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Comparative Evaluation of Remineralization Potential of Acidulated Phosphate Fluoride and Hydroxyapatite Nanoparticles with and without Iontophoresis on the Incipient Carious Lesion in Permanent Teeth

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Study Protocol

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ABSTRACT

Background: A non- invasive method used to improve the drug delivery with the help of electric arena is termed as Iontophoresis. The iontophoresis process may cause deeper penetration of ions using electric current. Remineralizing nanomaterial may be infused at higher concentrations in the deeper layer of incipient caries under the influence of iontophoresis. Hydroxyapatite crystals are the most stable form of calcium phosphate which is responsible for the mechanical strength of the dental tissues. Improvedre-mineralization occurs more with the apatite particle size of less than 4 µm. Due to the size of the nanocomplexes ofhydroxyapatite, there can be possibilities that they would enter the porosities and diffuse into the body of the subsurface lesion to enhance remineralization using iontophoresis technique.
Objectives: 1) To evaluate the remineralization potential of APF gel with or without iontophoresis on the incipient carious lesion in permanent teeth. 2) To evaluate the remineralization potential of

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hydroxyapatite nanoparticles with or without iontophoresis on the incipient carious lesion of permanent teeth. 3) To compare the remineralization potential of APF gel and hydroxyapatite nanoparticles with or without iontophoresis on the incipient carious lesion in permanent teeth.
Methodology: Forty extracted intact teeth will be taken and artificial caries will be induced. These specimens will be randomly divided into five groups as per the treatment - 1) APF gel application 2) APF gel with iontophoresis 3) Hydroxyapatite nanoparticles solution application 4) Hydroxyapatite nanoparticles solution with iontophoresis 5) Distilled water (control group). Remineralization will be analysis by using Vickers hardness test. The alterations in the carious lesion will be assessed by confocal laser scanning microscopy.
Expected Results: APF gel and hydroxyapatite nanoparticles with iontophoresis will give superior remineralization effect as compared to the conventional method.
Conclusion: The utilization of the iontophoresis with hydroxyapatite nanoparticle will provide improved mineralization of incipient caries and prove to be a better method for treatment.

Keywords: Incipient caries; remineralization; topical fluoride; hydroxyapatite nanoparticles; iontophoresis.

1. INTRODUCTION

Dental caries is a biofilm-derived oral disease. Due to deficient oxygen in deep biofilm layers, bacteria have the capacity to metabolize carbohydrates through the glycolytic pathways. The lactic acid formed as a by-product subsequently declines the pH level within the biofilm and results in subsurface demineralization. The repair of this subsurface demineralization at an early stage is advocated to reduce morbidity. Various methods and materials were utilized for the treatment of the non cavitated carious lesion with partial success. Fluoride decreases demineralization and increases remineralization by its inhibitory action on caries development [1]. Sodium fluoride, acidulated phosphate fluoride, stannous fluoride, amorphous calcium fluoride with casein phosphopeptide are used for topical application.

Remineralization of incipient caries occurs after the introduction of fluoridated restorative material. The combination of fluoride with different restorative materials has a better cariostatic effect than fluoride alone [2].

Among the fluoride-containing applications topical acidulated phosphate fluoride (APF) is used widely. Some earlier studies showed that acidulated phosphate fluoride (APF) is more efficient than neutral sodium fluoride in the healing of carious lesions. The acid present in APF dissolves the surface of the enamel and allows deeper penetration of fluoride into the enamel which leads to the formation of CaF_2 [3,4]. Thus it provides a significant effect in remineralization of enamel.

Studies have proved the deposition of the ions from subsurface areas into the superficial enamel leading to the smaller pore size on demineralization. In the superficial zone of the artificial carious lesion, the microchannels are found to be about 0.5-1.5 µm in diameter and approximately about 100 µm in length [5]. Hence materials with lesser particle sizes are required to allow the deposition at higher concentrations.

Enamel and dentin are mainly formed by hydroxyapatite crystal $[Ca_{10}(PO_4)_6(OH)_2]$, the building block of dental tissues representing 95% - 97% wt and 75 % in enamel and dentin respectively. Hydroxyapatite crystal $[Ca_{10}(PO_4)_6(OH)_2]$ constitutes the key mineral component of teeth and bone. Hydroxyapatite crystals are the most stable form of calcium phosphate which is responsible for the mechanical strength of the dental tissues. Synthetic hydroxyapatite nanoparticles were developed and about 20-100 nm in size. It was found that remineralization efficacy increases with lesser apatite particle size [6].

When compared to amine fluoride toothpaste, nano-HA showed more remineralization effects in dentin [7]. Nano-hydroxyapatite has been advocated for remineralization of teeth. It is hydrophilic and has greater surface area than the conventional hydroxyapatite crystals. Nano-hydroxyapatite is found to be hydrophilic with a larger surface area and better wettability than the conventional hydroxyapatite. Therefore it has been introduced as a remineralizing agent [8]. Because of its reduced size, nanocomplexes of hydroxyapatite will enter the porosities and diffuse into the body of the subsurface lesion to enhance remineralization [6]. Products with

nanoparticles have led to improved deeper precipitation of phosphate and calcium ions in the tooth structure. Therefore better techniques need to be utilized for the introduction of nanoparticles into tooth structure.

In the early 1960s, dentin hypersensitivity was reduced by using iontophoresis [9]. It utilizes a low ampere electrical current for the incorporation of drugs into the tissues. It works on the principle of repulsion of like charges and attraction of the opposite charges. It allows a concentrated form of the drug to be introduced into the needed localized area under an electrical gradient [10].

Thus, keeping this in mind, the present study will be conducted to evaluate the remineralization potential of APF gel and hydroxyapatite nanoparticles under the influence of iontophoresis. The null hypothesis is that there will not be any difference between the remineralization potential of the acidulated phosphate fluoride and hydroxyapatite nanoparticles with iontophoresis on the incipient carious lesion in permanent teeth.

1.1 Rationale

The topical application of fluoride leads to remineralization of the superficial layer of enamel without repairing subsurface lesions. The iontophoresis process may cause deeper penetration of ions using electric current. Remineralizing nanomaterial may be infused at higher concentrations in the deeper layer of incipient caries under the influence of iontophoresis. Therefore iontophoresis with nanoparticles might prove advantageous for subsurface remineralization.

1.2 Aim

To evaluate and compare the remineralization potential of acidulated phosphate fluoride gel and hydroxyapatite nanoparticles with and without iontophoresis on the incipient carious lesion in permanent teeth.

1.3 Objectives

1. To evaluate the remineralization potential of APF gel with or without iontophoresis on the incipient carious lesion in permanent teeth
2. To evaluate the remineralization potential of hydroxyapatite nanoparticles with or without iontophoresis on the incipient carious lesion of permanent teeth.
3. To compare the remineralization potential of APF gel and hydroxyapatite nanoparticles with or without iontophoresis on the incipient carious lesion in permanent teeth.

2. MATERIALS AND METHODS

2.1 Sources of Data

The present study will be carried out in the 'Department of Pediatric and Preventive Dentistry' of 'Sharad Pawar Dental College, Sawangi (Meghe), Wardha'.

2.2 Sample Size Calculation

Sample size formula for difference between two means:

$$n=\frac{(z\propto+z\beta)^2\left[\delta_1^2+\frac{\delta_2^2}{n}\right]}{\Delta^2}$$

Where,

Zα is the level of significance at 5% level of significance i.e 95% confidence interval =1.96
Zβ is the power of test=80%=0.84
δ_1=SD of VHN in negative control=17.41
δ_2=SD of VHN in conventional application=23
Δ= Difference between two means
=145.33-106.37
=39.07

$$n\ \frac{=(1.96+0.84)2[(17.41)2+(23)2/1]}{(39.07)2}$$

=4.27
=5 samples in each group

2.3 Materials Required

- Intact tooth
- 1.23%APF gel
- Hydroxyapatite nanoparticle solution
- 10% HCl as an etchant
- Iontophoresis device (ENDOEST 5F, Geosoft, Russia)
- Cold cure acrylic resin
- Plastic moulds
- Silicon carbide paper

2.4 Equipment for Analysis

- Vickers microhardness testing machine.
- Confocal Laser Scanning Microscopy.

2.5 Inclusion and Exclusion Criteria

2.5.1 Inclusion criteria

- Caries-free extracted permanent teeth.

2.5.2 Exclusion criteria

- Hypomineralized areas, fractures and distorted enamel structure.

2.6 Sample Preparation Procedure

Forty extracted caries-free permanent teeth will be collected and divided into two parts that are buccal and lingual. The specimen will be stored at 0.1% thymol solution. Removal of soft tissue remnants will be done, followed by cleaning with pumice without fluoride and a rubber cup. Each crown surface will be coated with double layers of acid-resistant varnish, parting an exposed window of approximately 4.0 X 4.0 mm on the middle third of buccal or lingual surface of the enamel.

2.6.1 Specimen will be allocated into five groups

Group 1: APF gel
Group 2: Iontophoresis with APF gel
Group 3: Hydroxyapatite nanoparticles
Group 4: Iontophoresis with hydroxyapatite nanoparticles
Group 5: Control group [distilled water]

2.6.2 Artificial carious lesion induction

Specimen will be immersed in lactic acid gel [0.1M lactic acid, 0.2% Carbopol ETD 2050, 50% saturated hydroxyapatite (calcium phosphate) pH-5 at 37^0 C] for 14 days for preparation of artificial carious lesion. Specimens will be washed with deionized water for 30 s after demineralization.

2.6.3 Analysis of surface microhardness

Measurement of surface microhardness will be done with the help of a Vickers microhardness tester under a 100g weight for 15 seconds. Differences in microhardness will be analyzed by measuring the microhardness post demineralization and remineralization treatment procedure. The mean of the three indentation readings at a distance of 100µm will be measured for assessment of the microhardness.

2.6.4 Remineralization method

Remineralizing agents will be applied to the 10 demineralized specimens in each group. In group 1 APF gel will be applied for 4 minutes. In group 2, APF gel application with iontophoresis will be carried out at 0.8 mA current for 4 minutes. In group 3 and group 4, etching with 10% HCl will be carried out for 1 minute followed by the application of Hydroxyapatite nanoparticle solution for 4 minutes in group 3. Whereas in group 4, Hydroxyapatite nanoparticles solution will be applied along with iontophoresis at 0.8 mA current for 4 minutes. In group 5, teeth will be placed in distilled water without any treatment. Post-treatment surface microhardness testing will be conducted under the same conditions as the pre-treatment test. The percentage of surface hardness recovery will be calculated.

2.6.5 Analysis by Confocal Laser scanning microscope

Six specimens will be included in each group. The teeth will be treated with the same method as mentioned above which includes carious lesion formation and treatment procedure. After treatment, the enamel specimens will be cross-sectioned using a microtome saw. The segmented specimen will be painted with 0.1 mM rhodamine B solution for 1 h to analyze the demineralized lesion area and red fluorescent images will be obtained. Then all the specimens will be washed in distilled water. Subsequently, all the specimens will be viewed under the confocal microscope for analyzing the demineralization and remineralization.

2.7 Statistical Analysis

Statistical analysis will be done using one-way ANOVA and Tukey multiple comparison test.

3. EXPECTED OUTCOME

APF gel and hydroxyapatite nanoparticles with iontophoresis will give a superior remineralization effect as compared to the conventional method.

4. DISCUSSION AND CONCLUSION

Various studies for the evaluation of iontophoresis effect on fluoride gain and remineralization was done which are discussed further:

Changes occurring due to iontophoresis employing various intensities of current on the

fluoride uptake in tooth enamel with carious-like lesions was assessed. The highest amount of Fluoro-hydroxyapatite was formed with a fluoride group treated at a 0.8 mA current, in contrast to the fluoride group treated without current and with the current application of 0.1 mA. Thereforeiontophoresis with more current that is 0.8 mA in conjunction with fluoridated gel application (2% NaF) results in improved uptake of fluoride by enamel with carious lesions, to form fluorapatite. Hence this study concluded that an increase in current intensity increases the deposition of fluoride [11].

Kim et al. compared the remineralization effect of fluoride with and without iontophoresis. There were three groups: no fluoride treatment, conventional fluoride application and fluoride iontophoresis. In the fluoride iontophoresis groups, an iontophoresis device (0.4 mA, 12V) was used. Fluoride iontophoresis groups showed a higher Vickers hardness number than the conventional fluoride application groups. No significant difference was observed between application methods of fluoride. Lesion depth was measured using Confocal Laser Scanning Microscope imaging, no remarkable difference was observed between the fluoride iontophoresis and conventional fluoride application groups. Therefore it was concluded that in the case of the remineralization effect fluoride iontophoresis group was not superior to the conventional fluoride group [12].

One study was done to evaluate the remineralization effects of an iontophoresis device named 'Flurinex', conventional acidulated phosphate fluoride gel (APF) treatment, and conventional iontophoresis device by measuring with a laser fluorescence device. The specimens with 60 immature, intact premolars and molars were taken and artificial carious lesions were created. These specimens were then randomly allotted into four groups as follows: i) The First group was 1.23% APF gel application by the conventional method for 4 minutes. ii) Second group was 2% sodium fluoride solution (NaF) application by a conventional iontophoresis device for 4 minutes. iii) The specimen in the 'Fluorinex' group were first treated with copper chloride ($CuCl_2$)for 1 minute followed by 1.23% Acidulated Phosphate Fluoride (APF) gel application for 4 minutes in a Fluoritray. iv) In the Control group, specimen were placed in distilled water for 4 minutes. Therefore it was concluded that fluoride application by 'Fluorinex' ensured a superior effect on remineralization of incipient caries as compared to APF gel application by conventional method and NaF iontophoresis [13]. Few of the related studies on caries and nanotechnology were reviewed [14-17].

5. SCOPE

It will be a new way of using an iontophoresis device with hydroxyapatite nanoparticles to improve subsurface remineralization.

6. LIMITATION

This is an in vitro study. The results may vary in in-vivo conditions depending upon the influence of saliva and oral microbial flora.

7. IMPLICATION

The utilization of the iontophoresis with hydroxyapatite nanoparticle will provide improved remineralization of incipient caries and prove to be a better minimal invasive method.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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