoft Excel version 13. The data was analyzed using IBM SPSS (Statistical Package for the Social Science) Version 21. The mean and standard deviation for continuous (in HV) between the 4 groups ANOVAwith Post Hoc Tukey Test was applied.

RESULTS

The microhardness was seen highest in Baseline followed with **Group A GC Tooth Mousse Plus**, then **Group B FAgamin**, then **Group D Conybio Plus** and then **Group C Enafix**. (Refer table and graph).

Group	Mean	Std. Deviation	Minimum	Maximum	Std. Error of Mean
Baseline	330.0000	16.49242	301.00	356.00	5.21536
Demineralized	283.7000	10.23122	271.00	301.00	3.23539

Table shows the mean microhardness in sound enamel (baseline values) and in demineralized tooth samples.



Comparison of mean microhardness (in HV) between the groups

DISCUSSION

Dental caries is an infectious microbiological disease of teeth that results in localized dissolution and destruction of calcified tissue (According to sturdevent) and WHO stated that dental caries is a post-eruptive pathological process of external origin involving softening of the hard tooth tissue & proceeding to the formation of cavity. It is one of the most commonly occurring oral disease[4]. The dynamic caries process consists of rapidly alternating periods of tooth demineralization and remineralization which, if net demineralization occurs over sufficient time, results in the initiation of specific caries lesions at certain anatomical sites on the teeth.

Margolis et al described white spot lesion as the first clinical presentation of dental caries which if left untreated may lead to cavitation[11].Enamel remineralization is not a new topic but nowadays remineralization of both non cavitated and cavitated lesions is being attempted. Calcium and phosphate ions must first penetrate surface layer of enamel, to bring about deposition of minerals through the body of the lesion, which confirms the reason for the Casein phosphopeptide (CPP) supported metastable calcium phosphate solutions of being such efficient remineralizing solution[12]. Majority of demineralizing solutions are composed of calcium and phosphate with acetic acid or lactate. The main variation lies in concentration of each component, which influences the final pH and the sample exposure time.

The pH employed varies from 3.5 to 5 and the time differs from 2 hours to 21 days[13,14]. In the present study solution was of **4.5-5.0pH** and composition of 2 mM Cacl, 2 mM phosphate, acetic acid 74 mM was employed for 4 days i.e., 96 hours this composition was similar to the one employed by **Featherstone in 1992**[15].

There are different methods for evaluation of demineralization and remineralization of enamel which may be direct or indirect. Direct techniques are longitudinal microradiography, transverse micro radiography. Indirect techniques are polarized microscopy, quantitative energy dispersive X-ray analysis, microhardness measurement methods [2]. Among the many methods to evaluate the remineralization of enamel Vickers, Knoop and brinnel hardness are used to measure the microhardness of the tooth surface[4]. In-vitro surface microhardness studies have the advantage of being simple, easy to conduct, noninvasive.In the present study, Vickers hardness (VHN) was adopted over Knoop's because the square shape of indent obtained in VHN is more accurate to measure[4,16,17,18].

The Vickers hardness values obtained during the baseline mean microhardness measurement in the present study, were in range of **333.00 ± 16.492 VHN**. These values satisfy the VHN range of normal enamel tissue according to studies done by **Priyadarshini Set al[19], Lata S et al[20], Srinivasan N et al[21]**.

In-vitro preparation of caries like lesion in enamel samples provides standard to compare between different remineralizing agents. In the present study, samples were stored in demineralizing solution for 96 hours at 37 degrees Celsius to create subsurface lesion range of **283.70 ± 10.231VHN**. After demineralization surface microhardness values decreased for all specimens of each group which was in accordance with studies conducted by **Priyadarshini S et al[19], Lata S et al[20], Srinivasan N et al[21], Featherstone et al[15]**.

Slurry solutions were prepared for the Groups A, B, C & D in 1:3 ratio of the remineralizing agent in deionized water in order to standardize the consistencies of the experimental materials. In the oral cavity, the paste will be quickly diluted by saliva. This effect is simulated by diluting the agents with water in this in-vitro study[14].

In the present study, **Casein phosphopeptide-Amorphous calcium phosphate (CCP-ACPF), silver diamine fluoride (SDF), calcium-sucrose phosphate and chitosan** were used as remineralizing agents and these agents were applied topically for 3 minutes twice for 28 days.

The values of surface microhardness indicate that remineralization of enamel is more in samples of **Group A GC Tooth Mousse Plus (CPP-ACPF)** followed by **Group B FAgamin (SDF)** and **Group D Conybio Plus (chitosan toothpaste)** and least with **Group C Enafix (calcium sucrose phosphate)**. The concept of using CPP–ACP as a remineralizing agent was introduced in 1998, using casein for caries prevention was addressed in the 1980s, and ACP technology was introduced in the early 1990s[20]. CPP-ACPF exhibited superior remineralizing property which could be attributed to the characteristic nature of CPP. CPP by stabilizing calcium phosphate in a metastable solution facilitates increased concentrations of calcium and phosphate ions, including dicalcium phosphate (CaHPO4), which can diffuse into the enamel subsurface lesion, while the fluoride synergistically enhances remineralization. Many studies have demonstrated that higher the CPP-ACP concentration, higher will be the remineralization[4]. In the present study, CPP-ACPF demonstrated increase in remineralization which was

in accordance to the study done by **Bhatt V et al[16], Shetty Set al[4].**

In Group B- FAgamin (Silver Diamine Fluoride) after remineralization the increase in mean microhardness value was **299.80 ± 24.193**. FAgamin, 38% SDF solution, consists of elevated concentrations of silver (253,870 ppm) and fluoride (44,800 ppm) ions. 38% SDF has shown to be biocompatible and safe for topical treatment with high effectiveness in caries prevention and arresting [22]. According to **Braga's study**, SDF was not only effective but also fast in arresting dental caries in partially erupted permanent molars pit and fissure caries[23]. It has also been found in literature that SDF is useful to treat tooth hypersensitivity, induce dentine desensitization and as a disinfectant during root canal treatment[24]. In addition, it is easy to use, has a relatively short treatment time, economical and has a wide application range [25].The most significant disadvantage of SDF was the aesthetic concern because SDF caused tooth surface discoloration[5]. Studies had shown the use of Potassium Iodide (KI) after SDF application reduced the discoloration. This was because KI reacts with the excess free ions to form silver iodide, which manifests white color.

In group C- Enafix (calcium sucrose phosphate) after remineralization the increase in microhardness values was **274.85 ± 6.360. Enafix - CaSP** is a specialized formulated toothpaste using Anticay® technology. The anticay in Enafix is Calcium sucrose phosphate which quickly breaks down and releases calcium, phosphate and sucrose ions. According to the manufacturers, it had the common ion effect where the rate of remineralization increases by decreasing the surface lesion depth. It was composed of 10-12% calcium and 8 - 10% phosphorous by weight[4.]

In group D Conybio Plus (chitosan) the mean values after remineralization were **286.85 ± 6.499.** Chitosan and its derivatives have been developed as novel biomaterial due to their low toxicity, biodegradability, biocompatibility, and biological activity. Among all the derivatives of chitosan, phosphorylated chitosan exhibited bactericidal properties along with other properties like biocompatibility and osteoinductive property[10,26]. It was observed that white spot lesions pretreated with chitosan bioglass slurry had superior biomechanical properties and a compact subsurface in comparison to other remineralizing actions of other agents. In a study conducted by **Simeonov et al.[27],** a new hybrid chitosan/calcium phosphates microgel was formulated to remineralize the demineralized enamel. The chitosan macromolecules had superior biological properties like antimicrobial property, bio-adhesiveness, and reservoir of Ca2+ and PO4 which aided in remineralization[28,29].

It is imperative to note that remineralization in in-vitro may be quite variable when compared to changes occurring in the oral cavity in-vivo. Further, long-term clinical trials should be conducted to prove the superiority of these materials in the vital teeth.

CONCLUSION

Within the limitations of the present study, following conclusions can be drawn all the four remineralizing agents showed improved surface remineralization. GC Tooth Mousse Plus (CCP-ACPF) showed highest remineralization potential and Enafix (CaSP) showed least remineralization potential. However, complete remineralization did not occur with any agents within 28 days.

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