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Comparative Study of Bioactive Glass and Calcium-Containing Fluoride Products on The Remineralization of Enamel White Spot Lesion: A Scanning Electron Microscopy (SEM) Study

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Abstract

Dental remineralization is ionic deposition in demineralized enamel, resulting in gain of minerals and limiting progression of early enamel lesions. Various approaches have been employed for enamel remineralization. This investigation aimed to measure the remineralizing efficiency both quantitatively and subjectively of bioactive glass powder (Sylc®), casein phospho-peptide-amorphous calcium phosphate with fluoride (CPP-ACPF) on white spot lesions (WSLs) using Scanning Electron Microscopy (SEM) combined with Energy Dispersive Spectroscopy (EDX) techniques. Methods: A sample of fifty enamels was obtained by extracting ten maxillary first premolars. Ten enamel samples were assigned to each group: Group I (Sylc®); Group II, CPP-ACPF; and Group III, artificial saliva (negative control), Group IV: WSLs, and Group V: baseline. All samples were kept for 72 hours in a demineralizing agent before being treated with remineralizing agents to form WSLs. Analyses of the morphology of enamel samples were performed by SEM and EDX. This study used one-way ANOVA and Tukey's HSD ($p<0.05$) as statistical analysis. Results indicate that the Mean±SD of Ca/P ratio of I (2.359±0.230), II (2.295±0.246), III (2.097±0.275), IV (2.068±0.471), V (2.00±0.16). SEM shows the morphology of the surface of enamel and the porous structure change to a homogenous enamel surface, most of the micropores were hidden as a result of using BAG and CPP-ACPF. Conclusion: CPP-ACPF and the Sylc® group may be thought to be beneficial for raising the Ca/P ratio.

Introduction:

White spot lesion is thought to be the initial indication of dental caries. Chalky white opacity is the clinical appearance and is thought to be the first macroscopic sign of enamel caries. The outermost layer of enamel covering the lesion is radiopaque and largely intact; however, the subsurface enamel is porous. To stop lesion activity and promote appearance, control of WSLs involves preventing demineralization and enhancing remineralization(1). White spot lesions affect the structural integrity of the tooth because demineralization can increase the holes in enamel, making it more porous and filled with water and air (2). Compared to the surrounding sound enamel, the enamel around the white spot lesion is tougher and may have a rougher surface (3). As the depth of the white spot lesion rises, the microhardness values drop. Following the treatment of WSLs, the microhardness values increased (4).

Remineralization is the most effective noninvasive therapy option for WSLs. Numerous therapeutic approaches have been suggested for WSLs, such as using fluoride-containing products to remineralize the lesion (5). Applying topical fluorides to WSLs is one of the remineralizing solutions utilized by many clinicians; this is the initial course of treatment (6). A crucial approach for managing WSLs is fluoride varnish. Fluoride varnishes function as a reservoir for fluoride reservoirs and release ions when the oral pH decreases, promoting the formation of calcium fluoride on tooth surfaces (7). Using fluoride varnish could return minerals to the subsurface enamel of WSLs (8).

Ca and P ions have been added by some manufacturers to their fluoride varnishes to increase their efficiency. This is done by improving the bioavailability of ions in the varnish and enhancing the precipitation of fluoro-hydroxyapatite (9). Amorphous calcium phosphate, calcium sodium phosphosilicate Bioactive Glass, and nano-hydroxyapatite are further remineralizing materials utilized in WSL treatment (10). There has been a suggestion that remineralization can be induced more attractively by using substances that transport phosphate and calcium ions to the enamel. Among these materials is CPP-ACP (11). CPP-ACP adheres to the surface of teeth and plaque to function. Such binding preserves large concentration gradients associated with soluble Ca and PO4 ions due to the stabilization of amorphous calcium phosphate (ACP), which is critical for remineralization (12). One further method of treating WSLs is the use of bioactive glass particles (NovaMin). The active component of these particles, calcium sodium phosphosilicate, is an inorganic chemical that binds to the tooth surface when it's in contact with watery substances like saliva. This action starts the remineralization process on the tooth enamel (13). Although various remineralizing agents are available, there is conflicting information regarding the superiority of a particular agent.

this study aims to identify the most efficient remineralizing method (Bioactive glass powder (Sylc), CPP-ACPF) in maintaining mineral content at different phases (treated WSL, Baseline, demineralization) using SEM-EDX analysis. The null hypotheses state that there are no appreciable differences in Ca/ P ratio for enamel WSLs between the three remineralizing procedures and phases.

Materials and Methods

Specimens Preparation

Based on an ethical protocol approved by the Health Research Council, ten sound maxillary first premolars were extracted from patients undergoing orthodontic treatment between the ages of 12 and 20 (Ref No. 724). The slurry that had not been fluoridated was used to clean and polish the samples. After that, the samples were kept for a week in a 0.1% thymol solution—an antimicrobial solution designed to limit the growth of bacteria. It was stored in distilled water prior to use (14). The buccal and palatal of the teeth were exposed by obtaining sections of them (15). Five enamel slab portions (3 × 3 mm) from the buccal and palatal surfaces were moulded in resin. Without polishing, the EDX samples were acquired in order to maintain the microstructure (16).

Sample grouping and study design

Group I (Sylc®): Enamel samples with existing WSL, then condition the samples with Sylc® powder

Group II (CPP-ACPF): Enamel samples were conditioned by CPP-ACPF (MI varnish, GC Company—Japan).

Group III (Artificial saliva): Enamel samples were merely stored in artificial saliva

Group IV (Baseline): Sound enamel samples without intervention

Group V (WSLs): Enamel samples without intervention.

Demineralization process

Enamel specimens were immersed in a demineralizing solution that had been pH-adjusted to 4.5 using a digital pH meter (China) to create an artificial white spot lesion. Over the course of four days at 37°C, the demineralization process (NaCl = 100 mM, NaF = 1 ppm, acetic acid = 50 mM, NaN_3 = 5 mM, NaH_2PO_4–$2H_2O$ = 10 mM, $CaCl_2$-$2H_2O$ = 2.2 mM) was carried out. The pH was adjusted to 4.5 using a 1 M NaOH solution. Samples will be rinsed with deionized water for three minutes to stop the demineralization process. After that, they will be ultrasonically cleaned in DW for five minutes and stored in deionized, distilled water (17).

Approach of remineralization

Sylc® group: An Aqua Care Air Abrasion & Polishing System (Velopex) with a Handpiece with a 0.8 mm diameter tip together with a disposable plastic tip was used 4mm from the teeth with 40-46 psi air pressure. The powder consists of calcium sodium phosphor-silicate. The application followed the manufacturer's instructions (Sylc; Denfotex Research Ltd). The air abrasion was conducted for 10 sec in wet abrasion mode fulfilled by shrouding the air stream with a curtain of deionized water. Followed by washing with running water, then, for fourteen days, storing with artificial saliva and changing it every twenty-four hours until the final measurement.

CPP-ACPF: A single unit dose packet contains 0.55g/0.5mL and 1 mL of MI. To make sure it was set and dried, a tiny layer was applied to each slab, and it was left for 20 seconds to air to dry. The single dose form was used to guarantee that each specimen received the same amount of fluoride varnish. The samples were then kept in artificial saliva in an incubator at 37˚C for fourteen days, with fresh saliva added every twenty-four hours.

Control group: slabs in this group were merely kept in saliva.

Scanning electron microscopy (SEM)\ Energy-dispersive X-ray spectrometry (EDX) analysis

The elemental compositions of the generated enamel samples were ascertained using EDX (count time, 60 s; working distance 10 mm) (18). Enamel slabs from each group received treatment before being air-dried in preparation for SEM analysis. The tooth sample was well cleaned with DW before imaging, let to dry at room temperature, and then coated with gold (Eindhoven, The Netherlands). As a result, following SEM imaging, the teeth could not be used. SEM photomicrographs at a 1300x magnification were taken. SEM-EDX was obtained at baseline and for the WSL group; finally, the third image and mineral content were obtained after the remineralization for each group (19).

Statistical analysis

Data description, analysis, and demonstration were achieved using (SPSS version 21) Statistical Package for Social Science (Chicago, Illinois, USA). The Shapiro-Wilk test was employed in the statistical analyses to ascertain whether the data had a normal distribution. ANOVA, or repeated measure analysis of variance, was applied for group comparisons. Tukey's honestly significant difference (HSD) test was applied post hoc to compare subgroups. Impact not statistically significant (P>0.05).

EDX Analysis

The data obtained by EDX analysis for all groups regarding the Ca\p ratio percentage of are shown in Table 1. The mean Ca\p ratio percentage was reduced after demineralization. After remineralization, the percentages of the Sylc® group and the CPP-ACPF group were higher than those of the control group and the WSLs group, respectively, with no discernible differences between the groups. Sylc® the group was used to record the maximum value. Comparing the groups treated with artificial saliva (control group) to the demineralization stage, a little rise in the ratio was seen.

Scanning Electron Microscopy results

Figure 1: baseline group. The microscopic inspection of the sound enamel surface revealed noticeable pitting spots that represented the enamel prism, and interprismatic material was discovered.

Figure 2: WSLs group. The keyhole-like appearance of the prisms in healthy enamel vanished after demineralization of the enamel surface, and the prism structure of the enamel was damaged along with heterogeneous pits and severe porosity of the enamel surface.

Figure 3: artificial saliva group. After demineralization and formation of artificial WSL, samples treated with artificial saliva without remineralizing agent treatment revealed many micropores on the enamel surface and exhibited a loss of enamel prism core but retained periphery.

Figure 4: CPP-ACPF varnish revealed regional crystal deposition was obtained by restoring the defect of the enamel prism and interprismatic substance.

Figure 5: the sylc group, the pores on the enamel surface were covered as more heterogeneous and globular structures.

Discussion

To preserve tooth enamel and improve dental caries resistance, the best course of action must be followed. Due to complex structural and functional connections, this can be challenging. This study examined the impact of Sylc® and CPP-ACPF on remineralization of enamel and mineral content. In vitro cariology research have mostly developed fake lesions using basic chemical models (20). It has definite advantages, including simple research, time, and controlled experiment settings (21). alterations in the mechanical characteristics and microstructure of enamel after being subjected to an acid challenge, wherein the intensity of phosphate was markedly reduced after the acid challenge (22).

Over time, this mineral loss causes cavitation by weakening the tooth's mechanical structure. The process of re-mineralization that follows is almost exactly the opposite. Salivary Ca2+ and PO43-ions combine to form new appetite, which is incorporated into the enamel's depleted mineral layers when the mouth's pH returns to a nearly neutral level. The new mineral deposition finds its nucleation sites in the demineralized zones of the crystal lattice. Ion gradients and enamel solubility are essential to this cycle. The abrupt pH decline that occurs after meals causes an under-saturation of those vital ions (Ca2+ and PO43−) in the plaque fluid concerning tooth minerals. This encourages the enamel to dissolve. Plaque's ironic super-saturation causes an equilibrium to be shifted in the opposite direction at high pH levels, which results in mineral deposition in the tooth. cycles of de- and re-mineralization throughout a person's life (23).

For many years, fluorides have been utilized as a preventative measure against the carious tooth process. But new assessments indicate that caries may be ending or even reversing, with some areas potentially seeing an increase in cases. Therefore, the development of novel biomaterials that can supplement the current fluorides or function independently to stop the carious and its remineralization is necessary (24). When the substance is first exposed to water, the release of sodium causes a temporary, localized elevation in pH. A calcium phosphate layer is formed when excess calcium and phosphate ions supplied by the BAG precipitate due to the increased pH. As these processes proceed, this layer crystallizes into hydroxycarbonate apatite (HCA) (25). In contrast to previous calcium phosphate technologies, BAG's ions generate HCA-a mineral that shares chemical similarities with natural tooth minerals—directly, bypassing the ACP phase that is an intermediary. After the initial treatment, these particles adhere to the tooth surface and keep releasing ions and remineralizing them. In spite of thorough cleaning and brushing, the residues hold fast. These particles have the ability to discharge ions and transform into HCA for up to two weeks, according to in vitro tests (26). According to Chabuk, using Sylc significantly decreased surface roughness. This enhancement may be related to the crystal growth of HCA, which successfully covers the porosities in the surface layer of WSL (27).

The most popular technique for comparing the morphologies of treated and untreated surfaces is the scanning electron microscope (SEM). The samples revealed plugging of porous flaws, resulting in decreased micro-pores and cavities in treatment groups. According to SEM analysis, the surface properties of sound and demineralized enamel matched those described in previous research (Milly et al. 2015; Dong et al. 2011; Gjorgievska et al. 2013) (28-30).

A quantitative assessment of the mineral changes on the enamel surface was conducted using the ratio of calcium to phosphorus. Calcium and phosphorus decreased during demineralization, indicating a quick minerals loss, and increased following remineralization, indicating a gain of minerals, according to the EDX data. WSL and the control groups had the lowest mean values of the Ca/P ratio, whereas the Sylc® group and CPP-ACPF groups had the greatest mean levels. The BAG group had a higher average ratio than the CPP-ACPF group. Narayana (31) discovered that bioactive glass was superior to CPP-ACPF in terms of remineralization. This clarifies why calcium and phosphate ions, which served as ions' repositories, were discharged into the environment for several days.

Burwell (32) revealed that because the BAG only released ions and changed into HCA for a maximum of 14 days, it did not exhibit significant CPP-ACPF action. Furthermore, the mean calcium and phosphorus readings of the CPP-ACPF group were higher than those of the control group. The ability of the CPP-ACPF complex to act as a transporter for ions to the teeth surface may help to explain this.

Because this is an in vitro study, it is challenging to replicate exactly the multifactorial influences of the oral cavity, which have a significant impact on the efficacy of the materials used and, ultimately, the results that are obtained. The length of the investigation and the sample size may have an impact on the statistical findings. To generalize the findings of this study, more research is necessary.

Conclusion:

In light of the findings, it can be said that the sylc and CPP-ACPF groups showed promise for boosting remineralization, as shown by their much superior capacity to do so in comparison to the WSLs group and the artificial saliva group. However, more in vivo research is required to corroborate it.

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Conflicts of interest:

The authors claim to have no conflicting interests.

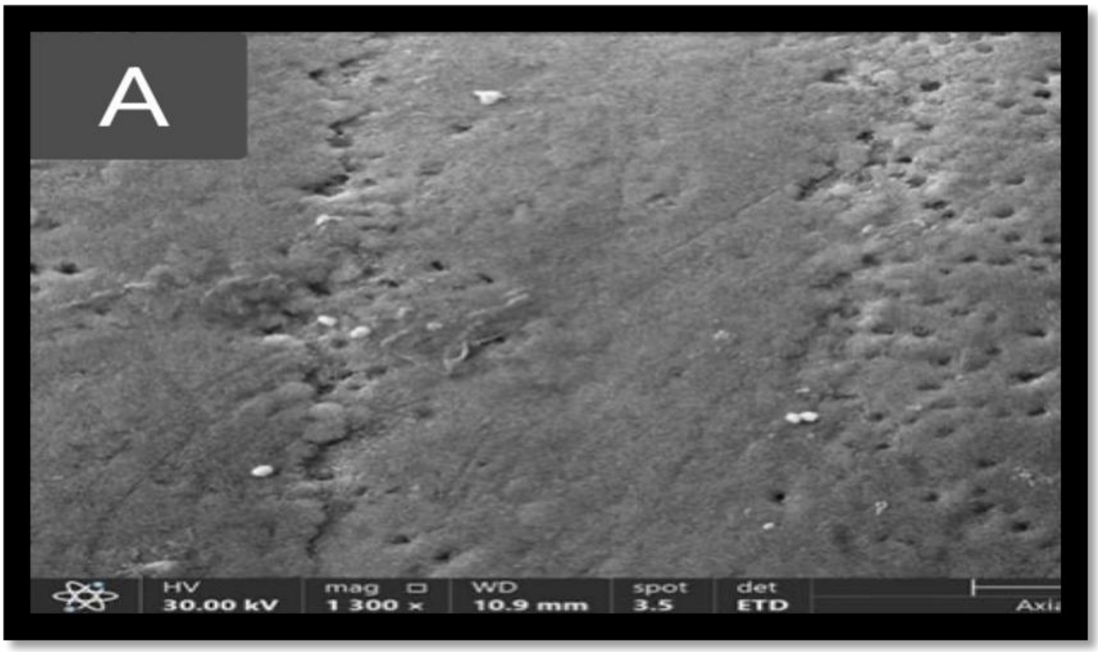

Figure 1: Baseline group

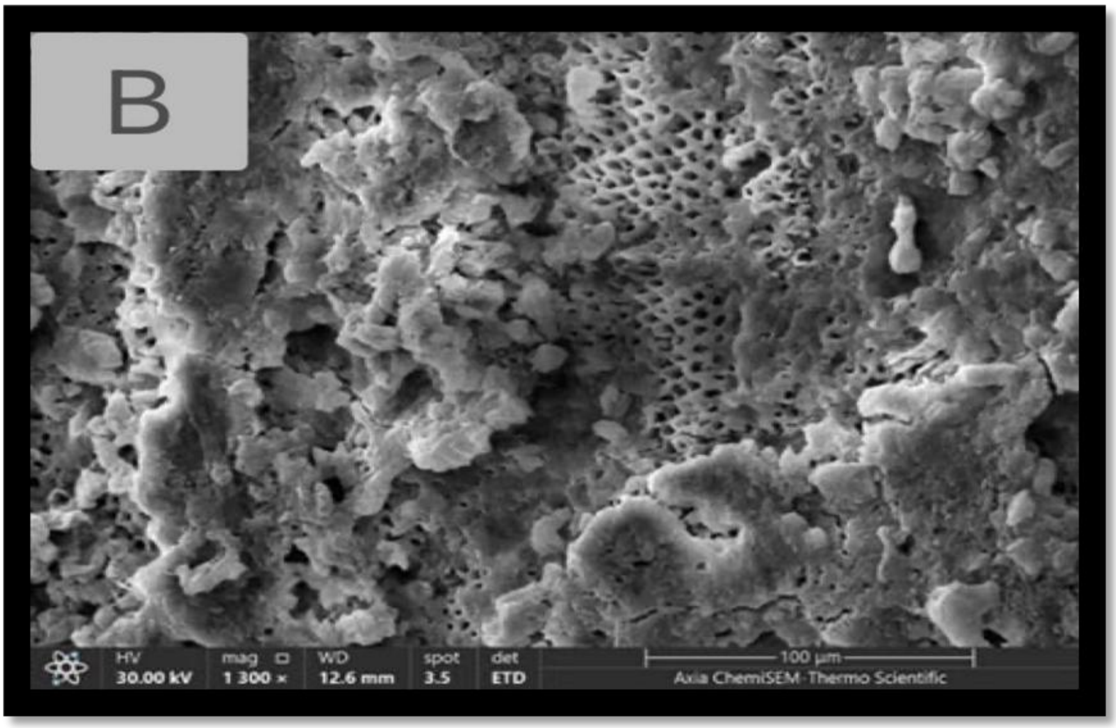

Figure 2:WSL group

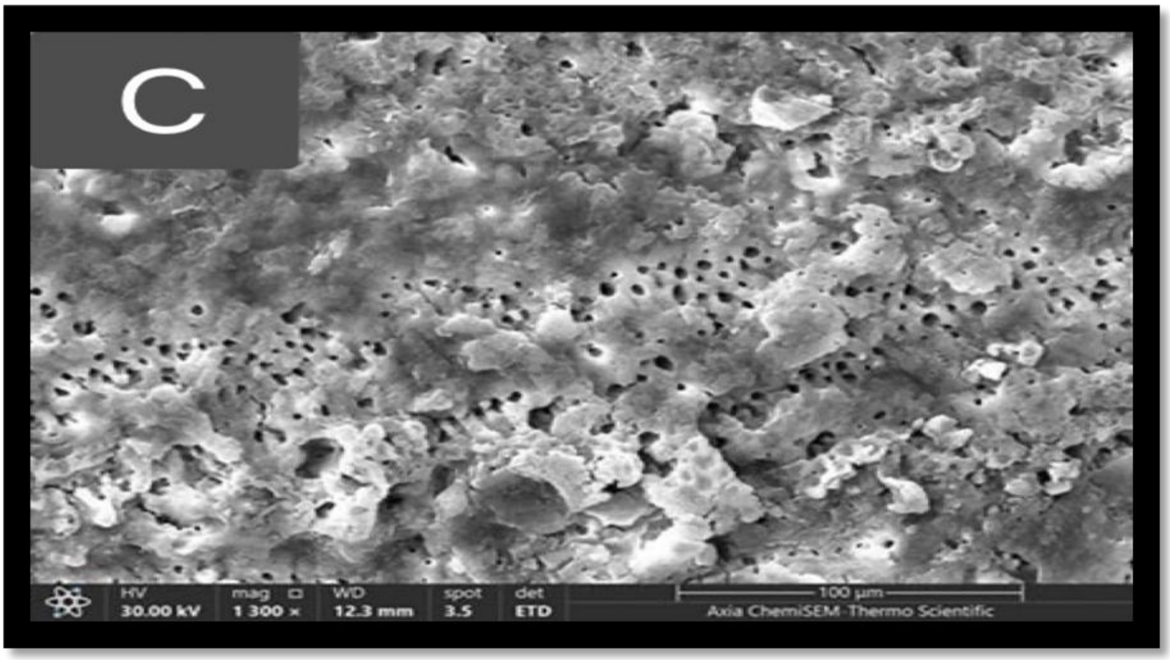

Figure 3:control group

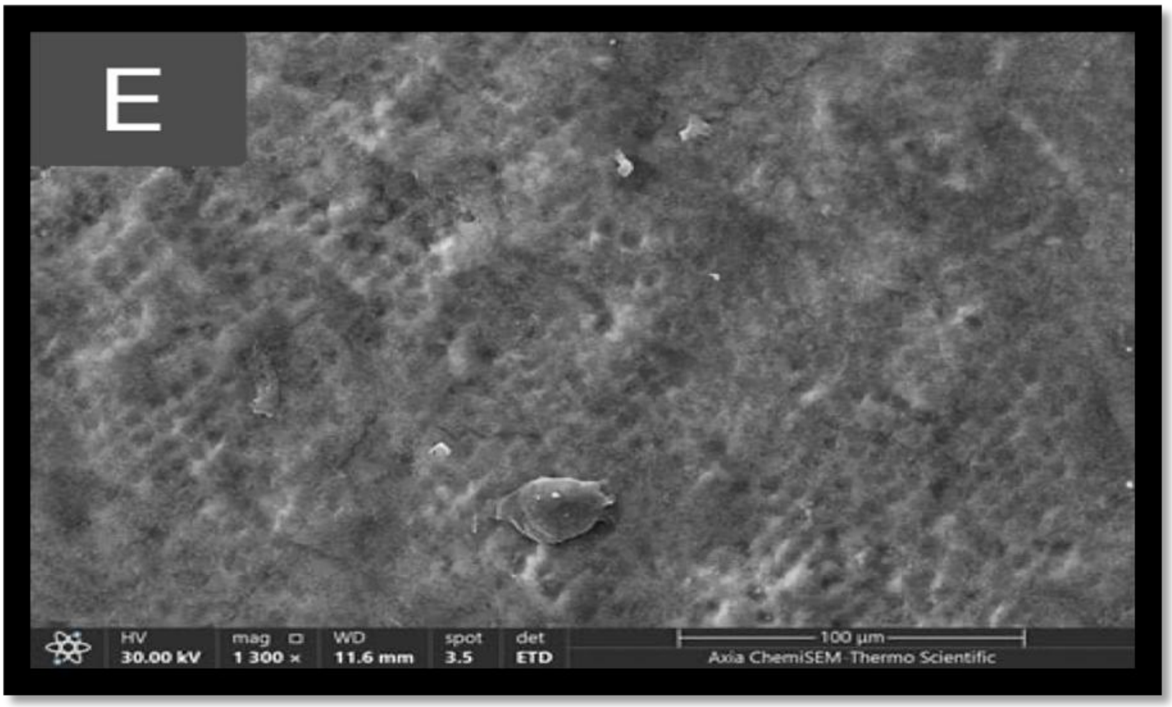

Figure 4:CPP-ACPF group

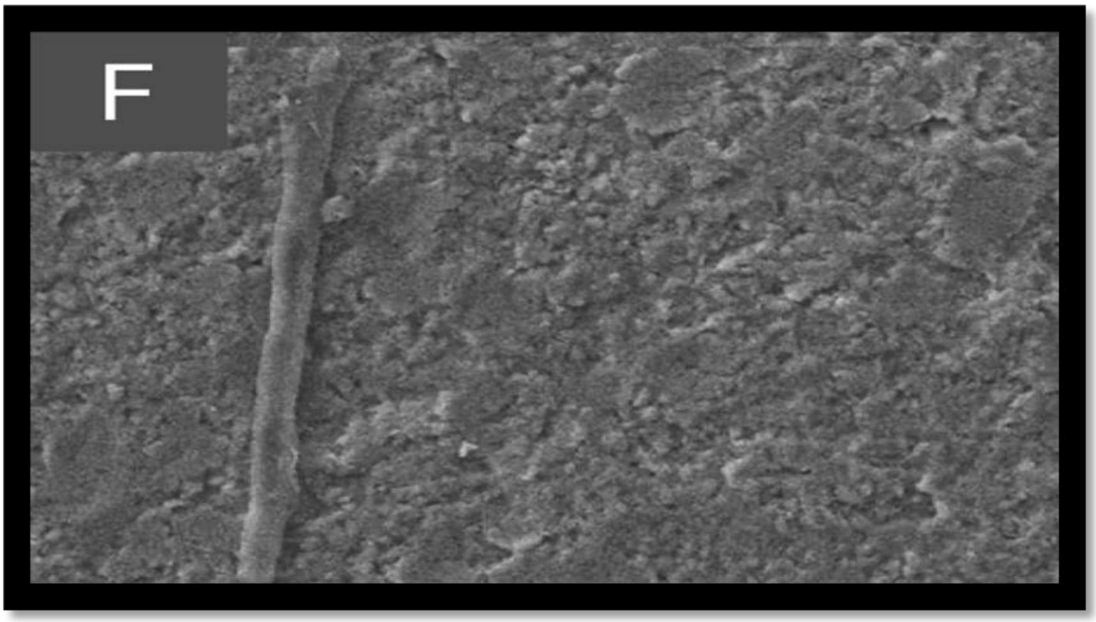

Figure 5:Sylc group

Table 1: Statistical and Descriptive test of Ca/p ratio among the groups

Groups	**Minimum**	**Maximum**	**Mean**	**±SD**	**F**	**P value**
Baseline	1.772	2.225	2.002	0.162	2.195	0.073 NS
WSL	1.526	2.760	2.068	0.471		
BAG	2.082	2.811	2.359	0.230		
CPP-ACPF	1.806	2.603	2.295	0.246		
Control	1.756	2.484	2.097	0.275		

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