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Effect of Water Soluble Carboxymethyl Chitosan and Chitosan Lactate on Enamel Demineralisation- An SEM Study.

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ABSTRACT

Mature enamel is a non-living tissue and any damage to the enamel is permanent as it cannot remodel itself. Enamel being the outermost layer of the tooth, is susceptible to dental caries and erosive wear (demineralisation). Conventionally, defective enamel is treated with dental restorative materials such as amalgam, composite resin, ceramics etc. The drawback of these restorative materials is that they differ from natural enamel in chemical composition, structure and physicochemical properties. This results in a compromised bonding with enamel at the enamel interface, which is susceptible to marginal leakage leading to hypersensitivity and secondary caries. Therefore, the search for an alternate strategy to repair enamel is needed. Chitosan, a naturally occurring polymer of chitin, seems to fill this void as an effective anti-cariogenic agent as it has both antibacterial and acid absorbing capacity (enamel protection capacity). This study was undertaken to assess the enamel surface changes by SEM on 40 premolar teeth pre-treated with water soluble Carboxymethyl chitosan and Chitosan lactate which were subjected to cariogenic acids.

Keywords: Chitosan, Enamel, Dental caries, Demineralisation

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INTRODUCTION

Enamel is an ectodermal derivative formed by the ameloblasts which are lost as the tooth erupts into the oral cavity and hence enamel cannot renew itself. This inherent deficiency is compensated by high degree of mineralization with almost absence of organic matrix in the mature state. [1] Enamel is both acellular and avascular and hence dental caries affecting the enamel is distinctive as it is not capable of healing itself by a cellular repair mechanism.[2]

The susceptibility of these crystals to dissolution by acid provides the chemical basis for dental caries.[1] Carious lesions are formed when the rate of demineralization exceeds the rate remineralisation.[3] As replacing the lost natural enamel is nearly impossible, products that are capable of preventing the process of demineralisation need to be used zealously to maintain healthy teeth.[4] Strategies in dental caries prevention mainly target the three contributing factors i.e. tooth, microorganisms and diet with varying degrees of success.[5]

Antimicrobials like chlorhexidine, cetylpyridinium chloride, sugar substitutes such as erythritol, xylitol, etc., limit the production of acids, while casein phosphopeptides remineralize enamel. Phosvitin, arginine and small peptides have also been used to increase the pH of the plaque as they accumulate on the surface and have a local buffering action. Fluorides have been incorporated into toothpastes and water supplies to inhibit demineralization.

These compounds, despite their widespread use, possess a high toxicological profile, develop microbial resistance, result in imbalance of oral microflora, are expensive, depend on patient co-operation, are not easily available and require application by professionally trained individuals to achieve optimum results. Chitosan is one such compound which has antibacterial property against biofilm pathogen (caries and periodontal microorganisms) and also has a enamel protection capability, is biocompatible, nontoxic and economical. [6]

MATERIALS

Carboxymethyl chitosan (CMC) and Chitosan lactate (CL) were procured from Everest Labs, Bangalore, Karnataka.

40 caries free human premolar teeth extracted for orthodontic purpose were procured from the Department of Oral Surgery, Farooqia Dental College, Mysore, Karnataka.

SEM analysis was done using Zesiss (EVOLS 15) microscope , Skanda lab, Bangalore.

1% formic acid was used to demineralize enamel. [7]

METHODS

40 premolars with sound enamel were divided into four main groups, A, B ,C and D, with each group containing 10 teeth. Group A was taken as control; Group B was subjected to formic acid (cariogenic acid). While Group C and D were pretreated with CMC and CL respectively and subjected to demineralisation.

All the teeth were maintained in an artificial saliva solution.

CMC and Chitosan lactate solution was prepared by dissolving 5gm of CMC powder and CL flakes in 10mL of water. All the teeth were sectioned and polished by a diamond disc using a Marathon micromotor fitted with a Marathon H37L1 straight fissure handpiece to obtain flat polished enamel surfaces.

Teeth in Group A were not subjected to any pre-treatment or demineralisation procedures.

Teeth in group B were subjected to demineralisation procedure.

Preparation of early artificial caries lesion: Specimens were exposed to a demineralization cycle with 1% acetic acid for one hour thrice daily for 5 days to cause demineralization

Teeth in group C and D were pretreated with CMC and CL solution thrice daily for 5 minutes for 5 days at an interval of 6 hours before exposure to each cycle of demineralization.

All the teeth were prepared for SEM analysis. The specimens were rinsed ultrasonically with water for ten minutes and dehydrated. Enamel surfaces were sputter coated with gold (~30-35 nm) and subjected to SEM analysis. Pre and Post treatment difference in the surface ultrastructure were noted. Photomicrographs of representative areas were taken at 1000x and 1500x magnifications.

RESULTS

Observable changes were seen in the SEM analysis of enamel before and after demineralisation with or without pretreatment with CMC and CL.

Fig -1 Group A: Enamel before demineralization reveals a smooth and intact enamel surface (keyhole pattern).

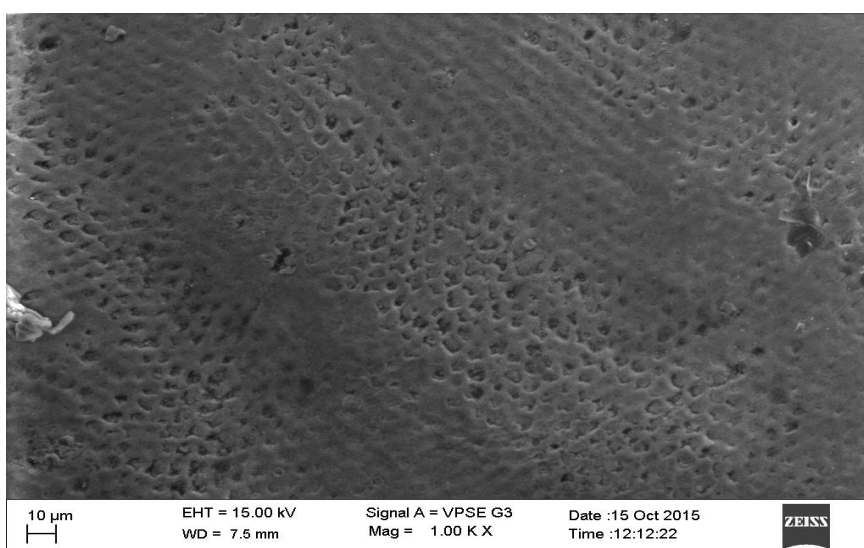


Fig -1 (Group A): Enamel before demineralization reveals a smooth and intact enamel surface (keyhole pattern) at x1000

Fig-2 Group B:–Untreated enamel post demineralization with organic acid exhibits many micropores and cellular structures.

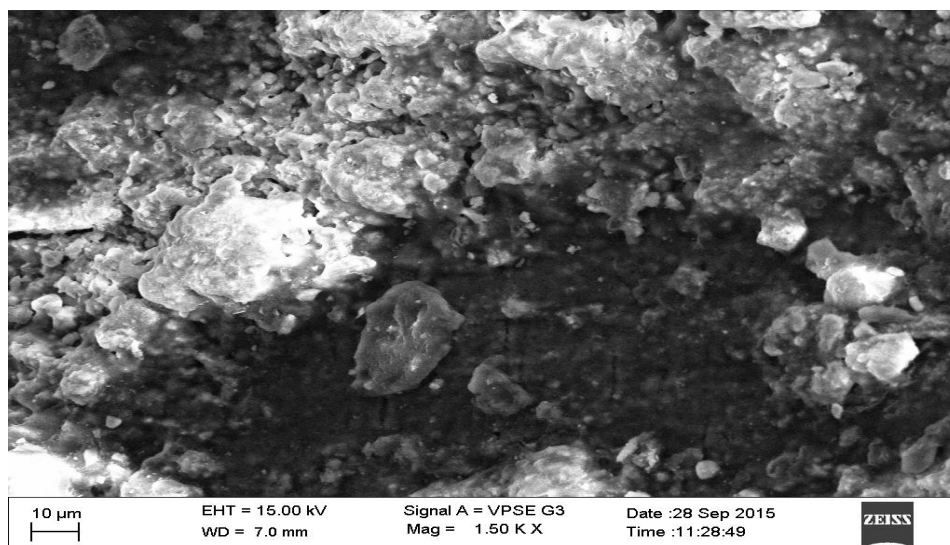


Fig 2 (Group B): Untreated enamel post demineralization with organic acids at x1500

Fig-3 Group C: Enamel pre-treated with CMC post demineralization with organic acid exhibits less micropores and cellular structures.

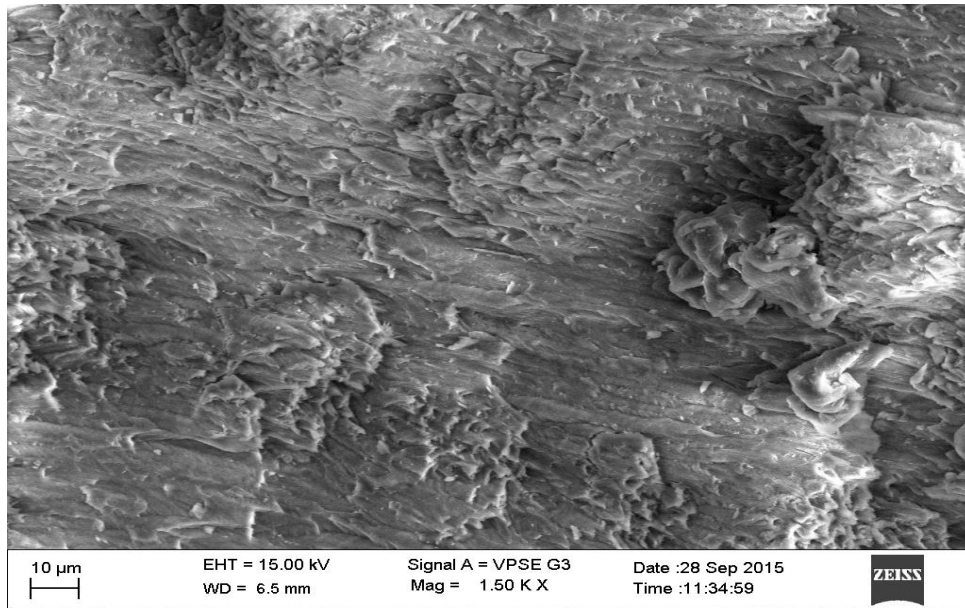


Fig 3 (Group C): Post demineralization enamel pre-treated with CMC at x 1500

Fig-4 Group D: Enamel pre-treated with CL post demineralization with organic acid exhibits decreased micropores and cellular structures.

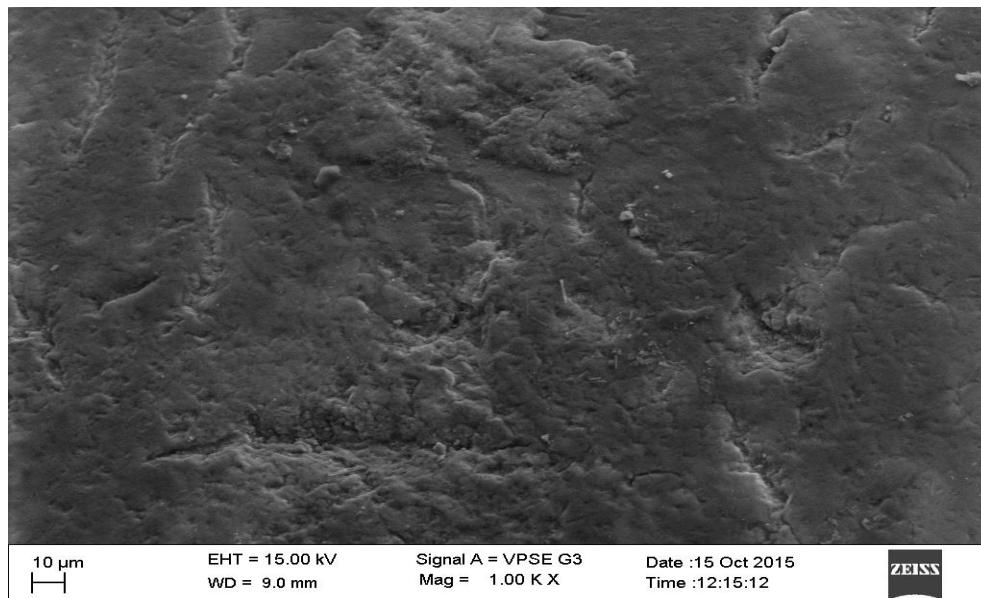


Fig 4 (Group D): Post demineralized enamel pre-treated with CL at x1500

The surface alteration in the chitosan untreated Group-B, was significantly greater than those of chitosan pretreated Groups -D, C, and the control group A. There were no significant differences between CMC pretreated group (B) and CL pretreated group (C). Thus SEM analysis in our study showed that both water soluble CMC and CL had the potential to protect enamel from organic acids.

DISCUSSION

Enamel is relatively stable in a healthy oral environment with the presence of saliva. The demineralisation and remineralisation of enamel is a continuous process and [4] repeated events of demineralisation will gradually surpass the capacity of oral fluids to demineralize, resulting in the formation of a frank carious lesion. [3]

The prevention of acid attack in the oral cavity is the most effective method in preventing demineralization of teeth. Various treatment modalities and preventive methods have been explored to protect the tooth enamel from acid attack with little success.

Alternate substances which protect enamel by overcoming the drawbacks of routinely used agents are necessary as demineralization is still a rampant problem. Chitosan is one such substance which is biocompatible, non-toxic, highly bioactive, biodegradable, selectively permeable, antimicrobial, mucoadhesive, anti-acid, forms gel and film, has ability to chelate and possess absorptive capacity. [8]

Chitosan is a deacetylated form of chitin, a natural polymer found in the cell walls of fungi and forms a major component of the exoskeletons of arthropods, such as the crustaceans (e.g. crab, lobster and shrimp), and the insects (e.g. ants, beetles, and butterflies) and of the beaks of cephalopods (e.g. squids, and octopi). Chitosan is composed of 2-amino-2-desoxy- D glycopyranose interconnected by glycosidic bonds -1,4 in variable proportions. It possesses highly reactive amino (-NH₂) and hydroxyl groups (OH). Chitosan contains amino groups with a pKa value of 6.2- 7 and it is considered as a strong base. Chitosan is soluble in dilute acidic solutions at pH below 6. It is insoluble in neutral and alkaline solutions which limits its potential usage. To overcome this problem, many water soluble chitosan derivatives have been developed by modifying the reactive functional groups of chitosan or by depolymerizing chitosan. Efforts to increase its solubility in water to broaden its applications have yielded CMC and CL.

Chitosan has been used as an antimicrobial agent against various cariogenic and periodontal pathogens. Chitosan-containing polyherbal toothpaste has shown to significantly reduce the plaque index by 70.47% and bacterial count by 85.29%. [9]

Antimicrobial activity of hydrosoluble chitosan has shown to be effective against S. mutans biofilms in vitro and in vivo.[10]

It has been shown that chitosan-containing dentifrice may reduce the enamel decalcification found in patients with poor oral hygiene around orthodontic brackets when compared with conventional nonfluoridated dentifrices. [11]

Chitosan maintains the integrity and structure of the tooth as well as the oral cavity by inhibiting dissolution of hydroxapatite by acids. The mechanism of action of the protective effect of chitosan can be enumerated in many ways;

- I. Maintaining the pH of plaque above the critical level of enamel demineralisation. The organic anions in chitosan hinder the rate of acid dissolution of hydroxapatite through rapid adsorption. The free amino (-NH₂) group in chitosan makes it highly reactive with dietary and cariogenic acids in the oral cavity and thereby reduces the acid and increases the pH to normal levels (Lee et al., 2012; Shetty et al., 2014)
- II. The cross-linking of chitosan and saliva with the physical adsorption of chitosan onto saliva prevents acid erosion of the hydroxyapatite surface (Lee et al., 2012).
- III. The penetration of chitosan into the enamel as far as the dentino-enamel junction has been demonstrated by Arnaud et al. It has been postulated that chitosan may act as a mechanical barrier for the acid penetration in the enamel and interferes in the process of enamel demineralization by inhibiting the release of mineral element (Arnaud et al., 2010).

- IV. Chitosan has been shown to scavenge free radicals (Liu et al., 2009). Free radicals liberated by hydrogen peroxide bleaching damage the structure of enamel and these form weak spots for demineralization and hence initiate dental caries. Free radicals are also genotoxic. The use of chitosan can limit the free radicals post bleaching treatments
- V. Chitosan is mucoadhesive which makes it highly bioavailable, ensuring a long period of action after the time of application. It also potentiates the action of chlorhexidine and has been used in the controlled delivery of fluoride (De Carvalho et al., 2011; Keegan et al., 2012; Bae et al., 2006; Pedro et al., 2009; Andrews et al., 2009).
- VI. vi) Nano-complexes of phosphorylated chitosan and amorphous calcium phosphate have been shown to remineralize enamel subsurface lesions at a rate significantly higher than that of fluoride treatment (Zhang et al., 2014). Apart from acid absorption and preventing demineralization of enamel, chitosan has a significant antimicrobial action against cariogenic and periodontal pathogen Enamel protected with acid soluble chitosan showed higher surface microhardness with Vickers test than enamel which was not treated with chitosan. Optical coherence tomography results indicate a correlation between the penetration of chitosan with its concentrations. At concentrations of 2.5 g/mL and 5.0 g/mL chitosan penetrates up to the dentin-enamel junction [12, 13,14,15]
- VII. Technically, chitosan controls the rate at which demineralisation occurs. Chitosan forms a veneer like coat on teeth and its presence in the plaque along with dissolved hydroxyapatite, at a higher pH precipitates the remineralisation/deposition of Ca and phosphate ions over the surface of the enamel. This veneer is much more acid-resistant than the original hydroxyapatite, and is formed more quickly than ordinary remineralised enamel.

Vickers hardness number of demineralised enamel pretreated with chitosan lactate showed a higher value than those of untreated enamel. This signifies the protective action of CL on enamel.[6]

In our study using SEM, teeth pre-treated with CMC and CL showed fewer enamel surface alterations when compared to untreated teeth. The results of our study are in concurrence with that of Arnaud T.M. et al, Arancibia et al, Uysal T et al and Visveswaraiah P.M et al where chitosan pretreated enamel exhibited decreased demineralisation when exposed to cariogenic acids and where chitosan pretreated enamel showed higher surface microhardness with Vickers test than enamel which were not treated with CMC. [6,11,14]

CONCLUSION

Prevention is better than cure. Preventing the process of demineralisation is equally significant as accentuating the process of remineralisation, hence proactive intervention is the need of the hour and not mere intervention after the diseases has set in. Water soluble CMC and CL are simple and safe alternatives that can be used in oral formulations to protect teeth against acid attack.

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