Minimal Intervention Approaches in Remineralizing Early carious lesions

[1]Hala M.Abbas, [2]Heba M.Hamza, [3]Hend M.Ahmed

[1]Associate Professor in Pediatric Dentistry& Dental Public Health Department, Cairo University.
[2]Professor in operative Dentistry Department, Cairo University.
[3]Lecturer in operative Dentistry Department, Cairo University
Corresponding author: dr.halaabbas@gmail.com

Abstract: Since the beginning if this Millennium information about the procedures and benefits of minimum intervention, an innovative, modern health care approach for dentistry has been increasingly disseminated. As with any innovation, wide adoption of Minimum Intervention (MI) by the dental profession is reliant upon factors related to the process of diffusion. Thus the goal of Modern Dentistry is to manage non- cavitated caries lesions non invasively through remineralization. Therefore, this in-vitro study will be carried out to apply and investigate three remineralizing agents fluoride, ACP-CPP+F and silica compounds on demineralized enamel. **Materials & Methods** This in vitro study was carried out on 45 teeth specimens carious like lesions will be induced and specimens were divided into three main groups of 15 teeth each, according to remineralizing agent applied, leaving the distal surface as a control group. Group I:using flor-Opal sodium fluoride gel (FG), Group II: using GC MI paste plus Tooth mousse (APP-ACPF) (GC), Group III: using bioactive silica suspension (S).The specimens were evaluated using a computerized method using Environmental Scanning Electron Microscope (ESEM) for measuring the surface roughness of the investigated samples. Also the mineral content was measured using Energy dispersive analytical X-ray (EDAX) for elemental analysis of (calcium, phosphate), showing the3D dimension of surface roughness. **Results:** revealed a high statistically significant difference between the control group and the three remineralizing agent but the highest mean of surface roughness was noticed in silica group also, this group showed the highest calcium wt .while the highest phosphorus wt was shown in the GC MI paste plus Tooth mousse (APP-ACPF). Flor-Opal showed the lowest statistical means .**Conclusion:** Minimal intervention dentistry will be promising spotlights on remineralization of early carious lesion. Flor-Opal showed the lowest remineralization while when used incorporation with ACP-CPP paste showed better results in reducing dissolution of enamel surface .Bioactive silica used as remineralizing agents showed a promising results further studies will be needed to be applied in vivo studies.
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1. Introudaction:

Minimal intervention is a key phrase in today's dental practice. Minimal intervention Dentistry (MID) focuses on the least invasive treatment options possible in order to minimize tissues loss and patients discomfort. Concentrating mainly on prevention and early intervention of caries. MID's first basic principles is the remineralization of early carious lesions, advocating a biological or therapeutic approach rather than the traditional surgical approach for early surface lesions, Frencken &Holmgren (2004).

Since the beginning of this millennium information about the procedures and benefits of minimum intervention, an innovation, modern health care approach for dentistry has been increasingly disseminated, White (2004). As with any innovation, wide adoption of minimum intervention by the dental profession is reliant upon factors related to the process of diffusion, Mickenautsch (2009)

As Dental caries is a highly prevalent disease and although in most developed countries, its prevalence has decline ,the disease remains a major public health problem ,Selwitz et al., (2007). Signs of the caries process cover a continuum from the first molecular changes in the apatite crystals of the tooth to a visible white -spot lesion , through to dentin involvement and eventual cavitations Progression through this stage requires a continual. imbalance between pathological and protective factors that results in the dissolution of apatite crystals and the net loss of calcium, phosphate, and other ions from the tooth (demineralization). The chemistry of this process has been reviewed by Robinson et al.,(2000).

As Tooth enamel is the hardest mineralized tissue in the human body. It's composed almost exclusively of apatite crystals that are biologically unique in terms of their crystalline and highly organized ultra-structure. Extensive researches have sought to understand the process of enamel formation which is important for advances in biomineralization. Dental enamel contains over 96 wt% inorganic mineral, and the main constituent is a single calcium phosphate phase, hydroxyappatite HAP {Ca10 (PO4) 6 (OH)2} crystallites. These hydroxyappatite crystallites are bundled together by organic molecules into organized larger-scale structures. (Dong et al, 2010)

The onset of dental caries requires the physiochemical conditions for mineral dissolution. Dissolution of enamel by caries process which is a gradual process of repeated acidic conditions below critical PH, where equilibrium between remineralization and demineralization tipped towards demineralization with development of clinically detectable white spot lesions ,Hicks et al., (2004).

One of the key elements for biomineralization of dental enamel are silica compounds, one of them is chemically prepared silica which is a highly porous structured material with an excellent capacity for dehumidification, absorbability and chemical stability so it is capable to form bone like apatite material on their surfaces when placed in human body environment in order to repair hard tissues ,Hassanein and El-Borolossy, (2006).

Also remineralizing agents containing Amorphous calcium phosphate casein phosphopeptide (ACP-CPP) have shown to produce beneficial effect to control dental caries progression but when used in paste form conflicting results have been obtained, however, it’s necessary to evaluate these potential remineralizing agents before recommending its use to patients, Neto et al, (2009)

Finally we can never ignore the cariostatic action of fluoride in initial caries lesions where it can form globules of calcium fluoride like material which is insoluble in oral environment; this explains how topical fluoride application inhibits caries progression, it’s important to determine fluoride uptake in carious like demineralized enamel after application of fluoride gels and their increasing the resistance of enamel against further demineralization ,Wigand et al.,(2005).

As the goal of Modern dentistry is to manage non-cavitated caries lesions non invasively through remineralization in an attempt to prevent disease progression and improve aesthetics, strength, and function ,Tung and Eichmiller(2004).Therefore, this study will be carried out to apply and investigate three remineralizing agents fluoride, ACP-CPP+F and silica compounds on demineralized enamel.

2. MATERIALS AND METHODS:

2.1. MATERIALS:

2.1.1.REMINERALIZING AGENTS:

a-Flor-Opal *

Flor-Opal is a sustained-release, 0.5% fluoride ion (sodium fluoride 1.1%) in a sticky, viscous gel to be used with a custom tray. It is a clear, nearly tasteless, odorless high-viscosity sticky gel with a pH of ~7; Dispensed in 1.2 ml unit dose syringes. (Fig.1).

Fig. 1 Floropal remineralizing agent

b-GC MI PASTE PLUS **

Is a water based crème containing Recaldent with incorporated fluoride (CPP-ACPF:casein phosphopeptide-amorphous calcium phosphate fluoride). Fluoride level is 0.2%w/w (900ppm), supplied in form of tube containing 40g. PH 7.8 it is derived from milk casein. (Fig 2)

Fig. 2 GC MI Paste plus

c-BIOACTIVE SILICA SUSPENSION ***

Chemically prepared in the lab then supplied in a powder form: 10 gm of sodium silicate was diluted up to 400 ml by deionized water. 200 ml of ethylene glycol were added to the above mixture. Precipitation of silica gel was performed by titration with formic acid till pH not exceeds 5. After centrifugation and washing for several times the resulted ppt. was collected and dried at 40 °C for six days.

The obtained solid was calcined at 600°C (i.e. 100°C every 40 minutes) the sample is nominated (S-Eg-FO). (Fig3).

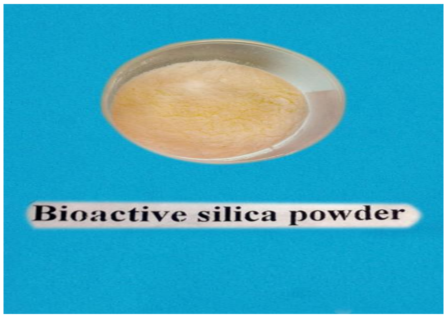


Fig.3 Bioactive silica powder

**DENTSPLY DETREY GmbH de-trey-str.1,78467 Konstanz Germany.*
***Ultradent Products, Inc. 505 West 10200 South South Jordan, Utah 84095 USA*
****Egyptian national research center, department of biomaterials*

d- -DEMINERALIZING SOLUTION

Chemically prepared solution containing 2.0 mM of calcium and 2.0 mM of phosphate in a buffer solution of 74 mM of acetate at pH 4.3

e- REMINERALIZING SOLUTION

Chemically prepared solution containing (1.5mM of calcium, 0.9 mm of phosphate and 150 of potassium chloride in a buffer solution of 20mM of Tris (Hydroxymethyl aminomethane) at pH 7.

2.2.METHODS

2.2.1.TEETH SELECTION:

A total of 45 freshly extracted sound human premolars teeth were selected. The teeth were extracted for orthodonitic reasons; they were cleaned from soft and hard deposits. Teeth were inspected for any enamel abnormality using stereomicroscope. They were then stored in distilled deionized water till later use.

2.2.2.TEETH GROUPING:

The 45 teeth specimens were divided into three main groups of 15 teeth each, according to remineralizing agent.

Group I: using sodium fluoride gel (FG) using flor-opal,
Group II:using GC MI paste plus Tooth mousse (APP-ACPF)(GI),
Group III: using biological silica bioactive silica suspension (S).

2.2.3. SAMPLE PREPARATION:

a. Induction of artificial caries lesions:

Specimens were subjected to pH cycling model. The pH cycling protocol consisted of immersion of teeth in 5ml of demineralizing solution for one hour at 37°C. Specimens were then washed with distilled deionized water for l minute under agitation followed by immersion into remineralizing solution for 22 hours at 37°C in an incubator. Specimens were then stored in distilled deionized water at 37°C till treatment.

b. preparation of molds:

Teeth were mounted in one inch cylindrical acrylic resin mold, 3mm below their labial cervical line, where each tooth was mounted so that its long axis was perpendicular to the base of the blocks and its incisal edge was parallel to the base of the blocks, this was done using a paralleling device surveyor.

2.2.4.Treatment of specimens:

The labial surface of each tooth was measured mesiodistally then divided into two equal halves using a digital caliber and graph paper, then marked using a very fine point marker, Distal half of each tooth was covered using an adhesive strip. The mesialhalf of each tooth from the labial surface was treated with the 3 remineralizing agents(Fluoride, APP-ACPF, Bioactive Silica) were carried out according to manufacturer's instruction.

a- **Flor-Opal**

The dematerialized enamel surface was covered by a single layer of approximately 2mm floro-Opal (sodium fluoride) gel using a minibrush, the gel was left in contact with tooth surface for 6 hours then washed under tap water till the surface is clean.

b- GC MI paste plus

The dematerialized enamel surface was covered by a single layer approximately 2mm of GC MI paste plus. Using a minibrush, the paste was left in contact with tooth for 30 minutes then washed under tap water.

c-Bioactive silica suspension

Prepared by addition of 2mg of bioactive silica powder to 2ml of distilled deionized water and stir well. Using a multi-tuffted microbrush, three coats of silica suspension were applied onto the bleached enamel surface to ensure coverage of the tooth surface by an adequate amount of suspension and left for 6 hours then thoroughly washed under tap water.

2.2.5.ENVIROMENTAL SCANNING ELECTRON MICROSCOPE (ESEM) ANALYSIS:

A. ASSESSMENT OF MINERAL CONTENT:

It was carried out after pH cycling, after remineralization with all types of remineralizing agents mentioned using the Energy Dispersive Analytical X-ray (EDAX) of the ESEM* (Fig.4).

Fig. 4 Photograph representing Environmental scanning electron microscope

EDAX performed quantitative analysis, available in the EDAX software, for all the component elements of the image of the sample that were seen on the monitor on the ESEM. The element composition automatically displayed, after the quantitative analysis of the scanned square area, in a spectrum curve showing peak identification and quantification results box which provided quantification for all of the elements displayed in the peak identification showing their weight percentages, taking an average reading of three different points in each specimen. This quantification results provided normalization to 100% for all elements in the peak identification list.
Energy dispersive X-ray analysis (EDAX) was used for elemental analysis at the ultra structural level. It is one of the latest micro-analytical techniques used in conjunction with scanning electron microscope (SEM). The principle is based on the energy emitted in the form of X-ray photons where the electrons from external sources hit the atoms in a material, thus generating characteristic X-rays of the element. When the sample is bombarded by the electron beam of the SEM, electrons are ejected from the atoms on the specimen's surface (secondary electrons). A resulting electron vacancy is filled by an electron from higher shell, and an X-ray is emitted (characteristic x-rays) to balance the energy difference between the two electrons. The EDAX X-ray detector measures the number of emitted x-rays their energy. The energy of the x-ray is characteristic of the element from which the x-ray was emitted. A spectrum of the energy versus relative counts of the detected x-rays obtained and evaluated for qualitative and quantitative determinations of the elements present in the specimen using a computer based program. Energy dispersive x-ray analysis was used to determine calcium and phosphate content in weight % of caries like lesions enamel, and after application of the remineralizing agents used on enamel surface in each group,Buzalaf,et al.,(2010).

* *(Quanta series ESEM, Quanta 200 Nertherland, FEI Company, Philips).*

B.SURFACE ROUGHNESS ASSESSMENT:

Also Measuring the roughness of the investigated sample take place by converting the captured image at the magnification wanted [1000X] into 3D [three dimensions] image in the form of peaks is there is roughness on that surface.

This process of conversion took place by using a software installed on the ESEM called X-T document this program able to measure the length, width and height of each peak by using three coordinates (X,Y,Z) the z axis represent the height of each peak, so it represent them in an excel sheet called data sheet.

From the data sheet we are able to get the mean of these heights, the highest peak, the lowest one, also the number of peaks that are measured on the investigated sample and represent them in another excel sheet called [statics sheet] by comparing between the mean of each investigated surface we can get the highest or lowest surface roughness

2.2.6.STATISTICAL ANALYSIS:

Data were collected, tabulated and statistically analyzed using mean and standard deviation (SD) values. Regression model with One-way Analysis of Variance (ANOVA) was used in testing significance for the effect of remineralizing agent and their effect on surface roughness and mineral contents (EDAX).The significance level was set at $P \leq 0.01$. Statistical analysis was performed with SPSS 16.0[8](Statistical Package for Scientific Studies) for Windows.

3.RESULTS :

This study was conducted to assess the progression of caries like lesions in terms of change in enamel mineral content (calcium and phosphate) using EDAX method, and surface roughness assessed utilizing 3D constructed images from the captured image by the ESEM, also the assessment of remineralizing effect of different remineralizing agents Fluoride (FG), amorphous calcium phosphate + fluoride (GC), bioactive silica (S).By using one way ANOVA, mean and stander deviation the results were shown in tables (1,2,3) for simplicity and ease of comparing the collected data the mean value of surface roughness and calcium and phosphate weight percent were plotted in a form of bar charts figures as shown (Fig 5,6,7)Representative samples of teeth in a 3D constructed images for surface roughness are represented in Fig(8,9,10 & 11) respectively .

Our results showed a highly significant difference between mean of the control specimens and the different remineralizing agents used regarding surface roughness. Silica showed the highest mean value where P<0.001,while the Fluoride group was the lowest (Table1,Fig5)

Table 2, Fig 6 showed the comparison between the control and remineralizing agents regarding Calcium wt percent , denoting a highly significant differences between groups but the highest mean was shown in the Silica group followed by Gc group then the Fluoride. Also when using the one way ANOVA the comparison between means revealed a significant difference where the Gc group was the highest, where P<0.01.

Table (1): Comparison between control and remineralizing agents regarding surface roughness

Surface roughness	Mean ± SD	F	P value	Sig.
Control	118.06 ± 18.23	4.74	0.001	Highly significant
FG	120.85 ± 17.02			
GC	128.98 ± 18.54			
Silica	138.85 ± 11.78			

One-Way ANOVA

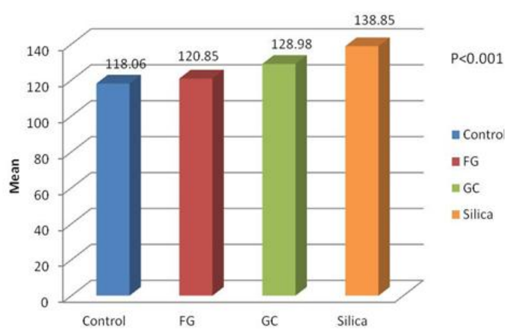


Fig (5): Bar chart representing comparison between control and remineralizing agent regarding surface roughness.

Table (2): Comparison between control and remineralizing agents regarding calcium weight percent

Calcium weight percent	Mean ± SD	F	P value	Sig.
Control	21.04 ± 0.71	269.61	0.001	Highly significant
FG	22.35 ± 0.01			
GC	29.42 ± 0.23			
Silica	31.23 ± 2.27			

One-Way ANOVA.

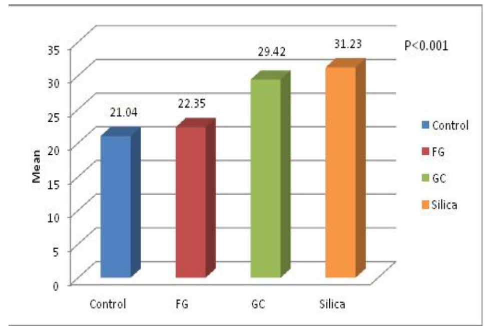


Fig (6): Bar chart representing comparison between control and remineralizing agent regarding calcium weight percent

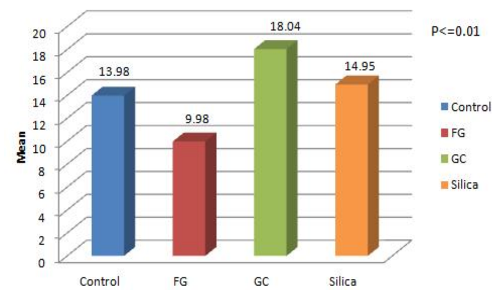
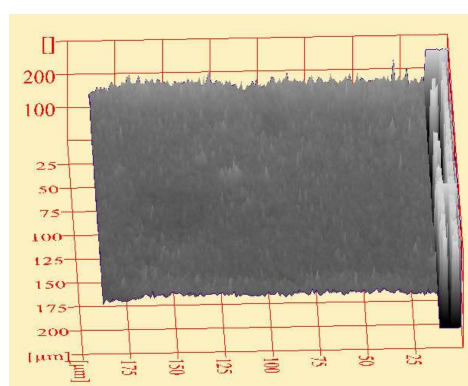


Fig (7): Bar chart representing comparison between control and remineralizing agent regarding phosphorus weight percent

Table (3): Comparison between control and remineralizing agents regarding Phosphorus weight Percent

Phosphorus weight percent	Mean ± SD	F	P value	Sig.
Control	13.98 ± 1.41	14.34	0.01	significant
FG	9.98 ± 1.74			
GC	18.04 ± 5.76			
Silica	14.95 ± 2.84			

One-Way ANOVA



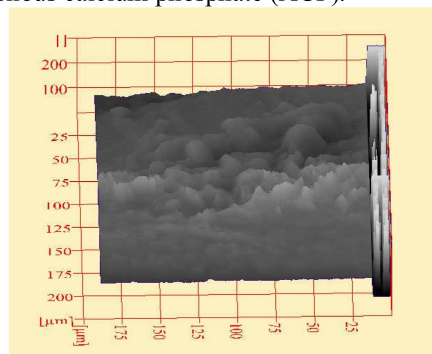
Fig(8):Representing 3Dimage of surface roughness of the control Gp

4. Disscusion:

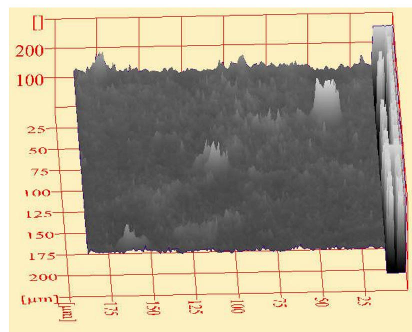
Dental caries is an important health problem; it is one of the most important problem in across the globe. The etiology of the dental caries is quite complex involving interaction between agent ,host and environmental factors. So, the Minimal intervention dentistry focuses on allows ultraconservative treatment that is more preventive than traditional dentistry. Successful diffusion of MI requires substantiation of its beneficial claims through remineralization of the demineralized carious lesions. This in-vitro study was to evaluate the effect of three different types of remineralizers; fluoride, ACP-CPP + fluoride and silica compounds studying their effect on induced enamel carious lesions, this was investigated in terms of measuring surface roughness and the changes in mineral content(ca & ph) of the studied specimens after application compared to control specimens.

It was found that enamel mineral loss was significantly reduced when topical fluoride was applied on enamel surface (Martin et al, 2010),it can be used in different forms solutions , gel &foam (Attin et al, 2007). Fluorides are important adjunct in the prevention of dental caries but it takes time for depositioning as it require about 3(ppm) shift the balance from net demineralization to net demineralization (Summit et al., 2001). Several mechanisms have been suggested to achieve the anticaries effects of fluoride, including the formation of fluoroapatite which is more acid–resistant than hydroxyapatite, increase enhancement of remineralization, interference of ionic bonding and also its effect extend when used in vivo where it inhibit the microbial growth and metabolism, Niessen (1997). The protective effect of fluoride is mainly attributed to the formation of a CaF_2 like layer on the tooth surface, which acts as a fluoride reservoir. This results come in agreement with ours where the fluoride group (floropal) showed the least findings . Exterkate et al.,(2007) were against this finding resulting that it enhanced the uptake of calcium and shows less loss of calcium during demineralization. also it was noticed that application of fluoride combined with ACP-CPP would give enhanced remineralization compared to individual application of fluoride, Cai et al, (2008); Borges et al., (2010) and El Zayat et al., (2012) this results agreed with our results as the least calcium and phosphorus depositioning compared with the two other groups was noticed when floropal was used . Better results were shown in this study when fluoride incorporated with another agents as similar as Borg et al., (2010).

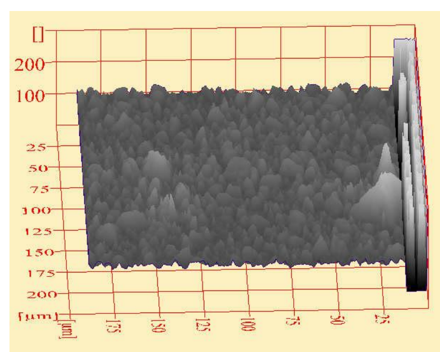
Other than fluoride an ACP formulation with incorporated fluoride to a level of 900 ppm GC MI Paste Plus was used in this study. This protein nanotechnology combines specific phosphoproteins from bovine milk with forming nanoparticles of amorphous calcium phosphate (ACP).



Fig(9):Representing 3Dimage of surface roughness of the Fluoride Gp



Fig(10): Representing 3Dimage of surface roughness of the GC



Gp

Fig(11): Representing 3Dimage of surface roughness of the Silica Gp

Under alkaline conditions the calcium phosphate is present as an alkaline amorphous phase complexed by the CPP. The nano-complexes form over a pH range from 5.0 to 9.0, while GC IM Paste Plus pH was 7.8, under neutral and alkaline conditions, the casein phosphopeptides stabilize calcium and phosphate ions, forming metastable solutions that are supersaturated with respect to the basic calcium phosphate phases. The amount of calcium and phosphate bound by CPP increases as pH rises, reaching the point where the CPP have bound their equivalent weights of calcium and phosphate, Walsh (2009). This finding augment our results in the high significance in the surface roughness of the Gc group as the particles is nano particles but it showed a calcium deposition less than silica and the highest significance was shown in phosphorus may be because its saturation level of phosphorus is greater than that of calcium as the calcium will be binded with fluoride in the CPP-ACP,since each molecule of casein phosphopeptides can bind to 15 calcium ions and 25 phosphate ions and 5 fluoride ions(Aimutis,2004) .This findings is also supported by Metz et al, (2007) who stated that fluoride incorporation had a beneficial and a protective effect on surface enamel after remineralization and demineralization cycles forming acid resistant fluoride rich crystal layer , also if enamel specimens where demineralized prior to application of fluoridated product, then the driving force will allow incorporation and attraction of fluoride ions resulting in further nucleation of calcium and phosphate ions and remineralization.

The calcium phosphate in these complexes is a biologically available for remineralization of subsurface lesions in tooth enamel ,Cross ,et al.,(2005)This can be attributed to the formation of a stabilized amorphous calcium fluoride phosphate phase. Our results come in agreement with several studies that proved that the combined effect of CPP-ACP and fluoride as two separate products was beneficial in enhancing remineralization and anti-cariogenic effect as well as improving acid-resisting effect,Lennon et al,(2006) ;Kariya et al, (2004).

Recently, bioactive silica materials have been introduced in many fields of dentistry .The compound is composed of minerals that naturally reacts when it comes into contact with water, saliva, or other body fluids,Liu Zhen-hua et al.,(2011). This reaction releases calcium, sodium and silicon ions in a way results in the formation of new hydroxyl carbonate apatite (HCA),Huang ,(2009) ,the crystals fuses with each other to form large one with large spherical outline . This finding was supporting our results where the silica group showed the highest mean of surface roughness and also the 3D image revealed the multiple spherical globule like appearance of silica with different geometries built up. Also it is ordered mesoporous silica which constitutes an amazing family of solids that show ordered arrangements of pores as channels and cavities. The pore sizes of these materials are always very homogeneous ranging from 2 to 50 nm and can be controlled and modified, in a reasonable extension, using several synthetic strategies.

This material has numerous features, of which are its ability to act as a biomimetic mineralizer, matching the body's own mineralizing traits Hassanein and El-Borolossy (2006). Our result showed a highly significant difference in calcium wt when silica is applied this may be due its adherence to the surface of the enamel supported by Hassanein and El-Brolossy in (2006). Also ,silica spheres have bioactive property where they can adsorb and bind their surfaces to calcium , phosphate and fluoride available ions so they are acting as foci for mineral aggregation and further crystal growth giving rise to hydroxyappatite molecules,Huang,(2009)

Bioactive Silica has been extensively studied the chemical modification of the surface of this mesoporous materials which is a common strategy for modulating the surface properties and the performance in the desired conditions (Ramalingam et al., 2005).This finding supported our results where the coarse particles of silica that adhere on the enamel surface showed the highest surface roughness,Table (1) fig,(5).

Dong et al., in (2010) demonstrated remineralization of human dental enamel by bioactive glasses. They found that after treatment with three bioactive glasses pastes and soaking in SOF for 7 days, new crystals formed on the enamel surfaces. The XRD patterns confirmed that the newly formed crystals were apatite crystals. The SEM micrographs of the polished original enamel surface were the densest. They concluded that definite compositions of silicate-based bioactive glasses have been effectively used as remineralizing agents, and the level of silicon content of bioactive glasses played a role in dental enamel remineralization.

In the current study surface roughness was measured by converting the captured image at the magnification [1000X] into *3D [three dimensions]* image in the form of peaks if there is roughness on that surface and then calculation of the highest and lowest surface roughness values through using a software installed on the ESEM called X-T document, this program able to measure the length, width and height of each peak by using three coordinates (X,Y,Z) the Z axis represent the height of each peak, so it represent them in an excel sheet called data sheet. This method doesn't require surface coating or complicated specimen preparation in addition to that it is computerized technique so it is more reliable.

In the present study enamel mineral content was tested using *Energy Dispersive Analytical X-ray (EDAX) of the ESEM.* (Quanta series ESEM, Quanta 200 Nertherland , FEI Company, Philips), A spectrum of the energy versus relative counts of the detected x-rays obtained were evaluated for qualitative and quantitative determinations of the elements present in the specimen using a computer based program. Energy dispersive x-ray analysis was used to determine calcium, phosphorous, fluoride and silica content in weight % of enamel caries like lesions, remineralized enamel in each group, supported by Mahoney et al., (2004); Hall et al., (2003); Lata et al.,(2010).

Conclusions:

1- Minimal intervention dentistry will be promising spotlight toward remineralization of early carious lesion .

2- Floropal showed the lowest remineralization but it had the lowest surface roughness so it can be used when smooth surface is needed .

3- When fluoride is used incorporation with ACP-CPP paste will be more effective in reducing dissolution of enamel surface. So it is better not to use sodium fluoride alone .

4- Bioactive silica used as remineralizing agents showed a promising results further studies will be needed to be applied in vivo studies .The use of new computerized technology in the assessment of the specimens showed more easier attempt and a reliable and un-bias approach.

Recommendations:

1- Minimal intervention dentistry should be targeted to infants and toddelers in the early beginning of Early Childhood Caries (ECC) and those children with special need for healing the demineralized lesions at an early stage.

2- The use of silica& ACP-CPPF for demieralized areas for example, after removal of orthodontic bracket , whitening , white spots caries , Hypersensitivity.

3- Bioactive silica could better be studied in vivo conditions, aided by calcium, phosphate and fluoride ions available in saliva to out get its ability to remineralize initial carious lesions.

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