



RESEARCH ARTICLE

COMPARATIVE ANALYSIS OF ENAMEL MINERAL PROFILE USING VARIOUS REMINERALIZING AGENTS WITH CONFOCAL LASER RAMAN SPECTROSCOPY - AN INVITROSTUDY

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Abstract

Aim: To evaluate quantitative changes in enamel mineral profile using CPP-ACP, ACP and Bio-active dentine with confocal laser raman spectroscopy.

Materials and Methods: Totally, 25 extracted intact pre-molar teeth were collected as specimens. Then the specimens were sectioned at CEJ. The sectioned teeth were evaluated for mineral content using Confocal Laser Raman Spectroscopy (Group -I). The specimens were placed in demineralizing solution for 4 days and were evaluated for mineral content using Confocal Laser Raman Spectroscopy (Group II). The teeth were then divided into other three groups (6 teeth each) and treated with MI Varnish (Group III), Enamel Pro Varnish (Group IV), and Bio-active Glass mixed with pro-fluoride varnish (Group V) and then dipped in artificial saliva for 5 days, remaining 6 teeth were dipped only in artificial saliva (Group VI) following which the teeth surfaces were studied using Raman spectroscopy to assess the remineralization potential of the three dentifrices. The data were recorded and analyzed statistically.

Results: Raman spectroscopic analysis revealed better remineralization with Bio-active Glass mixed with pro-fluoride varnish, from the groups treated with the MI Varnish and the Enamel Pro Varnish.

Conclusion: It can be concluded that the demineralized samples of teeth treated with Bio-active Glass mixed with pro-fluoride varnish, was most effective in remineralization of early enamel caries at surface level when analyzed using Raman spectroscopy.

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Introduction:-

Dental caries is initiated through the demineralization of tooth hard tissues, which is a “pH-driven phenomenon”. This occurs by organic acids produced from fermentable carbohydrates by dental plaque cariogenic bacteria. Demineralization and remineralization can be considered as a dynamic process, characterized by the flow of calcium and phosphate out of and back into the tooth enamel, which should be balanced to prevent the progression of caries.^(1,2,3) Tooth structure undergoes demineralization and remineralization in the oral cavity. If this balance is disrupted, demineralization will progress, leading to deterioration of tooth structure. Calcium and phosphate are lost from subsurface enamel, resulting in formation of demineralized enamel. This subsurface demineralization increases

porosity and changes the optical properties of enamel. Typically, the enamel surface layer stays intact during subsurface demineralization, but without treatment will eventually collapse into a full cavity.⁽⁴⁾

Remineralization is the natural repair process for caries lesions. This occurs when calcium and phosphate in water among enamel or dentin crystals recrystallize on the surface of existing crystal remnants. Remineralization requires saliva or some other liquid to facilitate the transport of calcium and phosphate ions into the tooth.⁽⁵⁾

There are increasing numbers of promising technologies aimed at enhancing tooth mineralization or preventing tooth decay, yet relatively few technologies are brought to market with substantiated claims of anticariogenic performances, even when favorable clinical performances have been established⁽⁶⁾. BAG is a unique material that has numerous novel features; the most important being its ability to act as a biomimetic mineralizer, matching the body's own mineralizing traits.^(7,8) Bioactive glass was originally developed as a bone regenerative material and recently has been used for oral care applications such as toothpaste, mouthrinse. Bioactive glass is made of synthetic mineral containing sodium, calcium, phosphorous and silica which also has remineralizing properties. It has been shown in vitro and in situ to reduce hypersensitivity by occluding exposed dentinal tubules, and decrease gingivitis and bleeding. It is hypothesized to form a mechanically strong hydroxyapatite-like layer on the dentine surface which is resistant to acid challenges.⁽⁹⁾

The concept of CPP-ACP as a remineralizing agent was first postulated in 1998. The CPP-ACP technology stabilizes high concentrations of calcium and phosphate ions at the tooth surface binding to pellicle and plaque, which provides a highly effective means for elevating calcium levels in dental plaque fluid, something which is desirable for enhancing remineralization.⁽¹⁰⁾ ACP is thought to be a precursor in the formation of hydroxyapatite, has also shown anticariogenic properties with remineralization potential.

Confocal Laser Raman Spectroscopy is considered as one of the useful techniques for assessing quantitatively the levels of demineralization and remineralization. This method allows the characterization, analysis of the concentration of ions, like carbonate, acid phosphate, as well as identifying other organic or mineral compounds.⁽¹¹⁾

Hence, this study was carried out to compare the remineralization potential of three different remineralizing agent using Raman's spectroscopy

Materials and Methods:-

Twenty five extracted intact premolar teeth were collected as specimens. They were cleaned of any debris, and stored in normal saline until further use. The teeth were preselected to exclude those with cracks or any external structural faults. Then the specimens were sectioned at CEJ.

Demineralizing Solution Preparation:

The buffered demineralizing solutions was made up of analytical-grade chemicals and distilled water. The demineralizing solution, which contained 2.2 mM CaCl₂, 2.2 mM KH₂PO₄, and 0.05M acetic acid, had the pH adjusted to 4.4 with 1 M KOH.

Grouping of specimens:

In Group I (Fig 1)- The sectioned teeth were evaluated for mineral content using Confocal Laser Raman Spectroscopy.

In Group II (Fig 2 & 3) - The specimens were placed in demineralizing solution for 4 days and were evaluated for mineral content using Confocal Laser Raman Spectroscopy.

In group III (Fig 4,5) – 6 specimen were treated with MI Varnish (Composition – 5%Naf with CPP-ACP). Thin uniform layer is applied and then placed in artificial saliva for 5 days

In group IV (Fig 6) –6 specimen were treated with Enamel Pro Varnish (Composition – 5%Naf with Amorphous calcium phosphate). Thin uniform layer is applied and then placed in artificial saliva for 5 days

In group V (Fig 7) - 6 specimen were treated with Bio-active Glass mixed with pro-flouride varnish of 0.4ml (consisting – 5% NaF). Thin uniform layer is applied and then placed in artificial saliva for 5 days

In group VI (Fig 8) - 6 specimen were placed directly in artificial saliva for 5 days

Then the specimens of Group III, IV, V and VI were evaluated for remineralization using Confocal Laser Raman Spectroscopy (Fig 9)

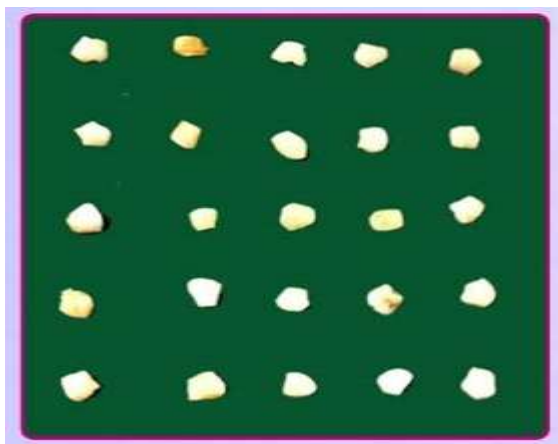


Fig 1:- (Group – I)

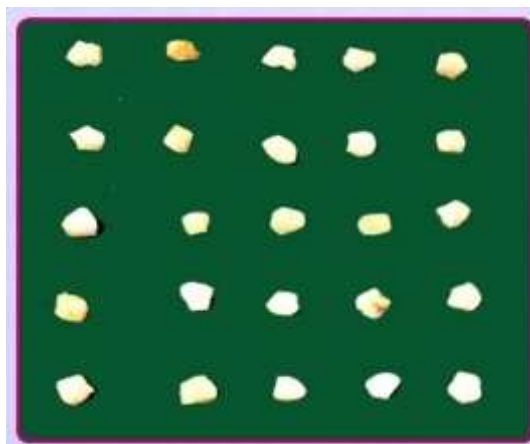


Fig 2:- (Group – II).



Fig 3:- (Demineralized Solution).



Fig 4:- (Group – III).



Fig 5:- (Group – III - treated with MI Varnish).



Fig 6:- (Group – IV).



Fig 7:- (Group – V).



Fig 8:- (Group – VI).



Fig 9:- (Confocal Laser Raman Spectroscopy).

Methodology:-

For Raman Spectroscopy:

Quantitative evaluation was done using Raman spectroscopy. The spectra were recorded with JobinYv on Horibra Lab RAM HR. The LabRAM HR systems provide high spectroscopic resolution and a unique wavelength range capability that offers both great flexibility and high performance. The laser source is an argon laser with a 488 nm wavelength. The output power was fixed to 100 mw so that the surface would not be overheated. The laser beam was focused onto the sample surface using the light-optical microscope of the micro-Raman spectroscope. The focal size was estimated to be 10 μ m in diameter. Moreover, a comparison is drawn between the three groups to assess the remineralizing potential of each remineralizing agent .

Results:-

1. Student T test revealed significant difference ($p < 0.05$) between Group I and Group II , Indicating samples were demineralized effectively.
2. In inter group comparison between Group III,IV,V & VI – the Group II revealed statistically significant difference with Group V ($p < 0.05$) , with mean values 37.2 and 56 respectively.
3. Bio-active glass was most effective in remineralization of early enamel caries at surface level



Intergroup comparison of the amount of remineralization.

Discussion:-

Crystals at the tooth surface regularly go through natural periods of mineral loss (demineralization) and mineral gain (remineralization), particularly surfaces covered by undisturbed (stagnant) films, i.e., dental plaque.

Several techniques have been developed to measure mineral changes on human enamel. These techniques include microhardness measurement of enamel surface, microhardness measurement of enamel cross-sections, polarized light microscopy, different microradiography techniques, iodine absorptiometry, and light scattering. All methods have their share of advantages and disadvantages. Only a few studies have assessed the remineralization of teeth using Raman spectroscopy taking into consideration the excellent remineralization capabilities of these remineralizing agent, this study was aimed at comparing the remineralization potential of the same using Raman spectroscopy.⁽¹²⁾

In the present study, the enhanced intensity in the peak of the Raman band following treatment with BAG corresponds to more hydroxyapatite formation, which explains the enhanced remineralization. BAG contains elements that release ions such as calcium, phosphorus, sodium, silica and when comes in contact with saliva helps in remineralization by formation of a crystalline hydroxylcarbonate apatite layer that is structurally and chemically similar to natural tooth mineral. Unlike other calcium phosphate systems, the ions that BAG release from hydroxycarbonate apatite (HCA) directly, without the intermediate ACP phase^(12,13,14)

Raman spectroscopy is a non-destructive method for identification of chemical bond in micron scale and assessing quantitatively the levels of demineralization and remineralization.

Conclusion:-

Within limits of the present study, Bio-active glass is effective in remineralization of early enamel caries at surface level.

However one must bear in mind , that remineralization in vitro may be quite different when compared to dynamic complex biological system which usually occur in oral cavity. Thus direct extrapolation to clinical condition must be exercised with caution because of obvious limitation of in-vitro study

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