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Original Article

Dentistry Section

Ultrastructural Morphological Evidence of Effect of Physical Barrier in Prevention of Dental Caries: An In-vitro Experimental Study

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ABSTRACT

Introduction: Dental Caries is primarily a disease of multifactorial origin. The *streptococcus mutans* the causative agent adheres to the tooth surface and breaks down the sugar to produce lactic acid as the by product which brings about demineralisation of tooth structure. There is a need to identify substance which prevents adhesion of the organism to the tooth surface thereby preventing colonisation.

Aim: To assess conditioning of the tooth surface with butter prior to subjecting the tooth to demineralisation.

Materials and Methods: An in vitro experimental trial was conducted on 45 non carious premolars. The teeth were divided in 3 groups of 15 each. Group I received no pretreatment. The group II and III received thin and thick layer of butter coating, respectively. The teeth were then immersed in demineralising solution and the effect of the of the same was assessed using Scanning Electron Microscope (SEM). Since the data obtained was qualitative, any statistical test were not applied. The observations recorded were based on the repetition of the similar findings in more than 50% of the samples.

Results: The results of the study indicated the presence of islands of protected enamel and limited destruction in the prismatic pattern on the samples with thin coating of butter. Samples with thick butter coating showed presence of intact prismatic substance in contrast to the unprotected teeth which showed loss of prismatic substance.

Conclusion: Pretreatment of the tooth surface with thin coating of butter prior to exposing the tooth to cariogenic environment protects the enamel from undergoing demineralisation.

Keywords: Adhesion, Adsorption, Buffering capacity, Butter, Demineralisation

INTRODUCTION

Dental caries is the most common preventable childhood disease; however, its occurrence is not restricted to the childhood, any individual can be susceptible to this condition during the lifetime. As per the Global Oral Health Data Bank, prevalence varies from 49% to 83% across different countries [1]. India also exhibited a similar trend with prevalence increasing from 49% among 5-12 years to 84% among 65-74 years of index age groups [2].

Microorganism in the dental biofilm produces acids that bring about the dissolution of the mineral content of the tooth thereby causing enamel demineralisation leading to initiation of caries. Since dental caries is disease of the microbial origin, caries prevention is often focused towards development of antimicrobial agents [3].

However, dental caries is a complex, multifactorial disease and hence a simple one-way approach may not be effective in caries prevention. Advanced microbial culture assays have proven that there are wide range of microorganisms apart from the *streptococcus mutans* and *lactobacilli* that can contribute towards caries initiation and progression and even fungi can significantly influence the cariogenic virulence of the oral environment [4]. Fluorides although most effective and economical caries preventive agent, may not be sufficient as its anticariogenic action is largely limited to the enhancement of remineralisation [5].

Hence, considering dental caries only as a microbiological disease and targeting caries prevention using antimicrobial agents alone may not be effective enough in the disease control. *Streptococcus Mutans* and other cariogenic organisms are harbored in the complex bacterial biofilm i.e., plaque. Although wide array of explanations has described oral biofilm, it basically adheres to non desquamating surface and consists of microcolonies of organisms adhered by means of physical appendages and extracellular polymeric substrates [6].

The initiation of plaque deposition occurs by adsorption of the organisms to the acquired pellicle. As described by Fletcher, formation and maturation of oral biofilm undergoes various stages namely: Adsorption, Attachment and colonisation. Hence, cariostatic process should aim at inhibition in each stage of biofilm formation thereby arresting the caries process. Moreover, the caries protective agent which are that are used are either antimicrobial or they focus towards remineralisation of the carious lesion. Also, financial, cultural and geographic barriers prevent the access of the vulnerable population to the caries prevention [7]. Hence, there is a need for exploring further into the materials that are available commonly and can be used as initial caries prevention modality without need of professional intervention.

Milk and its products have been traditional used as caries preventive agents because of benefits of presence of calcium, phosphorus and casein [8]. However, milk can be also cariogenic due to the presence of lactose. Milk products like cheese, butter and yogurt have been explored as cariostatic agents. These were known to release elements that can facilitate remineralisation [9]. However, there is sparse information available if these products can be used for prevention of demineralisation rather than enhancing remineralisation. Butter is a rich source of vitamin A and Activator X which has caries preventive benefits [10,11]. However, butter as a physical barrier in prevention of demineralisation has not been explored. Hence, this study was conducted to assess effectiveness of physical barrier method utilising the commonly available butter in preventing demineralisation.

Traditionally, the extract of the active ingredient and preparation of formulation that could prevent the dental caries is the technique that is commonly followed. However, this method may require a professional advice or professional application. It may not be accessible to the large group of population. Hence, this study utilised the benefits of the butter in its original form such that it is available to the large group of needy population.

MATERIALS AND METHODS

An in-vitro experimental study design was planned to assess the effect of butter as physical barrier in caries prevention. The study was conducted in Department of Paediatric and Preventive Dentistry, JSS Dental College and Hospital. The study duration extended from period of July 2020 to December 2020.

Ethical clearance was obtained from the Institutional Ethical Committee (JSS/ACP/Ethical/2018-2019). However, the study did not involve human subjects hence informed consent was obtained only for the purpose of extraction and storage of extracted teeth. Experimental study design was planned on the natural surfaces of extracted premolars. Total 45 non carious teeth extracted for orthodontics purposes were used for the study.

Inclusion criteria: Extracted non carious teeth were chosen to simulate the oral conditions. The teeth with no evident hypoplastic lesions or intrinsic and extrinsic stains as detected clinically as well as on Quantitative Light induced Fluorescence (QLF) based examination were selected for the study.

Exclusion criteria: Teeth with wasting diseases such as attrition and abrasion were excluded from the study.

Sample size calculation: The sample size was based on saturation sampling where in the inclusion of samples in the study is discontinued beyond a point wherein no new data/information can be derived from the samples [12].

The roots of the teeth were embedded in acrylic and only the crowns were exposed [Table/Fig-1]. The labial surfaces of the premolars were cleaned with pumice slurry and polishing cups prior to the commencement of the experimental trial.

Sample Preparation

For the purpose of standardisation, the sample preparation was done by single investigator. The area of interest was located using polyvinyl stencil of 3×3 mm dimensions on the labial surfaces of the teeth [Table/Fig-2]. The rest of the area was then coated with transparent nail varnish. The polyvinyl stencil was removed after drying of the nail varnish. This provided an area isolation of 3×3 mm on the buccal surfaces of the premolars which was the area of interest. Commercially available non salted butter was used for the purpose of the study. A thin layer of butter was coated on the tooth surface. The samples were then randomly divided into 3 groups:

Group 1: No pretreatment: Enamel without butter coating

Group 2: Pretreated with thin layer of butter: To ensure presence of thin layer, after uniform coat of butter, the surface was wiped with porous cellulose strips to simulate soft tissue movement.

Group 3: Pretreated with thick layer of butter: Enamel with thick layer of butter coating. To ensure presence of thick layer, after uniform coat of butter, the surface was not wiped with porous cellulose strip.

[Table/Fig-1]: Tooth mounted in acrylic block; **[Table/Fig-2]:** Isolation of area of Interest. (Images from left to right)

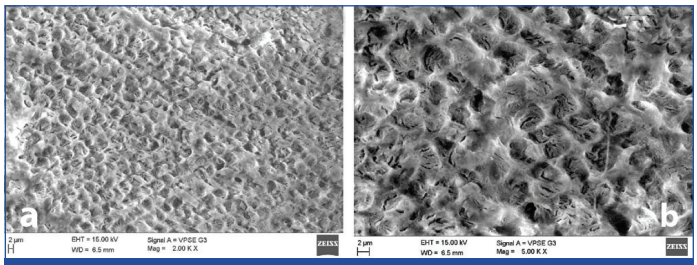
Artificial demineralisation: The demineralisation solution was freshly prepared as described by Mehta R et al., [13]. The samples were then immersed in the demineralising solution for 96 hours. The solution was replaced every day and at the end of 96 hours, the samples were washed to remove the demineralising solution and were subjected to Scanning Electron Microscopy (SEM).

STATISTICAL ANALYSIS

The present study is a qualitative study, describing the findings as observed in SEM, hence no statistical tests have been applied. However, the following observations have been recorded based on the repetitions of the findings. The overall observations were made when more than 50% of the samples provided the similar findings [14].

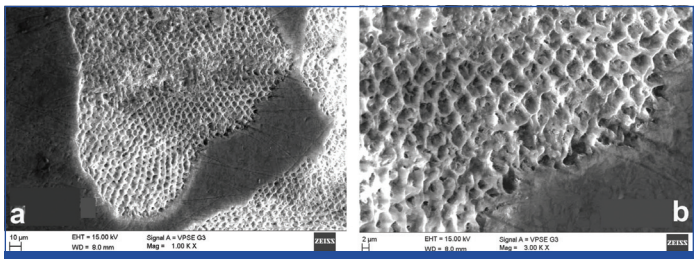
RESULTS

Several images were recorded with SEM on the area of interest from 16X to 3000X magnification. SEM of enamel without the butter coating at 2000X and 5000X magnification showed dissolution of enamel rods and the higher organic interprismatic substance with exposed fibers. Complete and uniform loss of enamel prismatic structure with increased surface microporosities is also evident thereby leading to enlargement of intercrystallite space [Table/Fig-3a,b].



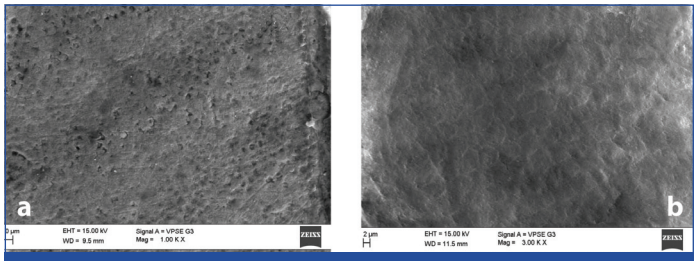
[Table/Fig-3]: Scanning Electron Microscopy (SEM) showed complete and uniform loss of enamel prismatic structure in Group I. a) 2000X and b) 5000X.

SEM of enamel with thin layer of butter coating at 1000X and 3000X magnification showed, enamel surface with islands of protected enamel and limited destruction in the prismatic pattern [Table/Fig-4a,b].



[Table/Fig-4]: Scanning Electron Microscopy (SEM) showed pretreated with thin coating of butter at a) 1000X and b) at 3000X.

SEM of enamel with thick layer of butter coating at 1000X and 3000X magnification, showed intact normal prismatic structure of enamel surface. Butter acts a physical barrier and prevents the demineralisation of the enamel rods. SEM shows uniform, well preserved surface enamel rods and interprismatic structure [Table/Fig-5a,b].



[Table/Fig-5]: Scanning Electron Microscopy (SEM) showed pretreated with thick coating of butter at a) 1000X and b) at 3000X.

DISCUSSION

Formation of dental plaque is a complex process involving 2 different stages. Stage 1 involves reversible attachment of the oral microbes to the tooth surface followed by sucrose dependent adhesion of microorganisms. The initial phase involves adsorption of the microbes onto the tooth surface. If this stage is completed successfully, it is followed by maturation of biofilm by passive transportation of other microorganisms [15].

There are several advantages of bacterial adhesion to the tooth surface. These bacteria utilise necessary metabolites and required co-factors from the adhered tooth surface and the metabolic end products thus produced by the microbes have direct influence on the tooth surface to which these organisms adhere. Considering this as the aetiopathogenesis of caries, research in management of dental caries has always been focused on remineralisation of carious lesions or towards development of antimicrobial products. Although *S. mutans* and *L. acidophilus* are commonly identified as specific cariogenic pathogens, there are many other pathogens capable of producing organic acids which can demineralise the tooth structure. The biofilms having such complex ecology may require wide spectrum antibacterial, which however may not be effective as these biofilms are also known to produce resistance to antibiotics. Bacteria having multidrug resistant pumps are identified which push away the antimicrobial agents from the cell. They are referred to as super resistant bacteria [9]. Also, the caries protective agents which are being prepared may not be accessible to the community due to various reasons. Hence, the anticariogenic agents should not only be effective but also be available easily for the community benefits.

This further justifies the need of approaching the condition from a different perspective. Hence, in this study commonly available dietary ingredient butter was used as surface barrier that would interfere with the process of demineralisation when exposed to cariogenic environment and also may interfere with the process of surface adsorption of the microorganism [16]. Butter was used in this study as this research focused towards identifying caries protective agents that are easily available to the community for routine use. Milk and its products have been long identified as anticariogenic agents as they contain calcium, phosphate, casein and lipids. Caseins are hydrophobic which may prevent pellicle formation on the tooth structure. It also further attracts calcium and phosphate ions onto the tooth surface [17,18].

In this study, butter was used as surface coating prior to exposing the tooth to demineralising solution. The hydrophobic property of casein present in the butter was utilised for preventing demineralisation. Moreover milk and its products are known to possess anticariogenic properties due to its buffering capacity [19]. Milk and milk products are also known to possess caries protective properties either through physical and microbial mechanism. Physical mechanism is through minimisation of adherence of food and microbial colonies to the tooth surface and this property has been assessed in the present study [20]. The other possible contributing factor for observation of reduced demineralised areas in the teeth coated with butter could have been due to the fact that the coated teeth remain supersaturated with calcium and phosphate ions thereby reducing demineralisation [21].

Similar observations were noted by Hegde M et al., [22]. This study reported that the administration of milk and its products would raise the salivary levels of calcium and phosphate and alkaline phosphatase levels thereby keeping the saliva supersaturated with respect to these ions [22]. SEM images clearly demarcated the areas of intact enamel when butter coated tooth was exposed to the cariogenic environment. This can be a simple and effective tool for the high caries risk children to coat the surface of the teeth with commonly available dietary component thereby minimising the risk of caries progression.

Limitation(s)

This present study did not assess the ability of the organisms to colonise after butter application. This can be considered as the future scope of the study.

CONCLUSION(S)

This article focuses on the use of commonly available ingredient butter as an effective mode of physical barrier that can prevent dental caries. Various effective caries preventive agents although existing may not be accessible to the population. Butter application to the tooth surface prior to exposure to the cariogenic environment is beneficial in prevention of the severity of demineralisation. It is known to act as a physical barrier for the prevention of enamel demineralisation. This modality can be considered as an effective caries prevention module for the community in large that has remote access to the therapeutic and preventive procedures.

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