# An Investigation about the Reminerlization Potential of Bio-active glass on Artificially Carious Enamel and Dentin using Raman Spectroscopy.

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The remineralization of artificially carious enamel as well as dentin using bioactive glass particles is investigated and compared to the application of mineralizing solutions. The role of Zeolite powder in enhancing the remineralization process is also studied. Four treatment combinations were done for each tissue. They were, the application of bioactive glass alone, Zeolite followed by bioactive glass, mineralizing solution alone, and Zeolite followed by mineralizing solution. All specimens were kept in a humidifier at 37$^{o}$C. Two studies were carried out, the scanning electron microscopy to study surface topography, and Raman spectroscopy to evaluate quantitatively possible chemical changes in mineral profiles. The results indicate that Bioactive glass has the potential for remineralizing artificially carious enamel and dentin, the application of Zeolite enhances the effect of the mineralizing agent and the remineralization ability of carious dentin is greater than that of enamel.

## 1. Introduction:

It is known that dental caries is a bacterially based disease. When it progresses, acid produced by bacterial action on dietary fermentable carbohydrates diffuses into the tooth and dissolves the carbonated hydroxyapatite mineral, a process called demineralization. Traditional caries pathology provides no definite guide for caries treatment, although textbooks advise complete removal of carious infected dentin showing both softening and discoloration. However, neither could indicate the depth of bacterial invasion [1].

On the other hand experiments to study remineralization have been carried out with acid-etched enamel using scanning electron microscopic technique [2]. It was proved that initially demineralized enamel could be remineralized, if there was no mechanical deformation of the enamel, i.e., there was no breakage of the surface layer [3].

Although gaining full understanding of the remineralization process may be difficult, since both physical and chemical processes are relevant. The site and the amount of mineral deposition are probably determined by the physical condition (mineral distribution profile and transport mechanism) and by the chemical process (deposition). There is little doubt that significant remineralization of enamel and dentin lesions can occur under both *invitro* and *invivo* conditions, and therefore, remineralization is increasingly accepted as a viable non-invasive approach for restoring carious teeth, at least during the earlier stages of the caries process [4].

Recently, bioactive glass materials have been introduced in many fields of dentistry. This unique material has numerous novel features, most important of which are its ability to act as a biomimetic mineralizer, matching the body's own mineralizing traits, while also affecting cell signals in a way that benefits the restoration of tissue structure and function. Bioactive glass is considered a break through advance in remineralization technology. This is because the current standard treatment for tooth remineralization and prevention of decay is a slow acting and is dependent on adequate saliva as a source of calcium and phosphorus [5]. Clark et al.[6], showed that occlusion and mineralization of open dentinal tubules occurs, when bovine dentin was treated with bioactive glass.

Furthermore, Zeolites are crystalline aluminosilicates with fully cross-linked open framework structures, made up of corner-sharing $SiO_4$ and $ALO_4$ tetrahedra with the ratio (Si & AL)/O must equal ½. Zeolites act as a "molecular sieve", as they have selective adsorption properties capable of separating components of a mixture on the basis of a difference in molecular size and shape. The primary building unit of a molecular sieve is the individual tetrahedral unit. Therefore, Zeolites are selective, high capacity adsorbents, because of their high intracrystalline surface area and strong interactions with adsorbate [7]. These special characteristics suggest that, treatment of caries lesion with Zeolites before bioactive glass or mineralizing solution, may play an important role in the reminerslization process, which will be investigated here.

The final efficacy of remaineralizing agent is assessed in terms of minerals lost during demineralization and minerals gained during remineralization. Raman scattering spectroscopy is considered one of the useful techniques for assessing quantitatively the levels of demineralization and remineralization [8]. It is a relatively recent approach for very fine analysis of chemical bonds of structures like, enamel, dentin, and bone, in a micron scale. This method allows the characterization, analysis of the concentration of ions, like carbonate, acid phosphate, as well as identifying other organic or mineral compounds. Furthermore, micro Raman spectroscopy can be used to study the chemical and physical properties of biomaterials and their evolution after implantation in a dental or bone site. It is the only non-destructive method for identification of chemical bonds in the micron scale and gives the fingerprint of the studied component.

The purpose of this research is to study the ability of bioactive glass to remineralize artificially carious enamel and dentin, with and without the use of Zeolite, using conventional mineralizing solution as a base line for the ability of remineralization. Three aspects were investigated in this study: a) the topography of the remineralized areas (using scanning electron microscope (SEM)), (b) the nature of the compounds deposited in the lesions, (c) the degree of remineralization, (both using Raman spectroscopy).

**Specimens Preparation and Experimental Methods**

Recently extracted human permanent molars were used in this study. Teeth were cleaned from soft deposits using non-fluoridated toothpaste, scaled and stored in isotonic saline till use. For the enamel samples, the buccal enamel was abraded using a fine diamond stone under adequate water coolant to produce flat surface and to remove the relatively inactive enamel. For the dentin specimens, also the buccal dentin was exposed 1 mm inside D.E.J. to produce a flat surface, in the same way mentioned for enamel. All flattened enamel and dentin surfaces were subjected to artificial caries lesion formation.

Teeth were suspended in jars containing a 12% solution of gelatin adjusted to pH 4 by using 0.1-M lactic acid for 2 weeks [9]. The acidified gel was renewed every 5 days, before the pH meter showed any deviation from 4. A pilot study was done using Raman spectroscopic analysis to determine the ability of the gel to create artificial caries lesion.

Two mineralizing materials were used, bioactive glass and ITS mineralizing solution. They were either applied directly over the artificially carious lesion or after the specimens were first treated with Zeolite powder. Therefore, four combinations of surface treatments were done for each artificially carious enamel and dentin. They were namely, application of bioactive glass, mineralizing solution, Zeolite powder followed by bioactive glass, and Zeolite followed by mineralizing solution. The composition and application time of each material are presented in table (1). After the designated treatments, excess materials were removed using air spray.

**Table (1):** Surface treatment materials used in the study:

| Material | Composition | Application technique | Manufacturer |
|---|---|---|---|
| Bioactive glass ($S53P_4$) | $SiO_2$ (53%), CaO (20%), $Na_2O$ (23%) and $P_2O_4$ (4%). | 1 gm of bioactive glass is mixed with 2 drops of distilled water using plastic spatula carried and applied over the tissue. Left for 10 days in humidifier at 37$^o$C | Apmin technologies, LTD, Turku, Finland. |
| Mineralizing solution (ITS) | $CaCl_2$ KCl, Mgcl. $6H_2O$,Nacl,$NHCO_3$, $NaH_2$ $PO_4$. $H_2O$ and glucose | Each specimen was immersed in a vial containing 2 ml of solution for 10 days at 37$^o$C | NOVRTIS Pharma AG., Basle, Switzerland |
| Zeolite | Me APSO-n (metallo silico-aluminophos-phates). Where the Me molecule is Fe. | Zeolite is applied with brush, agitated, left for 2 min and then mineralizing agent was applied. | ICN Biomaterials Inc Costa Mesa, CA 92626, No. 1318-02-1 |

In the scanning electron microscopic part of study, all designated artificially carious enamel and dentin specimens were gold sputtered and viewed under 30 kv JEOL scanning electron microscope.. Some specimens of each tissue were scanned immediately after artificial caries formation without any surface treatment.

As for Micro Raman spectroscopic study, profilometry of all artificially carious lesions of each group (enamel and dentin) was performed before any surface treatment. Each group was then subjected to its corresponding surface treatment and its mineral profilometry was then redone for comparison.

Raman spectra were recorded with upgraded Jobin-Yvon Ramanor HG25, equipped with a double monochromator and a photon counting module. As excitation source, an $Ar^+$ ion gas laser tuned to a wavelength of 514.5 nm was used. The output power was fixed to 100 mW so that the surface would not be over heated. The laser beam was focused on to the sample surface using the light-optical microscope of the Micro-Raman spectroscope. The focal size was estimated to be 10 μm in diameter. In all the specimens each recorded spectrum is an average of at least five times measurements.

## 2. Results:

The results of the scanning electron microscopic examination for the ability of bioactive glass and mineralizing solution with and without Zeolite to produce an effect on the surface topography and mineral content of artificially carious enamel and dentin are presented in Figs. (1-10). The corresponding Raman spectra are given in Figs. (11-12). Representative samples are selected from each group to be presented.

The scanning electron micrographs of artificially carious enamel are presented in Fig 1. It shows a rough surface with honeycomb appearance, characteristics of carious enamel. The effect of bioactive glass on artificially carious enamel is presented in Fig. 2. It exhibits a relatively smother surface compared to that of Fig. 1. The rod ends are almost smooth, with decreased inter-rod spaces. On the other hand, the SEM photomicrograph (Fig. 3) of bioglass treated, artificially carious enamel, following Zeolite application demonstrates a nearly smooth surface, with complete obtrusion of inter-rod spaces in some fields. The rods appeared as if they were fused together with some globules deposited on the surface. The effect of mineralizing solution on artificially carious enamel is presented in Fig. 4, which shows a smoother surface than that of artificially carious enamel of Fig. 1, with starting obliteration of inter-rod spaces. The porosity of the surface was greatly reduced, but a honey comb appearance is still recognizable. The mineralizing solution following Zeolite treatment is presented in Fig. 5 produced a smoother effect, yet some areas reveal a moderately rough surface. It is to be noted that, bioactive glass with zeolite (Fig. 3) produced a far smoother surface for the artificially carious enamel than that of mineralizing solution with Zeolite (Fig. 5). The SEM of artificially carious dentin is presented Fig. 6, which shows widely open dental tubules, with partially demineralized peritubular dentin. The SEM of artificially carious dentin, after bioactive glass treatment shown in Fig. 7, which reveals obtrusion of dentinal tubules with moderately smooth dentin surface. The SEM for the effect of bioactive glass following Zeolite application is shown in Fig. 8, which exhibits a smooth surface, with possible determination of the location of dentinal tubules. A distinctive feature, is that, some areas appeared to be folded over themselves. The mineralizing solution again produced obtrusion of dentinal tubules as presented by SEM in Fig. (9). The same effect was produced when Zeolite was first applied as seen in Fig. (10), but the inter tubular dentin surface exhibited a smoother surface.

Raman spectra of enamel and dentine in the region of 500-1800 $cm^{-1}$ with different treatments are shown in Figs. 11 and 12, respectively. The spectrum of the artificially carious specimen is shown together with the corresponding four cases of treatment that were mentioned previously. In all spectra for enamel and dentin, the strong characteristic peak at 962 $cm^{-1}$, which is assigned to the P-O totally symmetric stretching mode of the phosphate ion in hydroxyapatite. The enhancement of the intensity of this Raman line was used as a direct measure of the remineralization of enamel and/or dentine.

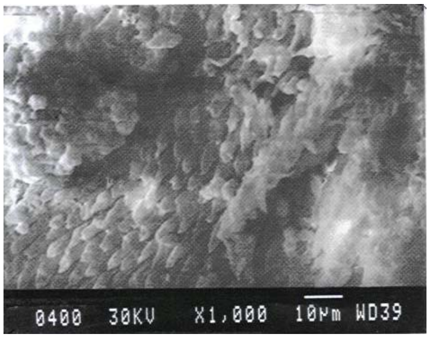


**Fig. (1):** SEM micrograph of artificially carious enamel.

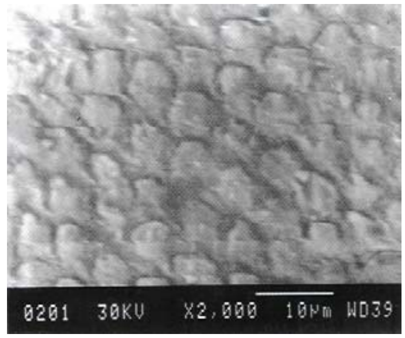


**Fig.(2):** SEM micrograph for the effect of bio-active glass on artificially carious enamel.

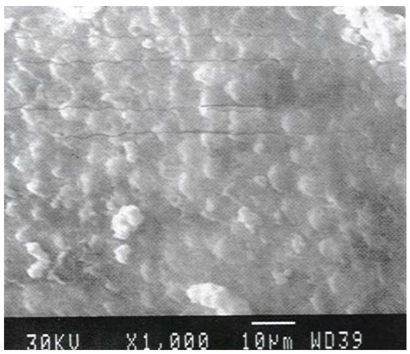


**Fig.(3):** SEM micrograph for the effect of bio-active glass on artificially carious enamel after zeolite application.

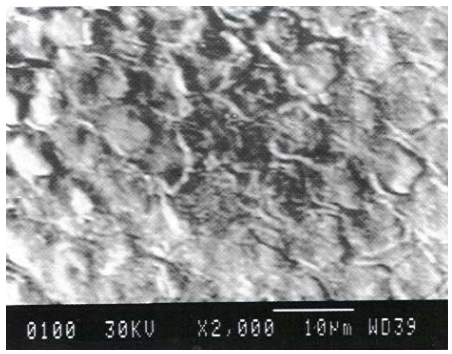


**Fig.(4):** SEM for the effect of mineralizing solution on artificially carious enamel

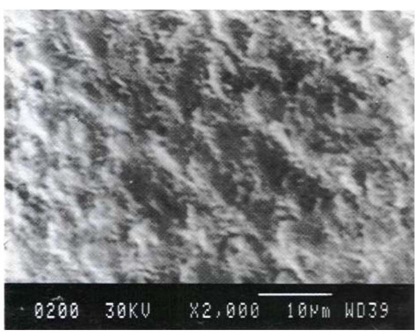


**Fig.(5):** SEM micrograph for the effect of mineralizing solution on artificially carious enamel after zeolite application.

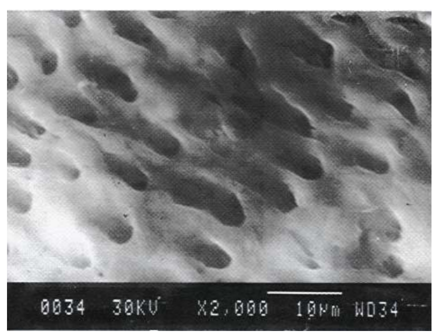


**Fig.(6):** SEM micrograph of artificially carious dentin.

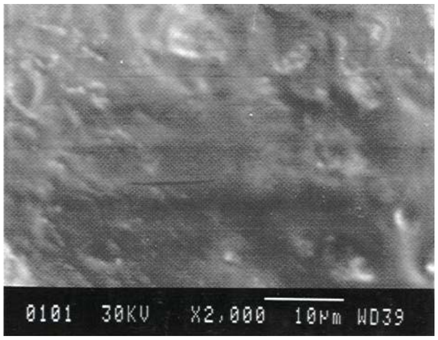


**Fig.(7):** SEM micrograph for the effect of bio-active glass on artificially carious dentin.

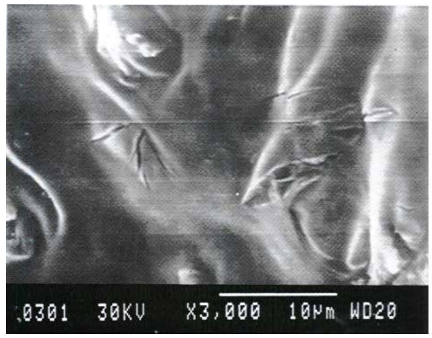


**Fig.(8):** SEM micrograph for the effect of bio-active glass on artificially carious dentin after zeolite application.

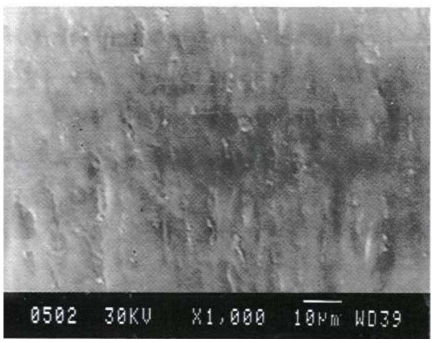


**Fig.(9):** SEM micrograph for the effect of mineralizing solution on artificially carious dentin.

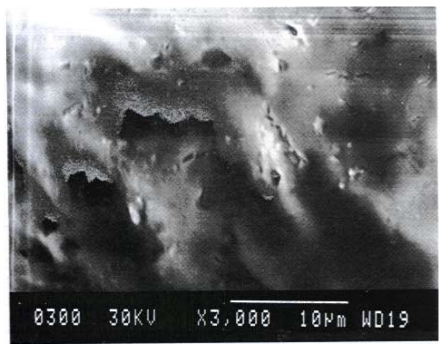


**Fig.(10):** SEM micrograph for the effect of mineralizing solution on artificially carious dentin after zeolite application.

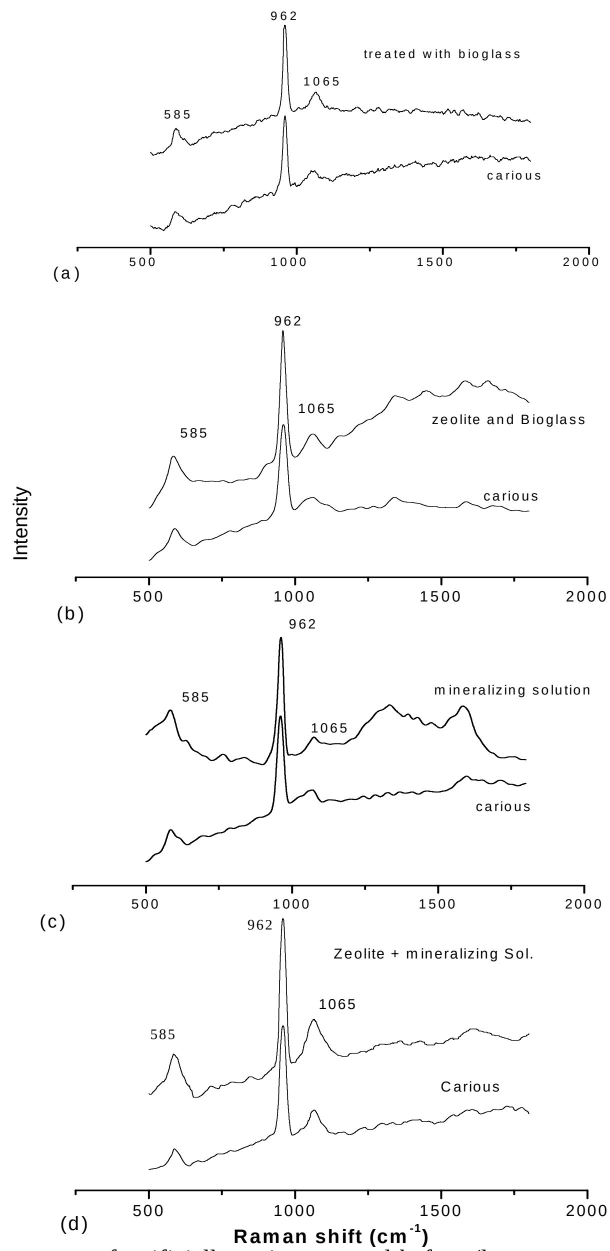


**Fig.(11):** Raman spectra of artificially carious enamel before (lower spectra) and after (upper spectra) treatment as indicated.

a. Bioactive glass treatment　　b. Bioactive glass & zeolite treatment

c. Mineralizing solution treatment　　d. Mineralizing solution & zeolite treatment

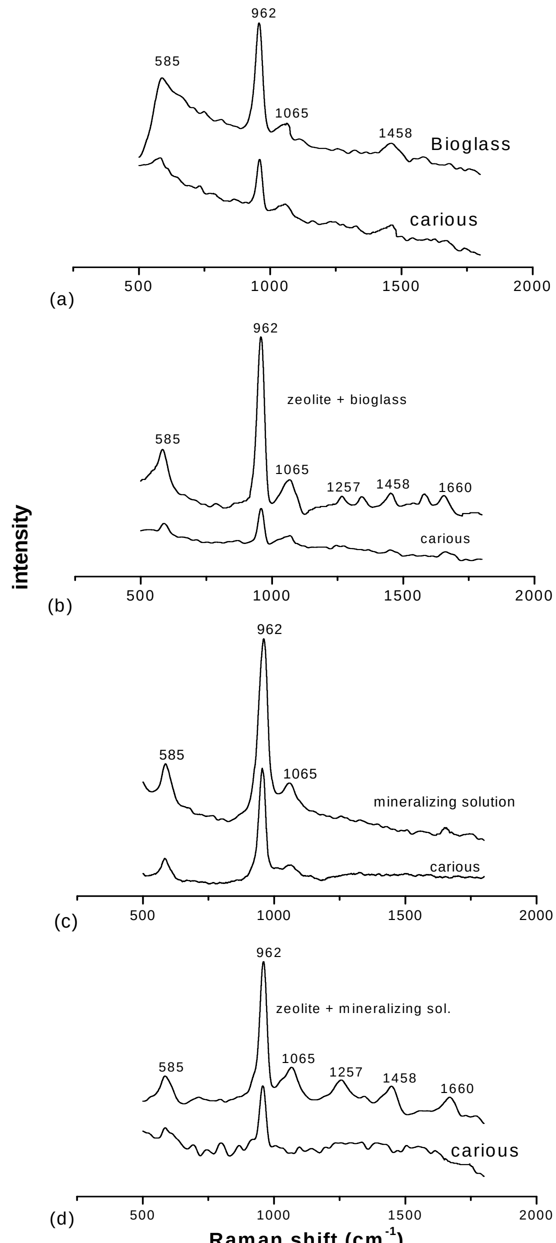


**Fig.(12):** Raman spectra of artificially carious dentin before (lower spectra) and after (upper spectra) treatment as indicated.

a. Bioactive glass treatment b. Bioactive glass & zeolite treatment

c. Mineralizing solution treatment d. Mineralizing solution & zeolite treatment

Fig. (11-a) shows the Raman spectra for the artificially carious enamel before and after treatment with bioglass. In addition to the principle peak at 962 $cm^{-1}$ there are two other lines at 585 and 1065 $cm^{-1}$ that are also assigned to the phosphate ion in hydroxyapatite, it is clear that there is an enhancement of the Raman intensity for the three lines.. When using mineralizing solution on the artificially carious enamel, also great enhancements of the Raman intensities are obtained for the same lines as shown in Fig. 11- c. The peak at 962 $cm^{-1}$ is enhanced by a factor of 28% in case of bioglass, which is close to the enhancement factor 30% in the case of mineralizing solution treatment.

Fig. 12 (a and c) shows the Raman spectra of the artificially carious dentin together with that after being treated with bioglass and the mineralizing solution respectively. It can be seen that the enhancement in the Raman intensity is larger in case of dentin than that in case of enamel for both bioglass and mineralizing solution. Where the enhancement factor of the characteristic peak (962 $cm^{-1}$) is 60% in case of bioglass and 50% in case of mineralizing solution treatment, showing that the two treatments having a greater effect in case of dentine than enamel.

The effects of applying Zeolite to the treated enamel with bioglass and mineralizing solution are shown through the spectra in Fig. 11 (b and d) respectively that show further enhancement of the phosphate ion lines. The same observations of a larger enhancement of the phosphate ion lines are obtained on applying zeolite to the treated dentin with bioglass and mineralizing solution as shown in Fig. 12 (b, d) respectively. Furthermore in these spectra it is easily observed that Raman lines 1257, 1458 and 1660 $cm^{-1}$ corresponding to the collagen that are completely within the noise of the spectra in Fig. 12 (a and c) are then clearly enhanced. From these results, it is obvious that, the application of Zeolite generally enhances the remineralization process. It is also, quite clear that the remineralization process due to applying Zeolite with bioglass is far greater than that in case of the application of zeolite with mineralizing solution especially for hydroxyapatite bands. Utilizing the intensity of 962 $cm^{-1}$ Raman line, the former treatment gives almost doubling of the mineralizing process (53: 28 %) for enamel and (240: 60 %) for dentin (Fig. 11-b and 12-b). The last treatment has a significant enhancement for collagen bands in dentine.

## 3. Discussion:

The modifications due to application of bioactive glass, mineralizing solution and Zeolite on artificially carious enamel and dentin, essentially concerned phosphate grouping $PO_4^{-3}$, which represents the mineral phase in enamel and dentin (hydroxyapatite). Therefore, changes in intensity of the

$PO_4^{-3}$ band were linked to the type of agent applied, the resulting spectral difference, between artificially carious and treated enamel and dentin, are used to measure enhanced remineralization. (Figs. 11, 12).

Both improvement in the surface topography of enamel and dentin, (in the form of sealing of enamel pores and plugging of dentinal tubules as observed from SEM, Figs. 2, 7) and the increase in the Raman intensity at 962 $cm^{-1}$ (corresponding to hydroxyapatite Figs. 11,12), following bioactive glass treatment, are due to its reaction mechanism. This involves dissolution, leaching and precipitation [10]. As bioactive glass is mixed with distilled water rapid dissolution and breakdown of silica network, accompanied by the release of $Ca^{2+}$, $PO_4^{3-}$ and $Si^{4+}$ occurs at the glass surface. Then, sodium ions are leached, leaving behind a silica-rich surface. Finally, a polycondensated silica-rich gel layer is formed on the glass bulk. The latter may act as a template for apatite nucleation [11] that grow by assuming more $Ca^{2+}$ and $PO_4^{3-}$ from the surrounding fluid. Therefore, the formation of apatite on glass surface is related to the concentration of the effective ions of $Ca^{2+}$, $PO_4^{3-}$ and $OH^-$ released in the reaction medium [12], which in turn depend on their concentration in the bioactive glass and its reactivity.

Also, the partial closure of surface pores following mineralizing solution application (Figs. 4, 9), as well as, increased peaks intensity of Raman band corresponding to more hydroxyapatite formation (Figs. 11c, 12c) can be explained in terms of enhanced remineralization, as its solution is saturated with calcium and phosphate, which might drive mineral back into the tooth [13].

Furthermore, the increased relative intensity in the band 962 $cm^{-1}$ (resulting from the increase of hydroxyapatite content), following Zeolite application before either bioactive glass or mineralizing solution (Figs. 11 b, d and 12 b, d) could be based on the role of Zeolite. It act as a molecular chemical sieve, which can adsorb molecules especially those with a permanent dipole moments. Therefore the enhanced remineralization following Zeolite application, could be attributed to the adsorption of $Ca^{2+}$, and $PO_4^{3-}$ to Zeolite from bioactive glass as well as from mineralizing solution. It first attracts $Ca^{2+}$, followed by $PO_4^{3-}$ leading to new mineral formation. The exchangeable cations in Zeolite particles create strong local electrostatic fields that interact with highly polar molecules, thus attracting more calcium and phosphorus [14].

The observed greater effects in all treatments in case of dentin, than that of enamel can be due to the structure of each tissue. Since dentin is less mineralized than enamel, so the diffusion through dentin is more than in enamel, owing to the higher porosity, resulting in larger mineralizing effect. In addition, the appearance of collagen Raman Lines (1257, 1458 and 1660 $cm^{-1}$)

of the dentin structure in the presence of Zeolite can be ascribed to the enhancement of the signal to noise ratio as a result of the great amelioration of the surface smoothness.

## 4. Conclusions:

It is concluded that bioactive glass has the potential for remineralizing artificially carious enamel and dentin. Furthermore, Zeolite powder, as a chemical molecular sieve enhances the effect of mineralizing agent either with bioactive glass or mineralizing solution. It is also observed that remineralization ability of carious dentine is greater than that of enamel.

## 5. References:


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