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Effect of Eggshell gel and CO_2 laser on microhardness of enamel white spot lesions in comparison to sodium fluoride gel

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Abstract

Objective: This research aimed to assess the impact of chicken eggshell, both with and without CO2 laser treatment, on humans enamel white spot lesions in comparison to fluoride sodium gels.

Material and methods: In order to produce white spots on the enamel of the teeth, thirty maxillary first the premolars were divided into three groups at random and submerged in a demineralising solution. Group 1 received treatment with eggshell gel for the demineralised white areas, group 2 had CO2 laser irradiation combined with eggshell gel, and group 3 was treated with sodium fluoride gel. Subsequently, the teeth were immersed in artificial saliva for a duration of 30 days. The hardness of the enamel surface was assessed using the Vickers method. A scanning electron microscope (SEM) was used to analyse a sample from each of the groups in order to identify the enamel surface's morphological characteristics. The data was assessed using Tukey's test and repeated measures one-way analysis of variance (ANOVA).

Results: In all three groups under study, the microhardness of dental white spot lesions increased. The use of eggshell gel combined with CO2 laser therapy (group 2) resulted in a greater increase in microhardness compared to sodium fluoride gel treatments (group 3) and was marginally superior to the use of eggshell gel alone (group 1).

Conclusion: The using of eggshell gel, eggshell gel with CO_2 laser, sodium fluoride gel are suggested to reharden the tooth enamel and the treatment with eggshell gel with CO_2 laser showed a higher elevation in microhardness than sodium fluoride gel.

Key words: CO_2 laser, eggshell gel, microhardness, white spot lesion

Introduction

"Subsurface enamel pores from serious demineralization" is the definition of the White Spot Lesion (WSLs), which manifests as "a milky white opacity when situated on smooth surfaces. (LeRoy, 2021). Clinically, an early caries lesion in enamel appears as a white, opaque area at first. It is distinguished by being softer than the nearby healthy enamel and becoming whiter as air dries it (Khurshid et al. 2015).

White spots on enamel might be categorized as WSLs, opacities, or dental fluorosis. Russell has created a set of standards to distinguish between opaqueness and fluorosis. Fluorosis was described by Russell's criteria as poorly defined white or yellowish lesions (Arya, 2019). Minerals are essential for remineralization in the treatment of white spot lesions. Minerals are abundant in chicken eggshells. Although chicken eggs are an essential source of nutrition, microbial activity in their shells pollutes the environment. The leftover eggshells were frequently thrown away as rubbish, which degrades the soil and produces stink. A natural bio-ceramic material, the outer coating of chicken eggs has been investigated since 1969. The fibrous tissue known as the eggshell membrane (ESM) contains a lot of collagen, structurally important proteins, and natural glycoproteins. The calcium carbonate, calcium dioxide, and calcium hydroxide found in the well-organized egg shell can have a beneficial impact on bone metabolism and oral health. The trash could reduce household waste and serve as a natural supply of calcium hydroxide, calcium carbonate, and calcium oxide (Niazy, 2021).

Lasers are light-amplification devices that were initially used in dentistry in the 1960s. They were originally presented in 1959. Coherency, monochromaticity, collimation, and high intensity are some of its physical characteristics. In 1989, the 3W Neodymium-Doped Yttrium Aluminum Garnet (Nd:YAG) laser became the first surgical laser. Erbium-Doped Yttrium Aluminum Garnet (Er:YAG) followed in 1989. The optical parameters of the tissue and the laser determine the laser-tissue interaction. Wavelength, power density, and application mode were three crucial aspects of laser application. The possibility of CO2 lasers, which produce light at particular wavelengths, to prevent dental cavities has been investigated. The caries prevention mechanism is yet unknown, though. There was no thorough analysis of research examining the benefits of CO_2 lasers in preventing dental cavities, according to a literature search (Luk et al., 2019).

According to References of these studies, CO_2 lasers had a major impact on the demineralization reduction, chemical alterations, and enamel morphology. Higher microhardness values, shallower lesions, and an 87% reduction in the development of caries were seen on laser-treated enamel surfaces. Analysis of cross-sectional microhardness revealed that groups exposed to laser radiation showed less mineral loss (Luk et al., 2019).

The aim of this study was to evaluate and compare the effectiveness of chicken eggshell–based remineralizing gel, used alone and in combination with CO_2 laser irradiation, on the rehardening of enamel white spot lesions, and to assess its performance relative to conventional sodium fluoride gel by measuring changes in enamel surface microhardness and morphological characteristics.

Materials and Methodologies

Sample preparation: After extraction due to orthodontic treatment, thirty maxillary fist the premolars with intact crowns were collected, cleaned, and stored at room temperature in thymol (Heravi et al., 2014). Ethical approval number is MUPRV005 All specimens were submerged in 10 millilitres of demineralising solution for 96 hours in order to create fake enamel white patches (Kamath et al., 2017). 0.05 M lactic acid, 2.2 mM of calcium chloride ($CaCl_2$), 2.2 mM sodium dihydrogen ions phosphate (NaH_2PO_4), and 0.2 ppm fluoride were all present in the demineralising solution. The solution's pH was lowered to 4.5 by adding 50% NaOH. Every day, the answer was changed.. Subsequent to immersion in the demineralising solution, the samples were preserved in an incubator at a temperature of 37°C (Lata et al., 2010).

Samples were cleaned using distilled water after the previously indicated process. Using a ruler, a 6 mm circle window was drawn on the buccal portion for every tooth to identify the centre region of the buccal surface. An imaginary line was traced across the distal and mesial surfaces of the teeth at their most pronounced curvature, and another line was drawn from the tip of the cusp of the buccal region to the cervical line. The tooth surfaces were coated with acid-resistant nail varnish after a circular adhesive tape measuring 6 mm in diameter was attached and smoothed over the buccal surface. After that, a circular hole was made in the tooth's lingual surface by removing the adhesive tape. After polishing and abrading each tooth to determine its microhardness, the teeth were repositioned in an acrylic model. The samples were kept at 37 °C in artificial saliva. Twenty white chicken eggs were gathered, their contents extracted, and filtered water was used to clean the eggshells. The eggshells were immersed in a hot water bath for 10 minutes prior to the internal membrane being removed. A household grinder was used to crush the dry shells into a coarse powder. After obtaining the treatment material, it was dissolved in distilled water at a 10% w/v concentration while being stirred. Polyethylene oxide (MW = 60,000) was then gradually added to the suspension to create a gel-like form of the treatment materials (Niazy, 2021).

Sample Distribution:

Three groups were picked at random from the samples: Eggshell gel (e) (n = 10), CO_2 laser radiation + Eggshell gel (L + E) (n = 10), and fluoride of sodium gel (F) (n = 10) are the first, second, and third groups, respectively.The enamel samples in group 1 received the treatment ingredient three times; it was put on with a microbrush for 20 seconds, left on for 10 minutes, followed by rinsed off with distilled water.
Dental air spray was used to sufficiently dry the teeth in group 2 . The CO_2 laser (MultiXel (DS-40UB), DAESHIN COMPANY, Seoul, South Korea) has a wavelengths of 10.6 μm, an oscillation rate of 20 Hz, an output power of 1 W, and a beam width of 0.6 mm. It uses a sweeping motion to operate for 30 seconds at an

offset of 2 mm. Its pulses last 10 and 15 milliseconds. Next, eggshell gel was used (Rafiei, 2020).

In group 3, the enamel blocks were treated with a pH 6.5–7.5a gel containing 2% NaF for four minutes over the course of three days. After using a cotton swab to remove any extra gel, they were rinsed with distilled water for about 30 seconds and dried with absorbent paper.

Microhardness Test: Prior to any sort of treatment, the enamel microhardness was first assessed for regular enamel as a baseline for microhardness testing. Following the production of a white spot lesion, the enamel microhardness was then assessed. Following treatment with the substance, the samples were tested for the microhardness test. A load of 200 grams was applied for 20 seconds in order to test the microhardness. Each specimen underwent three grooves and three records matching to these indentations in order to quantify the microhardness test. The mean of these three recordings was then computed. Using an optical microscope and a square-based diamond indenter with a 136° included angle between the opposing sides, the Vickers microhardness measurement was conducted.

software SPSS (V25) was used to evaluate the data and determine the microhardness mean and standard deviations for each group. The data was initially examined for normality using the Kolmogorov-Smirnov test, which verified that the distribution of the microhardness parameters was normal. To determine if the data had a distribution that was normal, a repeated-measures one-way ANOVA was employed. The group means were compared using Tukey's test.

Results

Table (1) showed the normality test of microhardness among study groups. The result reveled that microhardness was normally distributed among groups and phases using Shapiro Wilk test (p >0.05).

Table (2) displayed each testing group's baseline, demineralization, and treatment sample counts together with descriptive information such as averages, standard deviations, minimum and maximum values, and Findings show that in all groups surface microhardness decreased from baseline after demineralization then increase after treatment with significant change (p =0.000). In treatment phase, the high surface microhardness in CO_2 laser + ESP (231.0) followed by ESP (209.5) while the lower value in F. gel (176.8) with significant difference (p =0.033).

The mean microhardness values of intact enamel surfaces after demineralisation and treatment with eggshell particles, carbon dioxide (CO2) laser and eggshell particles, and fluoride gel are shown in Table 2. The findings indicate that the enamel microhardness values of all groups considerably decreased during the demineralisation phase in comparison to the microhardness values of intact teeth. After treatment with different agents during the remineralisation phase, all groups demonstrated a significant increase in microhardness values, with the fluoride gel group displaying the smallest enhancement in enamel microhardness, while the laser with eggshell group exhibited the greatest improvement.

According to the study, there were statistically insignificant changes in microhardness values between the groups during both the sound and demineralization stages ($p>0.05$). There were statistically significant differences between the groups for the remineralization stage ($p<0.005$).

Table (3) presents the multiple pairwise comparisons of surface microhardness across the groups (ESP, CO2 Laser + ESP, F gel) throughout the treatment phase, using Tukey HSD. The P value for the comparison between group 2 and group 3 was 0.048.

Table (4) shows the surface microhardness recoveries descriptively and statistically for the groups (ESP, CO2 Laser + ESP, F gel). A P value of 0.004 means that all groups showed a boost in microhardness after intervention.

Figure (1) illustrates the contrast of mean enamel microhardness after remineralisation, juxtaposed with baseline and post-demineralization measurements for each group. The group (L +ESP) had the greatest enamel microhardness after remineralisation. Figure (2) Scanning Electron Microscope (SEM) illustrated the microscopic features of the intact enamel surface prior to any intervention, revealing a clean enamel surface devoid of surface deposits. Following 96 hours of demineralisation using a demineralising solution, the Scanning Electron Microscope (SEM) showed microscopic changes in the enamel surface, including irregularities, fissures, and porosities over the affected area, as seen in Figure (3). In Figure (4), this sample was treated with eggshell particles, revealing a rather smooth and homogenous surface, accompanied by the presence of globules and crystals as seen in the SEM. After treatment with laser then application of eggshell particles the (SEM) shows in Figure (5) a somewhat smooth and homogeneous surface with the presence of some small globules and crystals deriving from eggshell particles, zones of demineralization like cracks and voids were still appearing in some regions on the enamel surface. In figure (6) showed the treatment material was used is sodium fluoride in which (SEM) showed Some fine globular structures probably derived from fluoride precipitate could be seen, zones of demineralization like cracks and voids were still appearing in some regions on the enamel surface.

Discussion

De-mineralization and re-mineralization cycles are ongoing in the hard dental tissues. When demineralization surpasses remineralization, caries continues to progress. Remineralization is the process of adding calcium, phosphate, and fluoride ions to the tooth surface's demineralized enamel from an outside source. Re-mineralization occurs when calcium and phosphate in saliva re-crystallize among the enamel or dentine crystals, making them more acid-resistant than they were initially (Mohamed et al., 2016).

Since enamel is the initial line of resistance against the advancement of caries, it was chosen as the tooth substrate for this investigation instead of dentin. Despite its hardness and density, enamel has local variations in porosity and acid solubility; instead of dissolving the enamel layer by layer, acids can dissolve tooth minerals regionally by penetrating deeply into the enamel. Thus, it is crucial to remineralize early enamel lesions in order to save other tooth tissues [Talwar et al., 2019].The present study's findings indicated that treatment of enamel samples with ESP resulted in a statistically significant increase in microhardness values. This may suggest the inclusion of calcium and phosphorus ions that diminish porosity and enhance the microhardness of demineralised enamel. Calcium ions are the primary constituents of enamel surface preparations; hence, their integration into the outer enamel surface increases microhardness values. The eggshell powder mostly consisted of calcium oxide and phosphorus. The increased concentration of bioavailable calcium ions was crucial for the remineralisation of enamel (Shen et al., 2011).

It has been shown that CO2 lasers enhance the microhardness of enamel surfaces (Rafiei, 2020). This research demonstrated that the application of eggshell, either alone or in conjunction with laser treatment, significantly enhanced the microhardness of white spot lesions (WSLs). The increase in microhardness within the combination treatment group may result from structural alterations induced by the laser. The laser's heat induces chemical alterations to the surface, resulting in the creation of more acid-resistant layers, such as calcium pyrophosphate, a more durable substance that exhibits lower solubility in acids compared to hydroxyapatite, so augmenting the enamel's hardness (Apel et al.,2005).

The study suggests that CO_2 laser irradiation before eggshell has a better effect on teeth when compared to the use of sodium fluoride gel and this preference might be due to its ability to penetrate deeper enamel layers and reharden demineralized enamel. Laser irradiation can also cause the formation of micro spaces in enamel, which has been confirmed in previous studies [9]. However, SEM images of samples showed cracks and fine scrapes in groups with laser irradiation, possibly due to the demineralized and weak surface of the lesions. The treated surfaces with eggshell showed a globular pattern of minerals, suggesting that covering the enamel surface with eggshell may create a protective layer against decay and demineralization. Also, the result of this study showed that the use of CO_2 laser before eggshell gel gave the same result as the studies that using CO_2 laser in addition to hydroxyapatite since that the main component of eggshell is hydroxyapatite (Tlotleng et al., 2014), (Al_Bazaz et al., 2023)

The CO2 laser had an inhibiting impact on enamel demineralisation [Raghis et al., 2018]. Post-CO2 laser treatment, microhardness values in group 2 exhibited a significant increase. Numerous investigations (Farhadian et al., 2017) (Rafiei, 2020) ,

(Fekrazad et al., 2017). have shown that CO2 laser irradiation improves the microhardness of demineralised enamel, corroborating this finding. These results are consistent with prior investigations [(Poosti et al., 2014) , (Chen & Huang, 2009),(Esteves-Oliveira et al., 2011).

Conclusions

The Using of eggshell gel, eggshell gel with CO_2 laser, sodium fluoride gel are suggested to reharden the tooth enamel and the treatment with eggshell gel with

CO_2 laser showed a higher improvement in microhardness than sodium fluoride gel.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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Table (1) Assessment of normality for surface microhardness across phases (baseline, demineralisation, and treatment) and groups (eggshell, CO2 laser, and eggshell with fluoride gel).

Groups	Baseline			Demineralization			Treatment			SMHR		
	Statistic	df	*p* value	Statistic	df	*p* value	Statistic	df	*p* value	Statistic	df	*p* value
ESP	0.956	10	0.742	0.946	10	0.620	0.903	10	0.235	0.888	10	0.161
CO_2Laser +ESP	0.930	10	0.449	0.895	10	0.191	0.856	10	0.069	0.894	10	0.190
F. gel	0.892	10	0.189	.870	10	0.101	0.921	10	0.365	0.923	10	0.384

ESP=Eggshell particle, F=fluoride

Table (2) Surface microhardness is evaluated and statistically examined among groups (ESP, CO2 Laser + ESP, and F gel) and phases (baseline, demineralisation, and treatment).

		ESP	CO_2 Laser +ESP	F. gel	F	*p* value
Baseline	Minimum	117.000	193.000	132.000	0.778	0.470
	Maximum	424.000	439.000	347.000		
	Mean	290.100	325.500	288.300		
	±SD	93.751	63.137	64.831		
Demineralizing	Minimum	82.000	68.000	103.000	0.099	0.906
	Maximum	238.000	286.000	222.000		
	Mean	146.100	155.100	147.300		
	±SD	45.931	61.167	37.331		
Treatment	Minimum	100.000	186.000	115.000	4.151	0.033
	Maximum	278.000	290.000	250.000		
	Mean	209.500	231.000	176.800		
	±SD	60.130	41.929	41.055		
F		20.618	29.252	16.950		
p value		0.000	0.000	0.000		

ESP=Eggshell particle, F=fluoride

Table (3) Numerous pairwise comparisons of surface microhardness across groups (ESP, CO2 Laser + ESP, F gel) were conducted throughout the treatment period using Tukey HSD.

Groups		MD	P value
ESP	CO_2 Laser +ESP	-21.500	0.589
	F. gel	32.700	0.303
CO_2 Laser +ESP	F. gel	54.200	0.048

Table (4) Descriptive and statistical analysis test of Surface microhardness recovery among groups (ESP, CO_2 Laser +ESP, F gel)

Groups	Minimum	Maximum	Mean	±SD	F	*p* value
ESP	24.189	75.221	46.678	19.430	6.940	0.004
CO_2 Laser +ESP	25.118	96.000	47.383	19.804		
F .gel	9.655	41.379	22.911	8.314		

ESP=Eggshell particle, F=fluoride

Table (5) Multiple pairwise comparison of surface microhardness among phases by groups (ESP , CO_2 Laser +ESP , F gel)using Tukey HSD.

Groups	phase	phase			
		Demineralization		Treatment	
		MD	p value	MD	p value
ESP	Baseline	144.000	0.000	80.600	0.001
	Demineralization			-63.400	0.000
CO_2 laser+ ESP	Baseline	170.400	0.000	94.500	0.000
	Demineralization			-75.900	0.000
F. gel	Baseline	141.000	0.000	111.500	0.000
	Demineralization			-29.500	0.026

Figure Legend

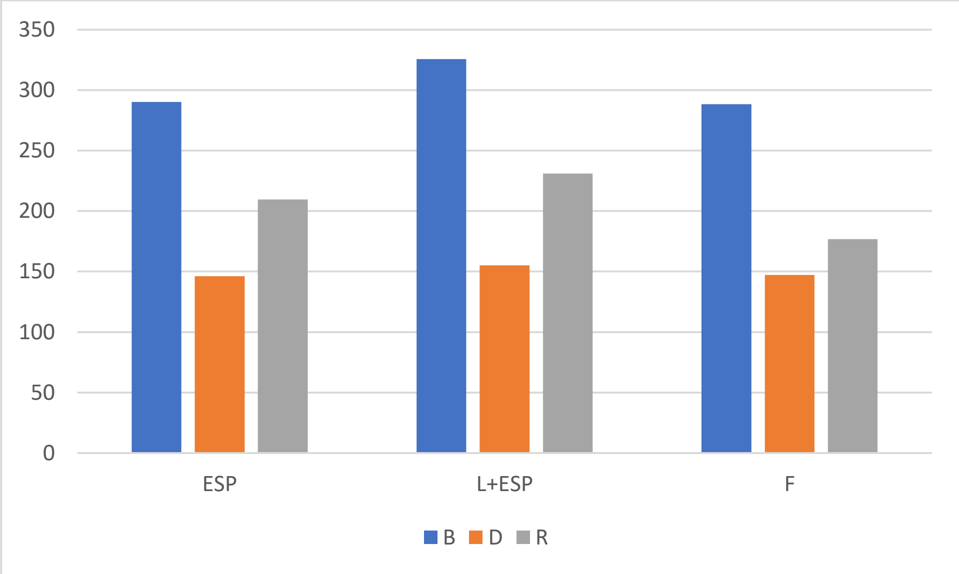

Figure (1) after ،Means of enamel microhardness at baseline **demineralization and following remineralization. ESP=Eggshell particle, F=fluoride, B=Baseline, D=Demineralization, T=Treatment**

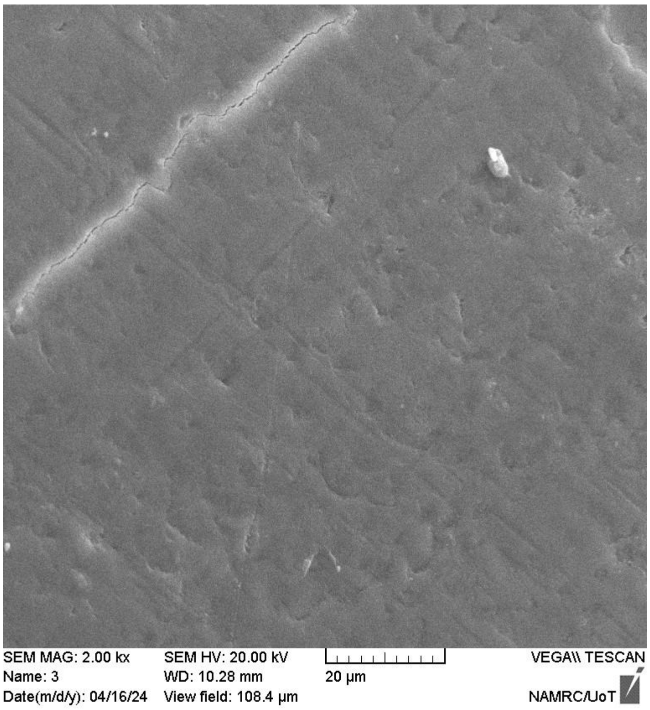

Figure (2) Scanning Electron Microscope (SEM) for normal sound enamel surface shows intact enamel with no cracks

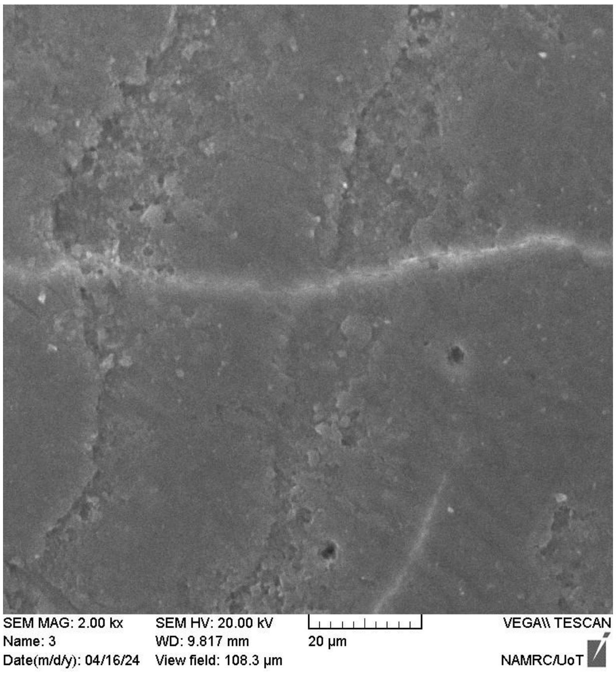

Figure (3) Scanning Electron Microscope (SEM) Enamel surface of tooth demineralized with demineralizing solution indicated voids and cracks for an affected enamel

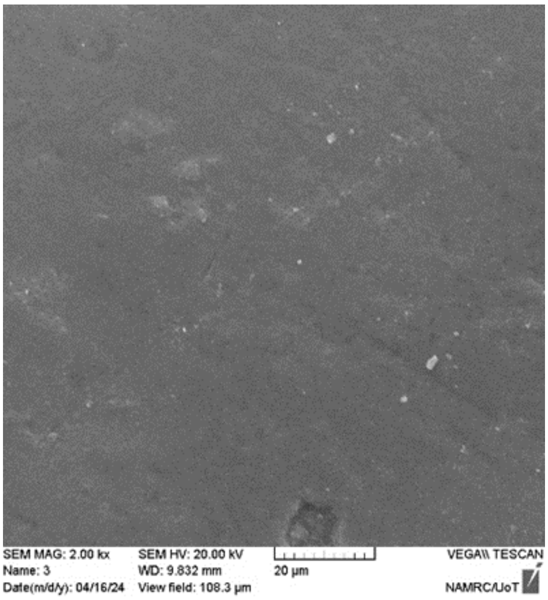

Figure (4) Electron Microscope (SEM) for Eggshell group shows presence of some globules and crystals

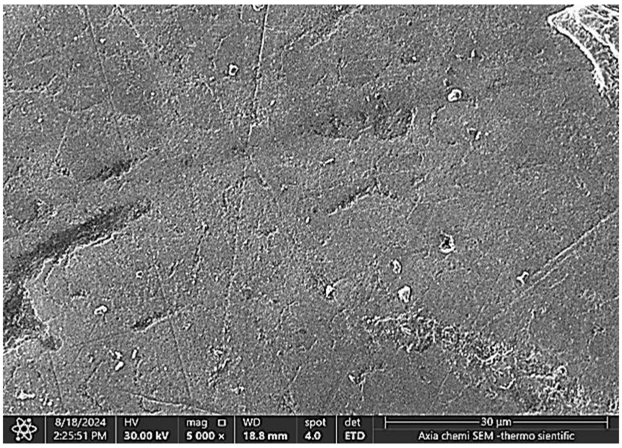

Figure (5) Scanning electron microscope (SEM) for group 2 (CO_2 laser and eggshell) shows surface with the presence of some globules and crystals

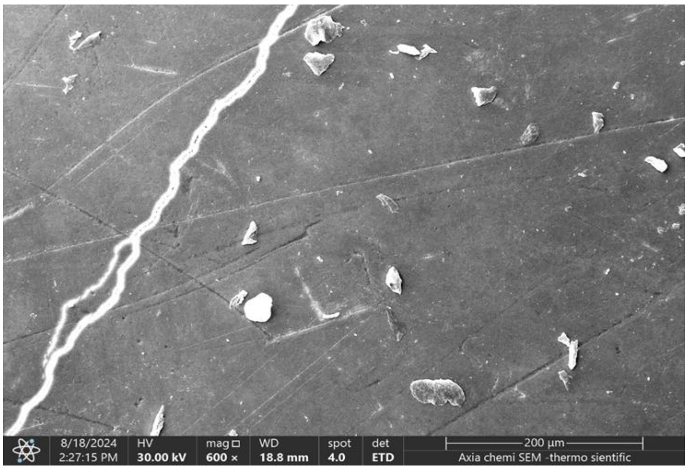

Figure (6) SEM image for group 3, enamel surface exposed to sodium fluoride where the presence of some gl